Molecular Mapping of the Stripe Rust Resistance Gene *Yr69* on Wheat Chromosome 2AS

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Abstract

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Wheat is one of the major food crops in the world. Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is an economically important disease that affects wheat worldwide. The discovery of novel resistance genes and the deployment of effectively resistant cultivars are important for the ongoing control of wheat stripe rust and the maintenance of the agricultural productivity of wheat. CH7086, a new stripe rust-resistant wheat introgression line, was selected by crossing susceptible cultivars with the resistant *Thinopyrum ponticum*-derived partial amphiploid Xiaoyan 7430. The resistance of CH7086 is effective against all current Chinese *P. striiformis* f. sp. *tritici* races. CH7086 was crossed with the stripe rust-susceptible cultivars to develop F_1 , F_2 , F_3 , and BC_1 populations for genetic analysis. Segregation in the F_2 and BC_1 populations and $F_{2:3}$

lines were tested for resistance against the *P. striiformis* f. sp. *tritici* race CYR32. This test showed that CH7086 carries a single dominant gene for stripe rust resistance, which was temporarily designated *YrCH86*. The closest of the eight simple sequence repeat (SSR) and expressed sequence tag-SSR markers flanking the locus were X2AS33, which is 1.9 cM distal, and Xmag3807, which is 3.1 cM proximal. The resistance gene and its polymorphic markers were placed in deletion bin 2AS-0.78-1.00 using the 'Chinese Spring' nullisomic-tetrasomic, ditelosomic, and deletion lines. The tests of both allelism and resistance specificity suggested that the resistance gene found in CH7086 was not *Yr17*, which was the only current formally named *Yr* gene on chromosome 2AS. Thus, *YrCH86* appeared to be a new locus and was permanently designated *Yr69*.

Food security currently depends on the production of wheat, corn, and rice, which are the three major cereals. Stripe (or yellow) rust of wheat, caused by the fungus *Puccinia striiformis* f. sp. *tritici*, is one of the major restraints to wheat production worldwide. It not only limits photosynthesis by reducing photosynthetic area but also secludes products for assimilation in spore production, which ultimately limits growth and yield, especially in moist and cool environments. The loss of crops to stripe rust is commonly in the range of 10 to 70%, but under extreme epiphytotic conditions, this disease can increase losses to 100% (Chen 2005). In terms of crop area, China is the largest stripe rust-affected region in the world, with more than 20 million hectares of wheat vulnerable. In the years 1950, 1964, 1990, and 2002, severe epidemics caused significant yield losses estimated at 6.00, 3.20, 2.65, and 1.40 million tons, respectively (Chen et al. 2009). The disease can be controlled economically through the use of foliar fungicides in high productivity areas. However, fungicides may add more cost, as well as potentially impacting the environment and human health. In addition, the use of fungicides continuously increases the selection pressure for potentially fungicide-tolerant strains of the pathogen. Accordingly, growing resistant cultivars is more effective for the control of stripe rust and can also have fewer detrimental environmental effects. However, the deployment of resistant cultivars depends upon

the availability of resistance genes and detailed knowledge of their inheritance.

Two types of disease resistance to stripe rust are widely recognized, seedling resistance and adult plant resistance. Seedling resistance, also called all-stage resistance, is effective at the seedling stage but is also expressed during all growth stages, and adult plant resistance is expressed at the postseedling stage (Chen 2005). Currently, more than 60 permanently designated resistance genes to stripe rust (*Yr1* to *Yr67*) have been cataloged in wheat (Cheng et al. 2014; <http://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2013.pdf>). Some of these genes have been widely used in wheat breeding worldwide (Chen 2005). However, genes for race-specific resistance are generally not durable in released resistant cultivars, due to the rapid selection through mutation or recombination and an increase of previously rare variants in the pathogen population. A good example of this occurring is *Yr9*. *Yr9* is a rye-derived resistance gene that was introduced and broadly used in Chinese wheat breeding programs in the 1960s. It became ineffective due to the appearance and spread of the stripe rust race CYR29 in 1985, which led to yield losses of 2.65 million tons in 1990. A more recent destructive epidemic happened in 2002 and was caused by race CYR32. It affected varieties with the so-called Fan 6 source of resistance and led to production losses of 1.31 million tons (Wan et al. 2007). CYR32 and CYR33 were identified most often in China among the races detected from 1997 to 2009, and they played a major part in causing the widespread epidemics of wheat stripe rust in recent years (Chen et al. 2009; Zeng et al. 2014). These races have combined virulence for wheat containing the resistance genes *Yr3b*, *Yr4b*, *Yr9*, and *YrSu*, one or more of which occurs in the cultivars covering 90% of the total winter wheat area in the entire country (Kang et al. 2010). Consequently, these races are major components of the pathogen population used by breeders when screening for stripe rust resistance (Wang et al. 2010). After the *Yr3b*, *Yr4b*, *Yr9*, and *YrSu* genes became

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ineffective against the predominant races of stripe rust, lines and cultivars with *Yr24* (= *Yr26*) were widely grown in Sichuan and Gansu provinces (Li et al. 2006). During the 2008-2009 crop season, a new race, CH42 (or v26), was detected in Sichuan on the previously leading resistant wheat cv. Chuanmai 42 containing the *Yr24* gene (Liu et al. 2010). This race is also virulent to wheat lines with *Yr10*, which is effective against all previously identified Chinese *P. striiformis* f. sp. *tritici* races (Zeng et al. 2014). Therefore, an effective strategy to counter the detrimental effects of the evolution of new rust pathotypes is to search for new rust resistance genes and, subsequently, strategically deploy these new genes in commercial cultivars.

