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Research Article

Identification and Validation of a Major Quantitative Trait Locus for Slow-rusting Resistance to Stripe Rust in Wheat

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Abstract

Stripe (yellow) rust, caused by *Puccinia striiformis* Westend. f. sp. *tritici* Eriks (*Pst*), is one of the most important wheat (*Triticum aestivum* L.) diseases and causes significant yield losses. A recombinant inbred (RI) population derived from a cross between Yanzhan 1 and Xichang 76-9 cultivars was evaluated for resistance to wheat stripe rust strain CYR32 at both the seedling and adult plant stages. Four resistance quantitative trait loci (QTLs) were detected in this population, in which the major one, designated as *Yrq1*, was mapped on chromosome 2DS. The strategy of using the *Brachypodium distachyon* genome, wheat expressed sequence tags and a draft DNA sequences (scaffolds) of the D-genome (*Aegilops tauschii* Coss.) for the development of simple sequence repeat (SSR) markers was successfully used to identify 147 SSRs in hexaploid wheat. Of the 19 polymorphic SSRs in the RI population, 17 SSRs were mapped in the homeologous group 2 chromosomes near *Yrq1* region and eight SSRs were genetically mapped in the 2.7 cM region of *Yrq1*, providing abundant DNA markers for fine-mapping of *Yrq1* and marker-assisted selection in wheat breeding program. The effectiveness of *Yrq1* was validated in an independent population, indicating that this resistance QTL can be successfully transferred into a susceptible cultivar for improvement of stripe rust resistance.

Keywords: Quantitative trait locus; slow-rusting; stripe rust; wheat.

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Introduction

Wheat (*Triticum* spp.) is one of the most important staple food crops, feeding about 40% of the world's population (Gupta et al. 2008). However, the stability of wheat production is challenged by numerous pathogens. Stripe (yellow) rust, caused by the obligate biotroph fungus *Puccinia striiformis* Westend. f. sp. *tritici* Eriks. (*Pst*), is one of the most damaging pathogens to wheat production worldwide, and is especially destructive in China (Chen et al. 2002; Yahyaoui et al. 2002; Wan et al. 2004). Widespread epidemics of stripe rust occurred in 1950, 1964, 1990, and 2002, caused wheat yield losses of 6.0, 3.2, 1.8, and 1.3 million tons, respectively (Li and Zeng 2002; Wan et al. 2004).

A total of 68 *Pst* races have been identified in China (Wan et al. 2007; Chen et al. 2009). Race CYR32 and CYR33,

which were first found in 1994 and 1997, respectively, are now the predominant races (Chen et al. 2009). Numerous studies have indicated that growing resistant cultivars is the most effective, economic and environmentally friendly way to control stripe rust of wheat (Line 2002; Chen 2005). So far, 48 officially named *Yr* genes (*Yr1*-*Yr48*) and many temporarily designated genes have been reported in wheat and its relatives, and some of them have been widely used in different areas worldwide (McIntosh et al. 2008, 2010; Marais et al. 2009; Cheng and Chen 2010; Bansal et al. 2011; Herrera-Foessel et al. 2011; Li et al. 2011; Lowe et al. 2011). However, cultivars carrying a single race-specific resistance gene often become susceptible in a few years after being released due to the rapid evolution of new races (Chen et al. 2002; Wan et al. 2004). Based on recent evaluations in China, genes *Yr5*, *Yr10*, *Yr12*, *Yr13*, *Yr14*, *Yr15*, *Yr16*, *Yr18*, *Yr24*/*Yr26*, *Yr30*, *Yr36*, *Yr39*, *Yr41* and some temporarily designated genes are still effective, whereas lines possessing *Yr1*, *Yr2*, *Yr3*, *Yr4*, *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr20*, *Yr21*, *Yr22*, *Yr25*, *Yr27* and *Yr29* are ineffective to the currently prevalent race (Wan et al. 2004; Wan et al. 2007; Kang et al. 2010). Hence, it is essential to identify new genes with resistance to races CYR32, CYR33 and newly emerged virulent races for wheat breeding programs.

Slow-rusting (Caldwell 1968) or partial (quantitative) resistance (Parlevliet 1975), is characterized by the combined components of a longer latency period, smaller uredinium size, lower infection frequency and reduced spore production (Wilcoxson 1981). This type of resistance is considered to be long lasting or more durable (Van der Plank 1964; Parlevliet 1975). Its longer durability for resistance may be due to the three reasons below. First, slow-rusting resistance which, shows compatible infection types exerts a lower selection pressure on pathogens. Second, due to the additive effects of several minor genes that slow-rusting resistance is based on, it is a greater difficulty for the pathogen to adapt by mutation to a multiple defense based on several (minor) genes. Third, the cloned two slow-rusting genes in wheat, namely *Lr34*/*Yr18* (encoding an ABC transporter) and *Yr36* (encoding a kinase-START protein) (Fu et al. 2009; Krattinger et al. 2009), suggest a different mechanism of slow-rusting resistance from the NBS-LRR (nucleotide-binding site-leucine-rich repeat) based *R*-gene resistance.

It has been difficult to use quantitative resistance in traditional wheat breeding programs, in part, due to both its polygenic nature and the presence of major genes in the germplasm. However, with the availability of tightly linked molecular markers to the resistant loci for use in marker assisted selection, usage of quantitative resistance in the breeding program would be more feasible. Linkage of molecular markers to quantitative trait loci (QTLs) for slow rusting and other yield-related traits (Zhang et al. 2011; Wu et al. 2011; Li et al. 2012) can be determined using comprehensive and dense genetic maps.