Molecular markers are useful in genetic mapping in wheat. Molecular markers are based on polymorphisms in DNA sequences and are independent of the environmental and developmental factors. Therefore, they facilitate the selection of the genes controlling a trait like disease resistance. Simple sequence repeats (SSR), or microsatellites, are one of the favored genetic markers due to their high level of polymorphism, genomic abundance, and codominance. Closely linked microsatellite markers have already been reported for many stripe rust resistance genes, including *Yr5*, *Yr15* (Murphy et al. 2009), *Yr10* (Singh et al. 2009), *Yr17* (Helguera et al. 2003; Jia et al. 2011), *Yr24/Yr26* (Wang et al. 2008), *Yr50* (Liu et al. 2013), *Yr64*, and *Yr65* (Cheng et al. 2014). Molecular markers closely linked to such genes have been useful in map-based cloning (Fu et al. 2009) and have facilitated the utilization and incorporation of multiple genes into breeding programs (Murphy et al. 2009).

In our previous screen of the wheat germplasm for resistance to stripe rust in the field nurseries at Chengdu, Sichuan province and under greenhouse conditions at Taiyuan, Shanxi province from 2005 to 2012, we identified a number of common wheat genotypes with effective resistance against the common Chinese races, including CYR32, CYR33, and CH42 (Zhijian Chang unpublished data). The objectives of the present study were to characterize stripe rust resistance in the wheat germplasm

line CH7086 and to identify molecular markers closely linked to it.

Materials and Methods

Plant materials and populations. The CH7086 line analyzed in this study is a homogeneous BC₂F₇-derived wheat line that was produced by crossing the hexaploid wheat (*Triticum aestivum* L.) cv. Zhong (Z) 8701 accession, as the female parent, with the Xiaoyan (XY) 7430 accession, as the donor of stripe rust resistance. The hybrid was backcrossed twice to another wheat accession, cv. Jimai (JM) 26, and the resulting resistant plants from the BC₂F₂ population were allowed to self-fertilize for five generations. The XY 7430 accession is a *Thinopyrum ponticum*-derived partial amphiploid with the pedigree of Misuzao (MSZ)/R431 (a *Thinopyrum ponticum* accession from the former Soviet Union) and Xiangyang (XY) 4 (Li et al. 1985).

Other plant materials including the *Thinopyrum ponticum* accession R431, XY 7430, and all the wheat parents involved in breeding XY 7430 and CH7086 (Table 1) were used in testing the reactions to the *P. striiformis* f. sp. *tritici* races to see if the resistance was derived from this wild species. Also included were the wheat genotype 'Chinese Spring' (CS) and various CS nullisomic-tetrasomic (NT) and ditelosomic (Dt) stocks and deletion lines obtained from B. Friebe, Wheat Genetic and Genomic Resources Center, Kansas State University (Manhattan, KS, U.S.A.).

CH7086 confers a high level of resistance to all the existing Chinese *P. striiformis* f. sp. *tritici* races, whereas Taichung (TC) 29 and Mianyang (MY) 11 are susceptible to most stripe rust races. To study the inheritance of the stripe rust resistance, CH7086 was crossed to the susceptible cultivars TC 29 and MY 11 to generate segregating populations. F₂, F₃, and BC₁ populations were tested for stripe rust response. A F₂ population and the derived F₃ families from the TC 29 × CH7086 cross were further used for molecular marker screening and genetic mapping. The mapping population was comprised of 205 F₂ plants and 198 derived F₃ families. The difference between the F₂ plants and the F₃ families is due to the loss of seven susceptible F₂ plants.

Table 1. Seedling infection types on selected donor lines, parents and controls using the nine Chinese races of *Puccinia striiformis* f. sp. *tritici*

Race	Virulent to		Infection type ^b									
	Differential line ^a	Known <i>Yr</i> gene	CH7086	TC 29	MY 11	MX 169	R431	XY7430	MSZ	XY 4	Z8701	JM 26
CYR23	1, 2, 3, 4, 6, 7, 8, 9, 11	1, 3, 6	0;	1	0;	4	0	0	3	0;	1	4
CYR29	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 16, 20, 21, 24	1, 2, 3, 3a, 4a, 6, 9, 13, 17, 25, <i>Vir1-2</i> , <i>A</i>	0	4	3	4	0	0	4	4	4	3
CYR31	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 16, 17,	1, 3, 4b, 6, 9, <i>H46</i> , <i>Su</i> , <i>Vir1-2</i> , <i>A</i>	0;	4	4	4	0	0, 0;	4	3	4	3
CYR32	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 20, 21, 24	1, 2, 3, 3a, 4a, 4b, 6, 9, 13, 17, 25, <i>H46</i> , <i>Su</i> , <i>Vir1-2</i> , <i>A</i> , <i>Ky1-2</i> , <i>Ju1-4</i>	0	4	4	4	0	0	4	4	4	4
CYR33	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 24	1, 3, 6, 9, 17, <i>Su</i> , <i>Vir1-2</i> , <i>A</i> , <i>Ky1-2</i> , <i>Ju1-4</i>	0	4	4	4	0	0, 0;	4	4	3	4
Su11-4	2, 3, 4, 6, 7, 8, 9, 10, 13, 14, 16	1, 3, 9, <i>Su</i> , <i>A</i> , <i>Ky1-2</i> , <i>Ju1-4</i>	0;	4	4	3	0	0	0;	3	4	0;
Su11-5	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 14, 16	1, 3, 6, 9, <i>Su</i> , <i>Vir1-2</i> , <i>A</i>	0;	3	4	4	0	0	1	4	3	3
Su11-7	1, 2, 3, 6, 7, 8, 9, 11, 14	1, 3, 6, 9, <i>Su</i> , <i>A</i>	0;	0;	3	4	0	0	4	1	0;	4
CH42	19, 22, 25, 26	10, 24/26	0;	0;	3	4	0	0	4	4	4	3