At present, more than 2 500 mapped genomic SSR (simple sequence repeat) markers are available in wheat, which will greatly facilitate the identification and fine-mapping of genes (Gupta et al. 2008). However, more markers are still needed, particularly for development of high-density markers in the target region for map-based cloning (Snape and Moore 2007). Resources are now available in public databases for the development of more DNA markers. There are 1 073 668 wheat expressed sequence tags (ESTs) represented in public databases (dbEST, 1 December 2010; http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). A total of 16 000 wheat EST loci have been assigned to individual chromosomal regions or bins (Qi et al. 2004). These deletion-mapped ESTs can be used to develop molecular markers such as SSR and STS (sequence tagged site) markers (Parida et al. 2006; Mohan et al. 2007). Besides, genomic sequence information from model species such as rice (*Oryza sativa* L.) whose genome has been sequenced, has been used for molecular mapping and gene isolation through comparative analysis in wheat (Liu and Anderson 2003; Distelfeld et al. 2004). However, numerous studies show that collinearity between rice and wheat frequently breaks down because of translocations, deletions and duplications of genes (Bennetzen 2000; Li and Gill 2002; Bennetzen and Ma 2003; Sorrells et al. 2003; Lagudah et al. 2006; Lu and Faris 2006; Valárik et al. 2006; Bossolini et al. 2007). These many exceptions found in micro-collinearity between rice and wheat led to increased interests in the genome of *Brachypodium distachyon*, which has been proposed as another model species of cereals (Draper et al. 2001; Vogel et al. 2010). Recently, the genomic sequences of *B. distachyon* have become available (http://ftp.brachypodium.org/files/8X_ASSEMBLY/). Many data suggested that collinearity between wheat and *B. distachyon* is better than between wheat and rice (Foote et al. 2004; Bossolini et al. 2007; Vogel et al. 2010). Thus, the genomic sequences of *B. distachyon* will be another useful resource for molecular mapping and gene isolation in wheat. Furthermore, a draft sequence of the 60 fold coverage of the D-genome (*Aegilops tauschii* Coss.), which was generated by Illumina Solexa next-generation sequencing platform (Jizeng Jia 2010, unpubl. data), will greatly facilitate the progress of marker development and map-based cloning.

The objectives of this research were to identify QTLs conferring slow-rusting resistance to stripe rust in wheat, to develop new markers for saturating a major QTL region, and to validate the major QTL in an independent population. This research will contribute to improvement of wheat resistance to stripe rust by providing tightly-linked markers for marker assisted selection (MAS) inbreeding program and to better understanding of molecular basis of slow-rusting by cloning of the major QTL.

Results

SSR markers and genetic linkage maps

A total of 1 000 SSR markers were screened for polymorphism between the wheat cultivars Yanzhan 1 and Xichang 76-9. Of them, 163 primer pairs (16.3%) showed polymorphisms between the two parents. One hundred and forty-four SSR markers were assigned to the 21 wheat chromosomes by referring to the previous map (Somers et al. 2004), while only 19 SSR markers were not assigned to any chromosome. The constructed SSR linkage maps contained 144 SSR markers and covered all 21 wheat chromosomes, with a total genetic distance of 1 433.4 cM and an average distance between markers of 9.9 cM (Figure S1).

Of the 163 polymorphic SSR markers, 41 markers (25.1%) showed a significant ($P \leq 0.05$) distorted segregation ratio from 1:1 for the two parental alleles based on the χ^2 analyses, and then 36 were mapped and the remaining five were not assigned to any chromosome. Twenty markers (57.1%) showed a segregation distortion in favor of Yanzhan 1. Nine SSR loci were clustered in the regions on chromosomes 2A and 2D (Figure S1). In these regions, all of the distorted marker loci showed an excess of alleles inherited from Yanzhan 1.

Phenotypic analyses

Yanzhan 1 was susceptible to the prevalent race CYR32 in China with a latency period ranging from 320 to 370 hai (hours after inoculation), whereas Xichang 76-9 was slow-rusting resistance to CYR32 with a latency period ranging from 410 to 470 hai in disease evaluation at the seedling stage (**Figure 1A–C**). The disease evaluation at the adult-plant stage showed that Yanzhan 1 and Xichang 76-9 are resistant to CYR32 with an infection type (IT) of 2 to 3 (**Figure 1D**). Disease resistance values of the 118 RILs measured by LP1S (latency period at which the first pustules became visible at the seedling stage), LP50S (latency period at which 50% of the final number of pustules became visible at the seedling stage) and ITA (infection type at the adult-plant stage) were not between that of the two parents, indicating transgressive segregation occurred in this population (**Figure 1B–D**). The latency period and infection type of RILs displayed an approximately continuous distribution and ranged from 290 to 560 hai and from 0 to 9 scales, respectively, indicating the polygenic characteristics of the slow rusting resistance.

Correlations among LP1S and LP50S values obtained from 3 years' experiments were significant ($r = 0.62 - 0.77$, $P \leq 0.01$) and the correlation of ITA between 2009 and 2010's experiments was also significant ($r = 0.74$, $P \leq 0.01$) (Table S1). LP1S and LP50S from the same experiment were highly correlated ($r = 0.96 - 0.98$, $P \leq 0.01$). Moderately negative correlations between ITA and LP1S or LP50S were observed ($|r| = 0.43 - 0.54$, $P \leq 0.01$) (Table S1).

ANOVA of the populations revealed significant differences ($P < 0.0001$) in LP50S, LP1S and ITA among genotypes in the RI population. Highly significant differences ($P < 0.0001$) were also observed for different environments and for genotype × environment interactions (**Table 1**). The broad-sense heritability (h^2) of LP1S, LP50S and ITA were 77.1%, 75.9%, and 83.7%, respectively.

QTL analysis

Quantitative trait locus analysis by composite interval mapping (CIM) methods using each phenotypic dataset (LP1S, LP50S) for each year detected four map positions with LOD (likelihood odds ratio) score exceeding the threshold values, which were obtained by the permutation tests, indicating the presence of at least four QTLs for stripe rust resistance at the seedling stage. These four QTLs were mapped to wheat chromosomes 2DS, 3AS, 6AS and 7BL, designated as *Yrq1*, *Yrq2*, *Yrq3* and *Yrq4*, respectively (**Figures 2** and S3, Table S2).

The largest effect QTL, *Yrq1*, is located in the short arm of chromosome 2D and is linked to locus *Xgwm455* (**Figures 2** and S3). At the seedling stage, *Yrq1* was consistently detected with very high LOD values (from 8.3 to 21.8) based on both LP1S and LP50S datasets from all 3 years' experiments, explaining 17.7 to 48.1% of the phenotypic variance (Table S2). At the adult-plant stage, the CIM analysis also revealed that 17.6% and 24.3% of the phenotypic variance were explained by this QTL in 2009 and 2010's dataset, respectively (Table S2).

Development of the region-specific markers for a major effect QTL

Comparative genomic analysis using sequences of restriction fragment length polymorphism (RFLP) probes flanking the *Yrq1* on chromosome 2DS showed that this region in wheat is syntenic to an interval of 0.93 to 2.15 Mb (covering a 1 220 000 bp region) in *B. distachyon* chromosome 5 and an interval of 0.47 to 5.8 Mb (covering a 5 330 000 bp region) in rice chromosome 4 (**Figure 3**). A total of 103 predicted genes in the homologous region of *B. distachyon* was used to search the homologous wheat ESTs. In total, 60 homologous wheat ESTs were identified. Twenty genomic DNA scaffolds from the draft sequence of the 60-times of genome-equivalent of the D-genome (*A. tauschii* Coss.) were identified through BLAST search by using the homologous wheat ESTs. Sixty one SSRs from homologous scaffolds were found, and six SSR markers were polymorphic between the parental genotypes (Yanzhan 1 and Xichang 76-9). The newly developed SSR markers were mapped slightly more distal to the *Yrq1* position (**Figures 4** and **5**). In order to develop more SSR markers in the

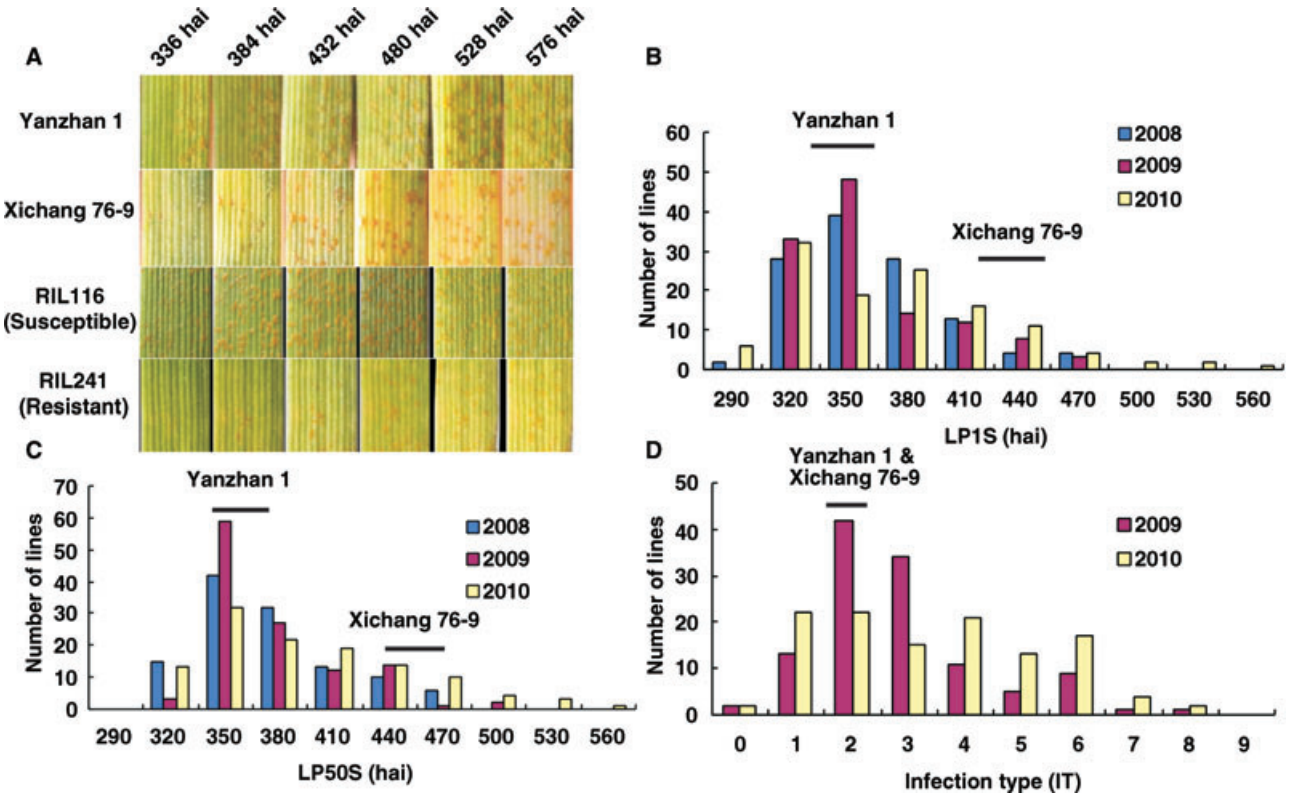

Figure 1. The phenotypes of 118 recombinant inbred lines (RILs) (F_8) derived from a cross between Yanzhan 1 and Xichang 76-9.