^a Differential lines: 1 = Trigo Eureka (*Yr6*), 2 = Fulhard, 3 = Lutescens 128, 4 = Mentana, 5 = Virgilio (*YrVir1*, *YrVir2*), 6 = Abbondanza, 7 = Early Premium, 8 = Funo (*YrA*), 9 = Danish 1 (*Yr3*), 10 = Jubilejina 2 (*YrJul1*, *YrJul2*, *YrJu3*, *YrJu4*), 11 = Fengchan 3 (*Yr1*), 12 = Lovrin 13 (*Yr9*), 13 = Kangyin 655 (*Yr1*, *YrKy1*, *YrKy2*), 14 = Suwon 11 (*YrSu*), 15 = Zhong 4, 16 = Lovrin 10 (*Yr9*), 17 = Hybrid 46 (*Yr4b*, *YrH46*), 18 = *Triticum spelta* album (*Yr5*), 19 = Guinong 22, 20 = Maris Huntsman (*Yr2*, *Yr3a*, *Yr4a*, *Yr13*), 21 = Heines VII (*Yr2*, *Yr25*), 22 = AvS Yr10NIL (*Yr10*), 23 = AvS Yr15NIL (*Yr15*), 24 = AvS Yr17NIL (*Yr17*), 25 = AvS Yr24NIL (*Yr24*), and 26 = AvS Yr26NIL (*Yr26*). Information on the virulence formula was based on Li et al. (2006), Chen et al. (2009), and Zeng et al. (2014).

^b Seedling infection types are as described by Li and Zeng (2002): 0 = no visible symptoms; 0 = hypersensitive flecks or necrotic areas without sporulation; 1 = necrotic and chlorotic areas with restricted sporulation; 2 = moderate sporulation with necrosis and chlorosis; 3 = sporulation with chlorosis; 4 = abundant sporulation without chlorosis. Scores of 0 to 2 were considered resistant and 3 and 4 susceptible. XY (Xiaoyan) 7430, the resistance donor of CH7086 is a partial amphiploid with 56 chromosomes and a genomic formula consisting of the wheat complement plus 14 chromosomes from the J and J^s genomic sets of *Thinopyrum ponticum*. R431: *Thinopyrum ponticum* accession of the wild parent of XY7430 with 70 chromosomes and a genomic formula of JJJJ^sJ^s. MSZ (Misuzao) and XY (Xiangyao) 4: the wheat parents of XY 7430; Z (Zhong) 8701 and JM (Jimai) 26: the wheat parents of CH7086.

Pathogen materials and stripe rust response phenotypes. Nine Chinese *P. striiformis* f. sp. *tritici* races (CYR23, CYR29, CYR31, CYR32, CYR33, SY11-4, SY11-5, SY11-7, and CH42) (Table 1) with various virulence gene combinations (Chen et al. 2009; Li et al. 2006; Zeng et al. 2014) were selected to test seedlings of the CH7086, TC 29, and MY 11 plants based on methods described by Li and Zeng (2002). CH42 is a new *Yr24/Yr26*-virulent race that is different from other currently known races in China (Liu et al. 2010). The remaining races cover all of the Chinese race groups and represent the most widely virulent and/or predominant races collected from 2003 to 2007 in the wheat-growing areas of 15 provinces (Chen et al. 2009).

Seedlings of each accession were grown in 70 × 45 × 18-cm flat plastic trays. The highly susceptible cv. Mingxian (MX) 169 was used as a control. Inoculations were performed when the first leaves were fully expanded. Inoculated seedlings were kept in a dew chamber at 17°C for approximately 24 h and, then, were moved to an environmentally controlled greenhouse with a daily cycle of 16 h of light at 18°C and 8 h of dark at 17°C. Reactions were scored on a 0 to 4 scale (Li and Zeng 2002) 18 to 21 days postinoculation, when the susceptible controls were heavily infected (Table 1).

Race CYR32, which is avirulent to CH7086 but virulent to TC 29 and MY 11, was used to test the F_1 , F_2 , and BC_1 populations derived from the TC 29 × CH7086 and CH7086 × MY 11 crosses (Table 2). Seeds from the parents, F_1 , F_2 , F_3 , and BC_1 populations were planted in the field nursery in Chengdu. Eighteen seeds for each parent and F_1 , 180 to 220 seeds of the F_2 , 120 seeds of the BC_1 , and 15 to 20 seeds for each F_2 -derived F_3 family were planted in a randomized design with 15 to 20 plants in a 1.6-m row, 25 cm apart. Susceptible spreaders, we used the cvs. MX 169 or Huixianhong, were planted in every tenth row for each population. The predominant *P. striiformis* f. sp. *tritici* race CYR32 was used for adult plant testing and the spreaders were artificially inoculated two to three times at the seedling stage. Epidemics were initiated by spraying aqueous suspensions of urediniospores of CYR32 with 0.03% Tween 20 onto the spreader rows at tillering. Adult plant reactions were scored at the soft dough stage using the 0 to 4 scale as described by Li and Zeng (2002). The $F_{2:3}$ plants were tested with the same *P. striiformis* f. sp. *tritici* race to determine the genotypes of the F_2 plants from the TC 29 × CH7086 and CH7086 × MY 11 crosses.