(**A**) Typical reactions of parental lines and RI lines (pictures taken in 2010).

(**B**) Frequency distribution of LP1S in the RI population.

(**C**) Frequency distribution of LP50S in the RI population.

(**D**) Frequency distribution of ITA in the RI population. RIL116 is a susceptible RI line (lack of *Yrq1*, *Yrq2*, *Yrq3* and *Yrq4* resistance alleles) with a latency period of 336 hai (hours after inoculation); RIL241 is a slow-rusting resistant line (contains *Yrq1*, *Yrq2* and *Yrq4* resistance alleles) with a latency period of 450 hai. The horizontal bars indicate the range of disease values of the parents Yanzhan 1 and Xichang 76-9. ITA, infection type at the adult-plant stage; LP1S, latency period at which the first pustule becames visible at the seedling stage; LP50S, latency period at which 50% of the final number of pustules became visible at the seedling stage.

region of the *Yrq1*, the 113 predicted genes from the extensive intervals of 0.63 to 0.93 Mb and 2.15 to 3.12 Mb were used to identify 153 wheat ESTs. Then 49 homologous scaffolds from the D-genome were obtained. Out of the 86 newly identified SSRs, 13 were polymorphic between the two parents. The primer sequences for markers detecting polymorphism between Yanzhan 1 and Xichang 76-9 are listed in **Table 2**.

Of the 19 polymorphic SSRs, 1, 5, 11 SSRs were mapped in the syntenic regions near *Yrq1* on the chromosome 2A, 2B and 2D, respectively, and only two were mapped on other regions or chromosomes (**Figure 4**). A linkage map of chromosome 2D with 22 markers covering a genetic distance of 93.4 cM was constructed. The average interval distance between markers was reduced from 8.7 cM to 4.2 cM (**Figure 4**). Within the 2.7 cM region of *Yrq1*, eight SSR markers were genetically mapped and were closely linked to the *Yrq1* (**Figure 5**), providing abundant SSR markers for fine-mapping of *Yrq1* and marker-assisted selection.

Validation of *Yrq1*

To validate the presence and position of *Yrq1*, an independent F_2 population was developed from the cross between Pinchun 16 and RIL290. Pinchun 16 is a highly susceptible line from the Chinese Academy of Agricultural Sciences with a latency period about 342 hai (**Figure 6**). RIL290, which carries only the *Yrq1* (i.e. it lacks the other three resistance QTLs) displays slow-rusting resistance with a latency period of about 440 hai (**Figure 6**). Of the 139 F_2 plants, 32 were found to be homozygous

Table 1. Analysis of variance of disease scores for the recombinant inbred (RI) population derived from the cross of Yanzhan 1 × Xichang 76-9

Trait	Source of variation	Degrees of freedom	Mean squares	TraitF value
LP1S	Genotype	117	12 860.1	26.8***
	Year	2	13 787	28.8***
	Line × Year	230	1 733.1	3.6***
	Error	674	479.409 2	
LP50S	Genotype	117	12 422.2	26.9***
	Year	2	35 658	77.1***
	Genotype × Year	230	1 710.2	3.7***
	Error	673	462.537 8	
ITA	Genotype	117	15	15.2***
	Year	1	54.8	55.6***
	Genotype × Year	116	2.4	2.4***
	Error	468	0.985 103	

***Significant at $P < 0.000\ 1$; ITA, infection type at the adult-plant stage; LP1S, latency period at which the first pustules became visible at the seedling stage; LP50S, latency period at which 50% of the final number of pustules became visible at the seedling stage.

for the resistant RIL290 genotype (based on the proximal flanking markers *Xgdm5* and *Xgwm455*), 60 were found to be heterozygous, and 47 were found to be homozygous for the susceptible Pinchun 16 genotype (**Table 3**). These 139 F_2 plants were inoculated with CYR32 spores and scored with LP50. The average LP50 value of the F_2 plants with the resistant genotype (BB) is significantly longer than the average LP50 values of heterozygous F_2 plants (HH) and the susceptible genotypes (AA) ($P < 0.01$) (**Table 3**), indicating the presence and effectiveness of *Yrq1* in Pinchun genetic background.

Discussion

In this study, four QTLs (*Yrq1*, *Yrq2*, *Yrq3,* and *Yrq4*) for stripe rust resistance were detected and mapped to the hexaploid wheat genome. The largest effect QTL, *Yrq1*, was consistently detected based on LP1 and LP50 values obtained at the seedling stage in all 3 years' experiments and also based on IT data for resistance at the adult plant stage from 2009 and 2010's experiments, indicating that this major QTL was stable across different experiments. Hypersensitive response was observed in many resistant RILs, which contain resistance alleles *Yrq1* at the adult-plant stage, indicating that the effect of *Yrq1* is enhanced during plant development. This result agrees with that of *Rphq3* in barley to barley leaf rust, which is effective

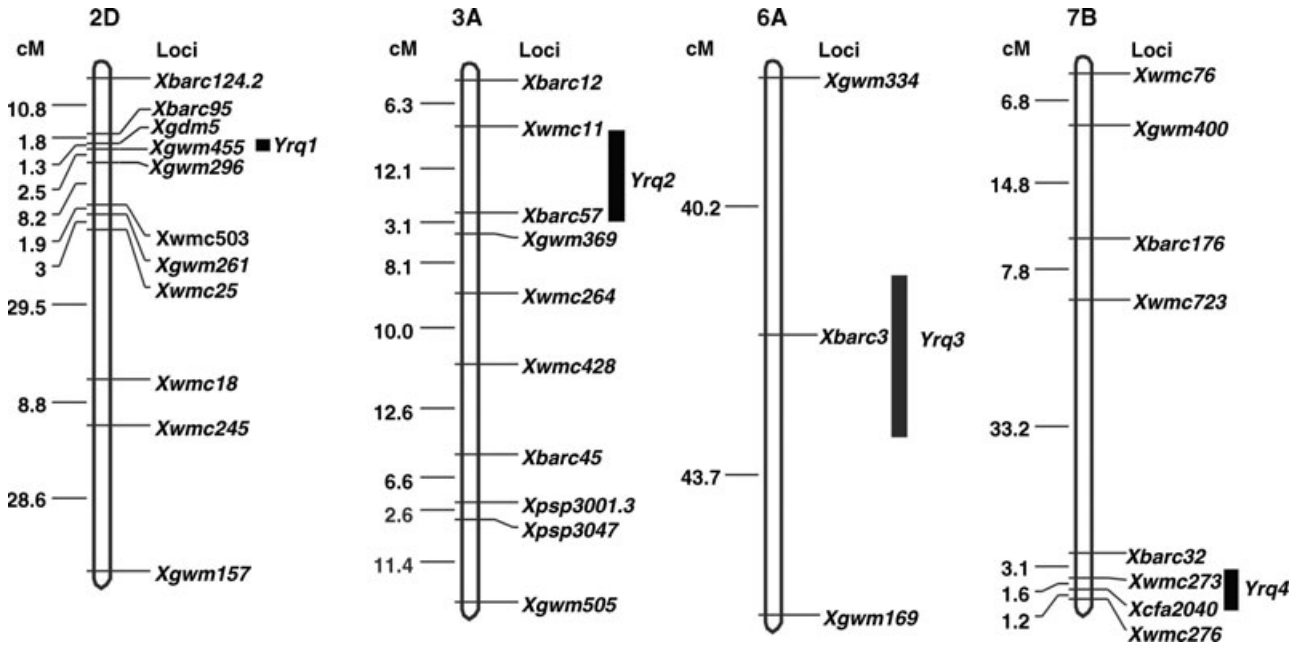

Figure 2. Quantitative trait loci (QTLs) for stripe rust resistance identified on wheat chromosomes 2DS, 3AS, 6A and 7BL.

Locus names of QTLs are indicated on the right-hand side of the chromosomes. Marker loci are listed to the right-hand side and centiMorgan (cM) distances are shown to the left. Length of black bars corresponds to 2-LOD support intervals (from peak) based on the results of the composite interval mapping (CIM) analysis.

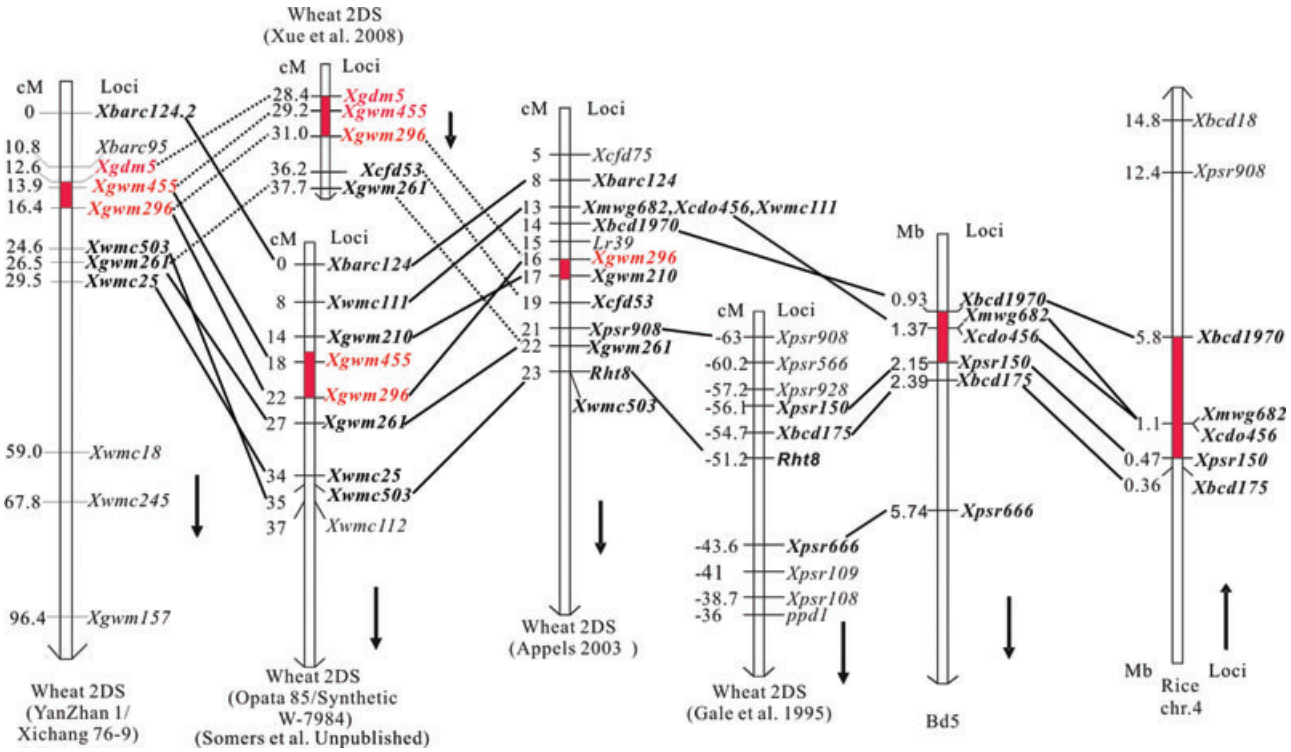

Figure 3. A diagrammatic prediction of the *Yrq1* collinear region in rice and *Brachypodium distachyon* genomes.