Additionally, the F_2 population of the AvS Yr17NIL × CH7086 cross was evaluated in the greenhouse, using race CYR23 to test for allelism of the stripe rust resistance genes in CH7086 and AvS Yr17NIL. Stripe rust inoculation, incubation of the infected plants, and rust scoring also followed Li and Zeng (2002).

Bulk segregant analysis. The parents and 198 $F_{2:3}$ families were subjected to SSR analysis. Total DNA was extracted from the leaves

following the protocol by Stein et al. (2001). Resistant (*Br*) and susceptible (*Bs*) bulks were made from equal amounts of DNA from 10 resistant and 10 susceptible F_2 plants, respectively. DNA bulks of 10 homozygous resistant (HR) and 10 homozygous susceptible (HS) $F_{2:3}$ lines were similarly prepared for bulk segregant analysis (BSA). Markers polymorphic between the resistant and susceptible parents and bulks were used to genotype the entire F_2 and $F_{2:3}$ populations.

Molecular marker analysis. For the initial survey of polymorphic markers, one primer pair from each of the *Xgwm*, *Xwmc*, *Xbarc*, *Xcfd*, *Xcfa*, and *Xgdm* SSR marker series was selected approximately every 10 cM along the chromosomes, according to the reported consensus map (Somers et al. 2004), and was used in BSA. Additional markers from the above series and the *Xgpw* chromosome 2A markers, which showed polymorphisms between the resistant and susceptible bulks, were used in genotyping individual plants.

PCR for each SSR marker was performed using a PTC200 Peltier thermal cycler (Bio-Rad Inc., Hercules, CA) in a total volume of 20 μ l containing 2 μ l of 10× buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂), 0.2 mM of each dNTP, 1 unit *Taq* DNA polymerase, 0.25 μ M of each primer, and 80 to 100 ng of total DNA. Each PCR reaction was performed at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, 50, 55, or 60°C (based on primer annealing temperatures) for 45 s, 72°C for 1 min, and a final extension at 72°C for 10 min, before cooling to 4°C. After amplification, 12 μ l of loading buffer (0.4 g of sucrose per milliliter, 1 mg of bromophenol blue per milliliter, and 1 mg of xylene cyanol per milliliter) were added to the PCR products; 4 to 6 μ l of each sample were loaded on an 8% nondenaturing polyacrylamide gels (Acr:Bis = 29:1) and were separated at 150 V for approximately 2 h, then, were visualized by silver staining.

The availability of the wheat chromosome shotgun survey sequences makes it possible to develop molecular markers. Wheat expressed sequence tags (ESTs) located in bin 2AS-0.78-1.00 were retrieved from GrainGenes 2.0 (<http://wheat.pw.usda.gov/>). These ESTs were blasted against 2AS survey sequences (International Wheat Genome Sequencing Consortium 2014) using BLASTN with an E-value of 0.00001. The contig sequences with the best hits to the ESTs in the blast results were further retrieved and were uploaded onto SSR Primer II (Jewell et al. 2006) to search for SSR markers. The PCR products amplified using the developed SSR markers were separated on 8% nondenaturing polyacrylamide gels.

Chromosome bin assignment and linkage analysis. Chromosomal locations and physical bin mapping of linked microsatellite markers were confirmed using CS nullisomic 2A tetrasomic 2B (N2AT2B), N2BT2D, N2DT2A, and ditelosomic 2AS (Dt2AS) stocks, and the CS chromosome 2AS deletion lines. Linkages between the DNA markers and the resistance gene were established

Table 2. Adult plant segregation of stripe rust responses in the F_2 and BC_1 populations and the F_3 lines when inoculated with the *Puccinia striiformis* f. sp. *tritici* race CYR32^a

IT	Parent			P ₂ /P ₁					P ₁ /P ₃					P ₁ /P ₃ /P ₃	
				No. of plants		No. of lines			No. of plants		No. of lines				No. of plants
						F _{2:3}					F _{2:3}				
	F ₁	F ₂	HR	Seg	HS	F ₁	F ₂	HR	Seg	HS	BC ₁				
	P ₁	P ₂	P ₃												
0	13			11	17	14	3	0	10	26	21	5	0	17	
0;	2			3	79	33	46	0	4	51	13	38	0	28	
1					40	3	37	0		33	9	24	0	9	
2					16	0	16	0		14	3	10	1	4	
3					10	0	2	8		11	0	3	5 ^b	8	
4		13	14		43	0	0	36 ^c		32	0	0	24 ^d	39	
Total	15	13	14	14	205	50	104	44 + 7	14	167	46	80	30 + 11	105	
				χ ² _(3:1) = 0.08		χ ² _(1:2:1) = 0.87			χ ² _(3:1) = 0.05		χ ² _(1:2:1) = 3.38			χ ² _(1:1) = 1.15	
				P = 0.78		P = 0.65			P = 0.82		P = 0.18			P = 0.28	

^a IT = infection type; P_1 = CH7086, P_2 = Taichung 29, P_3 = Mainyang 11; χ^2 value at $P = 0.05$, and 1 df and 2 df are 3.84 and 5.99, respectively; HR, Seg, and HS indicate homozygous resistant, segregating, and homozygous susceptible to the race CYR32, respectively.