Physical locations corresponding to the *Yrq1* on the genetic map of 2DS are indicated as million pairs on the genomic region of *B. distachyon* and rice. The red shaded region indicates the chromosomal interval harboring *Yrq1*. The *Yrq1* flanking markers are shown in red and other anchored markers are shown in bold. The arrow points to the long arm of chromosome.

at all developmental stages and its effect increases during barley development (Wang et al. 2010). Cloning of this QTL may reveal the molecular basis of this developmental stage-dependent disease resistance.

In this study, 25.1% of the SSR markers showed segregation distortions, and some of them were clustered mainly in two regions of the genome. Similarly, distorted segregation has also been reported by Cadalen et al. (1997) in an interspecific cross of wheat. Other studies also reported segregation distortion in intraspecific crosses (Messmer et al. 1999; Paillard et al. 2003; Sourdille et al. 2003; Zhang et al. 2008). The existence of markers or chromosomal regions showing segregation distortions has already been reported in other plant species (Jenczewski et al. 1997; Xu et al. 1997; Qi et al. 1998). In rice and maize, some segregation distortion regions were detected close to the location of known gametophytic factors (Xu et al. 1997; Lu et al. 2002). The aberrant genetic segregation might be due to linkage between the loci and sterility genes, due to gametophytic selection or due to physiological and environmental effects (Singh et al. 2007). Despite the high level of segregation distortion observed in the RI population derived from Yanzhan 1 × Xichang 76-9, the marker orders are similar to that of the previous map (Somers et al. 2004).

Several components, including the latency period, uredinium size, infection frequency and spore production, are involved in the slow development of the stripe rust disease. Longer latency periods for wheat stripe rust in seedlings may effectively retard disease development and reduce the number of urediniospores, delaying the onset of disease epidemics at the adult stage, and thus markedly reducing the final severity of the rust and associated yield losses. Previous studies (Zadoks 1971; Parlevliet and Ommeren 1975; Neervoort and Parlevliet 1978) revealed that the latency period explains most of the variation in partial resistance between cultivars and may be the most important component of disease resistance to study and use. There are two different accepted measurements of the latency period. The first has been used in barley and wheat leaf rust analysis (Parlevliet 1975) and is defined as the time period from inoculation to the point at which 50% of the final number of pustules appears (LP50). The second measurement is defined as the time period from inoculation to first pustule appearance (LP1) and has been used in wheat leaf rust analysis (Shaner et al. 1997; Xu et al. 2005). In this study, there is no significant

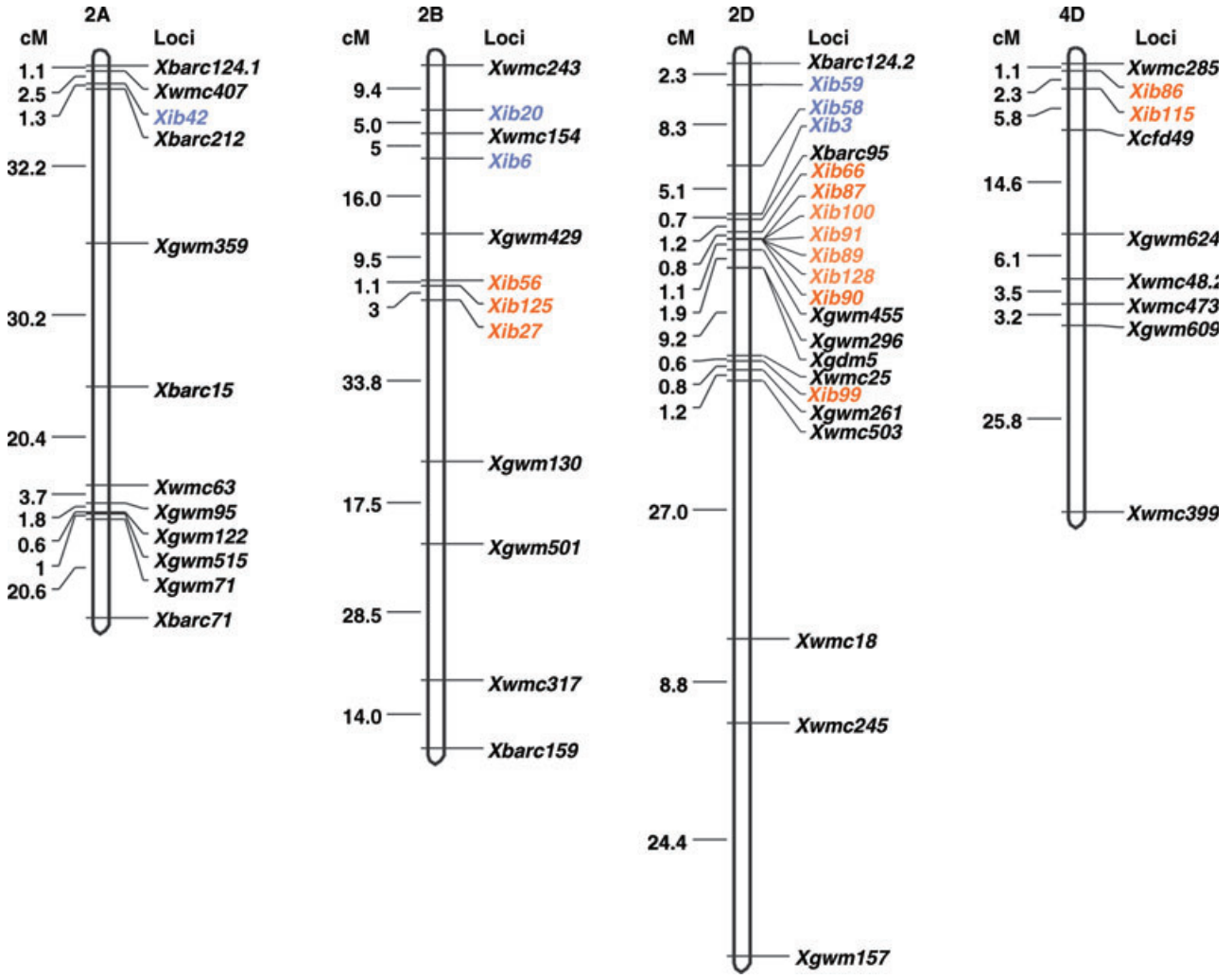

Figure 4. Map positions of the 19 polymorphic simple sequence repeat (SSR) markers in the mapping population containing 118 recombinant inbred lines (RILs) (F_8) from the cross of Yanzhan 1 × Xichang 76-9.

Marker loci are listed on the right and centiMorgan (cM) distances are shown to the left. The first and second sets of the developed markers are indicated in the blue and red, respectively.

difference between LP1 and LP50 in detecting QTLs, indicating that LP1 is also reliable and sensitive in detection of QTLs. Assessment of latency period by using LP1 is much less time and labor consuming than measuring LP50.

A large number of loci for resistance to stripe rust, including 48 formally designated (*Yr1* – *Yr48*) and many more temporarily named *Yr* genes, and QTLs (http://www.ars.usda.gov/SP2UserFiles/ad_hoc/36400500Resistancegenes/Yrgene.xls and http://www.shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp), have been identified in wheat. In addition to *Yrq1*, two *Yr* genes (*YrKat* and *YrCK*) (Bariana et al. 2001) and two QTLs for resistance to wheat stripe rust (Mallard et al. 2005; Lu et al. 2009) have been identified on chromosome 2DS. *YrKat* was an adult-plant resistance gene, flanked by markers of *Xwmc111* and *Xwmc25* on chromosome 2DS in the cultivar Katepwa, while *YrCK* was a temperature-sensitive resistance gene, which falls within the marker interval between *Xgdm005* and *Xwmc190* (Bariana et al. 2001). According to the wheat consensus maps (http://www.shigen.nig.ac.jp/wheat/komugi/maps/markerMap.jsp), the map intervals of these two genes likely overlap with the map position of *Yrq1*. Further genetic analysis such as the allelic test is required to establish a direct relationship among *YrKat, YrCK* and *Yrq1*. *QYr.inra-2DS* was identified from cultivar Camp Rémy as a QTL locus and mapped on chromosome 2DS by Mallard et al. (2005). Comparative analysis of wheat consensus maps indicated that *QYr.inra-2DS* is more than 18 cM away from *Yrq1*. *QYr.caas-2DS* was recently identified as a QTL for reduced