^b Three plants dead in the field. They were assumed to be HS.

^c Seven plants dead in the field. They were assumed to be HS.

^d Eight plants dead in the field. They were assumed to be HS.

with JoinMap version 4.0 software (van Ooijen 2006) with a logarithm of odds threshold of 3.0. Map distances were determined using the Kosambi mapping function.

Results

Phenotypic characterization and inheritance of stripe rust resistance. Seedling infection type (IT) data for CH7086, TC 29, and MY 11 tested using the nine Chinese *P. striiformis* f. sp. *tritici* races are shown in Table 1. CH7086 was resistant (IT 0-0;) to all races at the seedling stage, whereas MY 11 was susceptible to all races except CYR23, and TC 29 was resistant to races CYR23, SY11-7, and CH42. These results demonstrated that CH7086 conferred a high level of resistance to all the Chinese *P. striiformis* f. sp. *tritici* races tested.

The adult plant IT data for the parents, F₁ plants, F₂, and BC₁ populations are summarized in Table 2. When inoculated with race CYR32 at the adult plant stage, CH7086 was highly resistant (IT 0), whereas TC 29 and MY 11 were highly susceptible (IT 4). F₁ plants from both crosses were highly resistant and showed ITs (IT 0-0;) similar to the resistant parent CH7086, indicating that the resistance was completely dominant. Some plants in the F₂ and BC₁ populations (Table 2) had intermediate responses. When the numbers of F₂ plants with IT 0 to 2 and IT 3 to 4 were pooled as separate resistant and susceptible groups, respectively, the ratios conformed to those expected for segregation at a single locus. When tested with the same race, the F₃ lines from the TC 29 × CH7086 and CH7086 × MY 11 crosses segregated 1:2:1 HR:segregating (Seg):HS, confirming the segregation of a single gene (Table 2). In addition, the pooled numbers of resistant and susceptible plants in segregating F₃ lines from the Taichung 29 × CH7086 cross were 1,327 resistant and 424 susceptible ($\chi^2_{(3:1)} = 0.58$, $P_{1df} = 0.45 > 0.25$). These results suggested that a stripe rust resistance gene, provisionally designated *YrCH86*, was present in CH7086.

Almost all of the 17 plants scored IT 0 in the F₂ population from the TC 29 × CH7086 cross were HR in tests on the F₃ lines. Of the 56 plants scored IT 1 or 2, 53 produced Seg F₃ lines, and three were HR in the CH7086 × MY 11 cross, 21 of 26 plants that were scored IT 0 were HR and five segregated as F₃ lines. Thirteen of 51 plants that were scored IT 0 were HR and 38 segregated as F₃ lines, whereas among the 47 that scored IT 1 or 2, 34 segregated and 12 were HR. In both populations, the F₃ distributions of HR, Seg, and HS showed a good fit to a 1:2:1 ratio, indicating a single dominant gene could confer the resistance in CH7086. Again, very low proportions of the plants from the two crosses (two plants from TC 29 × CH7086 and three plants from CH7086 × MY 11) that were scored IT 3 proved to be heterozygous rather than HS upon F₃ progeny testing (Table 2).

Identification and mapping of the markers linked to *YrCH86*.

To identify markers linked to *YrCH86*, primer pairs for the SSR markers distributed on all of the chromosomes were used in BSA, using the 198 F₂ plants from the TC 29 × CH7086 cross. The rust response genotypes were inferred from segregation of the F₃ families. Of the 608 GWM, WMC, BARC, CFD, CFA, and GDM microsatellite primers chosen for initial screening, 211 (34.7%) were polymorphic between the parental lines. Only markers *Xwmc382* and *Xgwm636*, which were previously located on chromosome arms 2AS and 2BS, respectively (Somers et al. 2004), were polymorphic between the contrasting parental and bulk populations. The fragments amplified by WMC382 were approximately 160 and 148 bp, and those amplified by GWM636 were approximately 95 and 110 bp in the resistant and susceptible parents, respectively, indicating that these markers are associated with *YrCH86*. Therefore, an additional 76 markers on chromosome 2A were examined, and four polymorphic SSR markers, *Xgpw7101*, *Xgwm210*, *Xwmc25*, and *Xbarc124*, and one sequence-tagged site (STS) marker, *Xmag3807*, were associated with resistance in both the bulk segregant pools and the parents (Fig. 3). These markers were used for genotyping in all 198 surviving plants of the F₂ population. Linkage analyses confirmed the genetic associations of the six SSR and one STS markers with stripe rust resistance. The F₂ population segregated either 3:1 or 1:2:1 for all seven markers (Table 3). Analyses with JoinMap also showed linkage between the markers and *YrCH86*. *Xgwm636*, *Xgpw7101*, *Xwmc382*, and *Xgwm210* were proximal to the resistance gene and *Xwmc25*, *Xmag3807*, and *Xbarc124* were distal. A linkage map of gene *YrCH86* and the closely linked markers with a total map length of 28.2 cM was constructed (Fig. 2).

YrCH86 was bordered distally by *Xgwm636* and proximally by *Xbarc124* (Fig. 2). Both of these markers are located in deletion bin 2AS5 FL 0.78-1.00 (<http://wheat.pw.usda.gov/ggpages/SSRclub/GeneticPhysical/>). A total of 104 SSR markers that were designed based on the sequences of the wheat ESTs assigned to this chromosome region were further tested for the discovery of polymorphic markers. Among them, only the EST-SSR marker X2AS33 (F: attgtttacgccgagca, R: atccagcattacattacatca) was associated with *YrCH86* (Fig. 1A). Based on the linkage analysis, *YrCH86* was placed on chromosome 2AS in bin 2AS5-0.78-1.00 and was flanked by markers X2AS33 and *Xmag3807* at genetic distances of 1.9 and 3.1 cM, respectively (Fig. 2).

Chromosome regional assignment and the possible source of *YrCH86*. Based on the reported chromosomal locations of the linked markers (Eriksen et al. 2004; Marone et al. 2012; Somers et al. 2004), *YrCH86* was putatively assigned to the short arm of chromosome 2A (2AS). However, microsatellite markers are not

Table 3. F₂ genotypes of the Taichung 29 × CH7086 cross inferred from the stripe rust reactions of F₃ families and the corresponding alleles at the eight marker loci^a

Genotype	Total	<i>Xbarc124</i>		<i>Xwmc25</i>		<i>Xwmc382</i>			<i>Xmag3807</i>	
		A- ^a	aa	A-	aa	AA	Aa	aa	A-	aa
<i>YrYr</i>	40	30	10	34	6	33	6	1	35	5
<i>Yryr</i>	82	6	76	5	77	5	68	9	5	77
<i>yryr</i>	36	1	35	0	36	0	6	30	1	35
Total	158	37	121	39	119	38	80	40	41	117
$\chi^2_{(3:1 \text{ or } 1:2:1)}^b$	0.430	0.211		0.008		0.076			0.076	
<i>P</i>	0.806	0.646		0.927		0.962			0.783	

Genotype	Total	<i>Xgwm210</i>			<i>Xgpw7101</i>		<i>Xgwm636</i>			<i>X2AS33</i>		
		AA	Aa	aa	A-	aa	AA	Aa	aa	AA	Aa	aa
<i>YrYr</i>	40	34	6	0	35	5	32	7	1	36	4	0
<i>Yryr</i>	82	4	70	8	8	74	5	67	10	2	77	3
<i>yryr</i>	36	0	6	30	0	36	0	4	32	0	2	34
Total	158	38	82	38	43	115	37	78	43	38	83	37
$\chi^2_{(3:1 \text{ or } 1:2:1)}^b$	0.430	0.228			0.414		0.481			0.418		
<i>P</i>	0.806	0.892			0.520		0.786			0.812		

^a A- = AA or Aa; aa = homozygous for the Taichung 29 allele; AA = homozygous for the CH7086 allele, Aa = heterozygous.

^b Table values of χ^2 for significance at $P = 0.05$, and 1 df and 2 df are 3.84 and 5.99, respectively.

always chromosome-specific (Plaschke et al. 1996). Among the SSR markers linked with *YrCH86*, only *Xbarc124* and *Xgwm636* had previously been mapped solely to chromosome arm 2AS. The remaining markers were reported to occur at more than one location in the genome. For example, *Xgwm210* was assigned to all three chromosomes in the homeologous group 2S, *Xwmc382* to 2AS and 2BS, and *Xgwm7101* to 2AS and 2DS (Somers et al. 2004; Xue et al. 2008). Although most of the markers detect multiple loci, 2AS is a common location for all of them. The locations of the linked marker loci were further verified using CS nullisomic-tetrasomic and ditelosomic lines. Microsatellite primer pairs GWM636, GPW7101, WMC382, GWM210, WMC25, and BARC124 amplified products of the expected size in the CS, CS N2BT2D and N2DT2A, and CS Dt2AS lines, but no PCR products were amplified in the N2AT2B, N2AT2D, and Dt2AL lines (Fig. 3). These results further confirmed the positioning of the linked microsatellite markers to chromosome 2AS.

The microsatellite markers *Xgwm636* and *Xbarc124* linked with the resistance gene were 25.2 cM apart on chromosome arm 2AS (Fig. 2). Because these markers are located in deletion bin 2AS5-0.78-1.00 (Sourdille et al. 2004), we bin-mapped all our markers using the CS deletion lines. All of the markers linked to the resistance gene, including *Xgwm636*, *Xgwm7101*, *Xwmc382*, *Xgwm210*, *Xwmc25*, and *Xbarc124*, and the EST-SSR markers *Xmag3807* and *X2AS33* were located in the 2AS5 deletion bin. *YrCH86* was placed in the interval flanked by *X2AS33* and *Xmag3807*, and the physical location of *YrCH86* was positioned in the 2AS5 deletion bin. The physical and genetic maps of *YrCH86* and the four codominant and four dominant markers are shown in Figure 2.

In the genomic in situ hybridization (GISH) experiment (data not shown) and our earlier work (Zhan et al. 2014), no signal could be detected in CH7086 when using the total genomic DNA of

Fig. 1. A, Silver-stained polyacrylamide gels showing the simple sequence repeat (SSR) markers X2AS33 and B, *Xwmc382* associated with the resistance locus. *P_R* and *P_S* denote CH7086 and Taichung 29, respectively; *B_R* and *B_S* denote the resistant and susceptible bulks, respectively. Selected homozygous resistant (R), homozygous susceptible (S) and heterozygous (H) *F₂* plants from the Taichung 29 × CH7086 cross are included. M, 100-bp DNA ladder; arrows on the left side indicate the fragment linked to the resistance gene.

Fig. 2. The map position of *Yr69* on chromosome 2AS and comparison with other *Yr* genes on this arm. The genetic maps of *Yr17*, *YrSph*, and *YrR61* were from Jia et al. (2011), Chen et al. (2012), Hao et al. (2011), respectively. The order of the simple sequence repeat markers in the physical map for hexaploid wheat was from a USDA GrainGenes database (<http://wheat.pw.usda.gov/cgi-bin/cmap>).

Thinopyrum ponticum as a probe. This indicates that if a translocation was present, its size was too small to be detected through cytology. To determine the possible origin of *Yr69*, the linked markers *X2AS33*, *Xgpcw7101*, *Xmag3807*, and *Xbarc124* were also screened for polymorphisms among the *Thinopyrum ponticum* accession R431, CH7086 and its resistance donor XY7430, as well as their wheat parental lines Z 8701, JM 26, MSZ, and XY 4. The genotyping analysis revealed that no allele in CH7086 was found to be inherited from *Thinopyrum ponticum* at the four marker loci linked to the *YrCH86* gene (data not shown). However, the stripe rust evaluation showed that, when tested at the seedling stage, CH7086 had the same or similarly low reactions to the nine Chinese *P. striiformis* f. sp. *tritici* races as R431 and XY7430, whereas all the wheat parents were susceptible to most *P. striiformis* f. sp. *tritici* races (Table 1). This suggests that a cryptic introgression from *Thinopyrum ponticum* appears to be present in CH7086 and to be associated with rust resistance.

Relationship between *YrCH86* and *Yr17*. *YrCH86* was assigned to the distal part of chromosome 2AS within a region flanked by *X2AS33* (distal) and *Xmag3807* (proximal) (Fig. 2). *Yr17*, derived from *Aegilops ventricosa*, was also assigned to the distal part of 2AS (Helguera et al. 2003) and is the only formally named *Yr* gene in this region of chromosome 2A. A carrier of *Yr17*, AvS Yr17NIL, was resistant (IT 0;) to races CYR23 and CH42 and susceptible (IT 3) to races CYR29, CYR32, and CYR33, at the seedling stage (Zeng et al. 2014), whereas CH7086 was highly resistant (IT 0-0;) to all the races tested (Table 1). This indicated that *YrCH86* differs in specificity from *Yr17*.

In order to further clarify the genetic relationship of *YrCH86* and *Yr17*, 318 F₂ plants from the AvS Yr17NIL × CH7086 cross were inoculated with CYR23. Four susceptible plants were identified, and the frequency of resistant and susceptible plants deviated from the 15:1 ratio expected for independent segregation of dominant alleles at two loci ($\chi^2_{15:1} = 13.53$, $P_{df1} < 0.01$), confirming that *YrCH86* and *Yr17* were neither allelic nor genetically independent. Assuming linkage, the frequency of susceptible plants in a repulsion phase cross is $p^2/4$, where p is the recombination value. The recombination value for *YrCH86* and *Yr17* was estimated to be 0.224. After converting the recombination value, using the formula $d = -1/2 \ln(1 - 2p)$, the map distance was determined to be 29.8 cM.

Discussion

Potential role of *YrCH86* in the improvement of stripe rust resistance. In China, some stripe rust resistance genes have been incorporated into commercial wheat cultivars, but few remain effective against the *P. striiformis* f. sp. *tritici* races CYR32 and

CYR33 (Wang et al. 2010). In our search for new sources of resistance, CH7086 showed high levels of resistance to all current Chinese *P. striiformis* f. sp. *tritici* races in the greenhouse (Table 1) and in the field nurseries. This line also exhibits excellent resistance to powdery mildew (conferred by *Pm51* [Zhan et al. 2014]), but these resistances are not associated. Markers that cosegregate with or are closely linked to *YrCH86* can be used to accelerate the incorporation of this gene into commercial cultivars by marker-assisted selection. Therefore, there is a high likelihood that the material described in the present study is a valuable source for resistance breeding and can be utilized by breeders, especially in southwestern China.

Chromosomal location of *YrCH86* and comparison with other *Yr* genes on 2AS. So far, more than 60 formally designated stripe rust resistance genes have been reported (Cheng et al. 2014; <http://wheat.pw.usda.gov/>). It is essential that wheat pathologists and breeders continue to search for new sources of resistance because of the ongoing emergence and spread of new races virulent to currently resistant varieties. CH7086 is one such source. To investigate the inheritance of the stripe rust resistance in CH7086, we developed segregating populations. The segregation patterns showed that the resistance was controlled by a single dominant allele located on chromosome 2AS. Eight molecular markers on chromosome 2AS were linked to *YrCH86*. We verified the chromosomal locations of the linked microsatellite loci, using the Chinese Spring NT and Dt stocks and 2AS deletion lines (Fig. 3), because molecular markers are not always chromosome-specific (Plaschke et al. 1996). The presence of PCR products of the same size in CH7086 and in the euploid Chinese Spring and the absence of the PCR products in certain deletion lines confirmed the physical locations of the linked markers in deletion bin 2AS-5 (FL 0.78-1.00).

Previously, stripe rust resistance genes *Yr17*, *YrSph*, and *YrR61* were located on chromosome 2AS. *Yr17*, the only formally named *Yr* gene in this region of chromosome 2A, originated from *Aegilops ventricosa* ($2n = 4x = 28$, D^VD^VM^VM^V) (Helguera et al. 2003). More recently, Jia et al. (2011), through a comparative genomics analysis using EST-SSR and the syntenic region in rice, developed a genetic linkage map of the *Yr17* locus. *Yr17* (or the translocation breakpoint) mapped to 9.0 cM distal to *Xgwm636*, whereas, in the present study, *YrCH86* was 13.9 cM proximal to *Xgwm636* (Fig. 2). This indicates that *YrCH86* is proximal to *Yr17* (or the breakpoint) with an estimated genetic distance of 22.9 cM. A test of the allelism of *YrCH86* and *Yr17* indicated a low level of recombination and an estimated genetic distance of 29.8 cM. In addition, the resistance conferred by *YrCH86* to the Chinese *P. striiformis* f. sp. *tritici* races CYR29, CYR32, and CYR33 was also different from that of AvS Yr17NIL (Table 1).

Fig. 3. Amplification patterns of the linked markers in CH7086 (*P_R*), Taichung (TC) 29 (*P_S*), resistant bulk (*B_R*), susceptible bulk (*B_S*), Chinese Spring (CS), nullisomic-tetrasomic (NT) 2A, ditelosomic (Dt) 2AS stocks, and the homozygous deletion line 2AS-5 of CS. Arrows indicate the critical bands.

The recessive *YrSph* gene was located in the region between *Xwmc246* and *Xwmc149*, approximately 13 cM distal to *Xwmc149* (Chen et al. 2012). Because *Xbarc124* was situated 23 cM distal to *Xwmc149* on the consensus map (Somers et al. 2004), *YrSph* is predicted to be approximately 10 cM proximal to *Xbarc124*. In the present study, *YrCH86* was placed 11.3 cM distal to *Xbarc124* (Fig. 2), thus the genetic distance between *YrSph* and *YrCH86* should be approximately 21.0 cM. *YrR61*, a major quantitative trait locus for adult plant resistance explaining 56.0% of the total phenotypic variation in stripe rust response in a RIL population, was also mapped to the terminal bin of chromosome 2AS, approximately 5 cM proximal to *Xbarc124* (Hao et al. 2011), whereas *YrCH86* was placed 11.3 cM distally of that locus (Fig. 2). Therefore, *YrCH86* and *YrR61* seem to be different loci. Based on the combined results, it appears that *YrCH86* is different from the other known *Yr* genes on chromosome 2AS and represents a novel *Yr* locus and, thus, was permanently designated *Yr69*.

Possible origin of the stripe rust resistance in CH7086. Genetic variation is essential for developing new plant varieties. It can be achieved by introducing genes from a related species or, sometimes, from a relatively distant species or even an unrelated species. *Thinopyrum ponticum*, an important perennial Triticeae species, is immune to stripe rust, and certain wheat derivatives of *Thinopyrum ponticum* are also highly resistant. For example, a resistance gene was recently found in the partial amphiploid XY 7430 and the substitution line 6J*(6B), in which the J*-chromosome of *Thinopyrum ponticum* was substituted for chromosome 6B in wheat (Hu et al. 2011). However, there is no published report of the transfer of stripe rust resistance to a wheat chromosome from this species. CH7086, a putative derivative of *Thinopyrum ponticum*, was produced by crossing and backcrossing the XY 7430 line with susceptible wheat cultivars and selecting for stripe rust resistance. However, there was no documented record of the chromosome status of the individual plants involved in each step. CH7086, R431, and XY7430 had very similar reactions to each of the nine *P. striiformis* f. sp. *tritici* races, while all common wheat parents in the pedigree of the line are highly susceptible (Table 1). This indicates that the resistance in CH7086 is assumed to derive from *Thinopyrum ponticum*. Genetic segregation data clearly indicate the presence of a single dominant resistance gene in CH7086 (Table 2). None of the permanently named wheat genes for stripe rust resistance is derived from *Thinopyrum ponticum*. The resistance gene in CH7086 seems to involve a novel locus, as its origin and chromosomal location indicate. However, GISH and Giemsa C-banding analyses of CH7086 have produced no cytological evidence for an alien translocation (Zhan et al. 2014) (data not shown), suggesting that the translocated *Thinopyrum ponticum* chromosomal fragments may be too small to be detected by GISH. Such cryptic alien transfers have been reported in other studies (He et al. 2009; Huang et al. 2014; Kuraparthi et al. 2007; Lu et al. 2015). To determine the origin of the gene, four linked markers around *Yr69* were screened, and a specific marker inherited from *Thinopyrum ponticum* was not found in CH7086; it is possible that we have not tested a sufficient number of markers. More available markers that could have been transferred can be used to provide information on this introgression. The gene *Yr69*, based on its widespread effectiveness and unique chromosome location, must be either present in an intercalary cryptic translocation from *Thinopyrum ponticum* or a wheat gene derived from an unknown source. More detailed studies are needed to better elucidate the source of *Yr69*.

In conclusion, we found a new stripe rust resistance gene, formally named *Yr69*, which was putatively derived from *Thinopyrum ponticum*. It was mapped distally on chromosome 2AS and flanked by the closely linked markers *X2AS33* and *Xmag3807*. These markers, especially if used jointly, are adequate for use in marker-assisted selection aimed at combining multiple resistance genes into a variety to achieve more durable resistance.

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