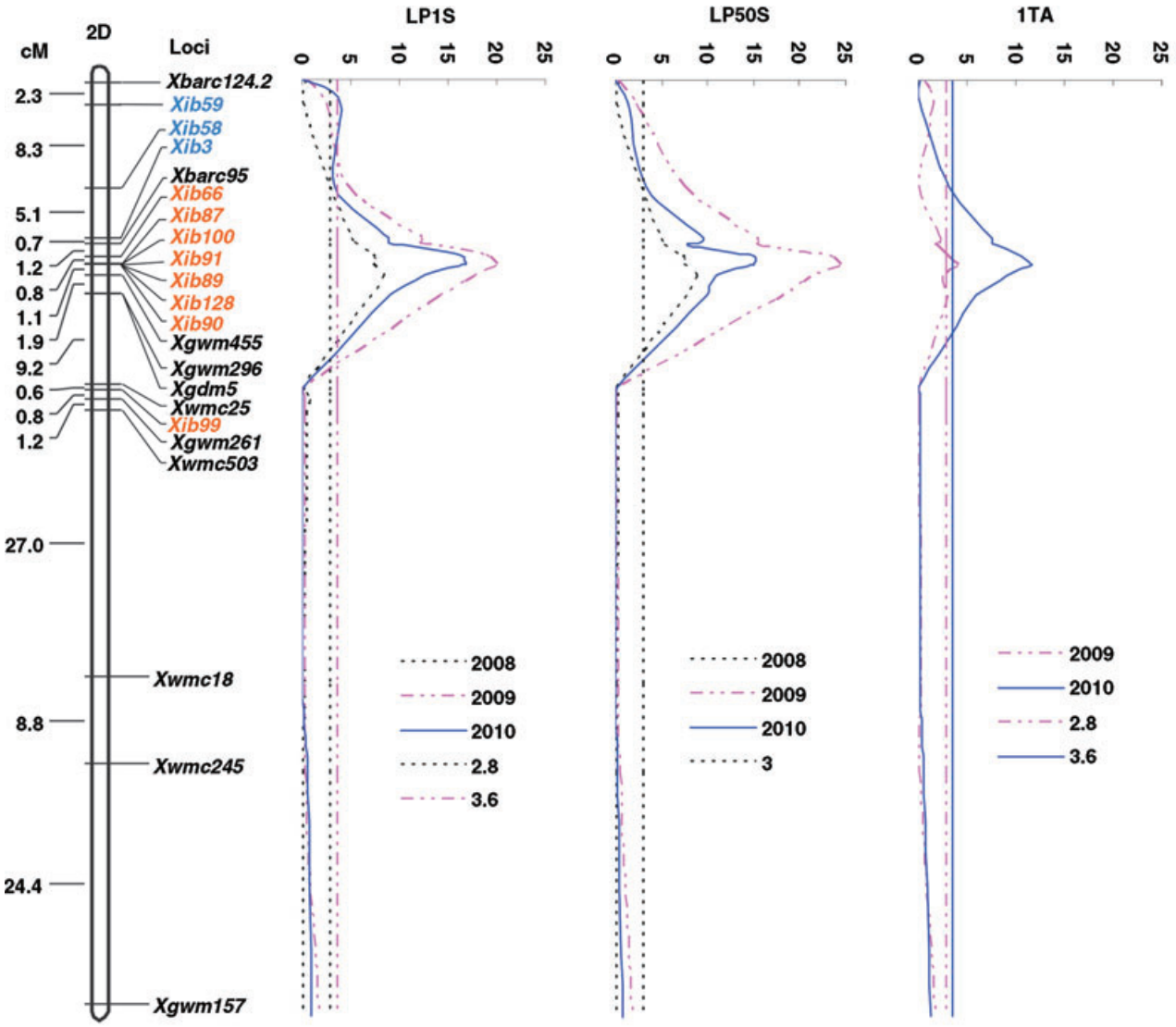

Figure 5. Likelihood plots of *Yrq1* for slow-rusting resistance on chromosome 2DS with 11 newly developed simple sequence repeat (SSR) markers.

Data from composite interval mapping in the cross of Yanzhan 1/Xichang 76-9 was shown. The LOD plot of each trait (LP1S, LP50S and ITA) is represented separately. The LOD score is the log base 10 of the likelihood ratio under the hypotheses of linkage and non-linkage. LOD threshold for each dataset was established by conducting a permutation test with 1 000 permutations. Marker loci are listed to the right and centiMorgan (cM) distances are shown to the left. The first and second newly developed markers are indicated in blue and red, respectively.

stripe rust severity in cultivar Libellula (Lu et al. 2009). This QTL was mapped between markers *Xcfd51* and *Xgwm261*, explaining 8.1–12.4% of the phenotypic variance. The map position of *QYr.caas-2DS* is estimated to be at least 12 cM away from the *Yrq1* locus (**Figure 2**). *Yrq1* is likely located at a different position from the previously identified QTLs, *QYr.inra-2DS* and *QYr.caas-2DS*.

The order of the newly developed SSRs in wheat matches the overall order of the corresponding DNA sequences (scaffolds) of *B. distachyon* (Figure S4), indicating that the level of macro-collinearity between *B. distachyon* and wheat is high. This is similar to the results of Bossolini et al. (2007), who found a perfect collinearity between a 371 kb *B. distachyon* sequence and wheat. The good collinearity between *B. distachyon* and wheat indicates that the *B. distachyon* genome sequence is an excellent resource for wheat genomic studies. Of the 19 polymorphic SSRs developed based on the genomic sequences of *A. tauschii* Coss. (the D-genome), 17 SSRs were mapped

Table 2. Newly developed markers that detected polymorphism between Yanzhan 1 and Xichang 76-9

Marker	Sequence source	Forward primer	Reverse primer	Annealing temperature (°C)
Xib3	scaffold4026_19.8	GGAGGGACTATTTCTGTATT	ATTTTGTCAGTTCTGTTGTC	49
Xib6	scaffold4026_19.8	TAATCTTACCAGAGCTTGAG	CAAGTTCTATCTCGTCTTGA	49
Xib20	scaffold9124_19.8	TCTAGATACGTCGTCCTTTA	GCTTTTCCTCTATCTCTCTC	49
Xib27	scaffold21839_18.7	GCTAAGTGTCTGATGACTGT	ATGATGAAGATGTTGATTTC	49
Xib42	scaffold50000_18.8	GATACCATCTCAATAACCAA	ATGTCTTGTATGGGTAACAC	49
Xib56	scaffold119080_18.7	GTCTGTGTAAAATAAATGCC	GCTAGTCAAGTCATAATTGC	49
Xib58	scaffold139119_19.3	GTGTGTGCTTATTTCTTTTT	TTACTGCAACCTATTCATTC	49
Xib59	scaffold169442_19.8	GAGGTTTAGAGACATTAGCA	TGATCTTTTCTGCATCTATT	49
Xib66	scaffold4026_19.8	GGAGGGACTATTTCTGTATT	ATTTTGTCAGTTCTGTTGTC	49
Xib86	scaffold26841_19.0	ATTTGCAGTATACACAGCTT	TGTCATTTTTGAAAAGAAGT	50
Xib87	scaffold30717_17.3	ATGATGATTAAAGTACGTGG	GCAACTTTTCTCTTCTCTTC	50
Xib89	scaffold30717_17.3	AACCTGAACTACCACACATA	ACACAAGAAACACACAAGAT	50
Xib90	scaffold30717_17.3	ATCTTGTGTGTTTCTTGTGT	TCTATCTTTGCAATGAATTT	50
Xib91	scaffold30717_17.3	AGCTCCTCTTTAGAAACAAC	TTACAACCTCTTGTTGAAAT	50
Xib99	scaffold42040_17.1	TATAAAGCTCTGCTCTCAAC	GCCATACTTTGAGACTTGT	50
Xib100	scaffold50771_18.7	AAACAACTTTTGGTGATAGA	TAGAACAAAGCACCTTTTAG	49
Xib115	scaffold116088_18.5	ATATATTCTGTTCCTCCTCC	AATTATGTGATTATTGGTGG	49
Xib125	scaffold116885_17.3	AATAGCCCTAAAAAGAGAAC	TGTGGATCTTGATAGATTGT	49
Xib128	scaffold127482_17.6	CTTACATTGTTTCCACTCAT	GTACAAAAATGTTTATTGCC	50

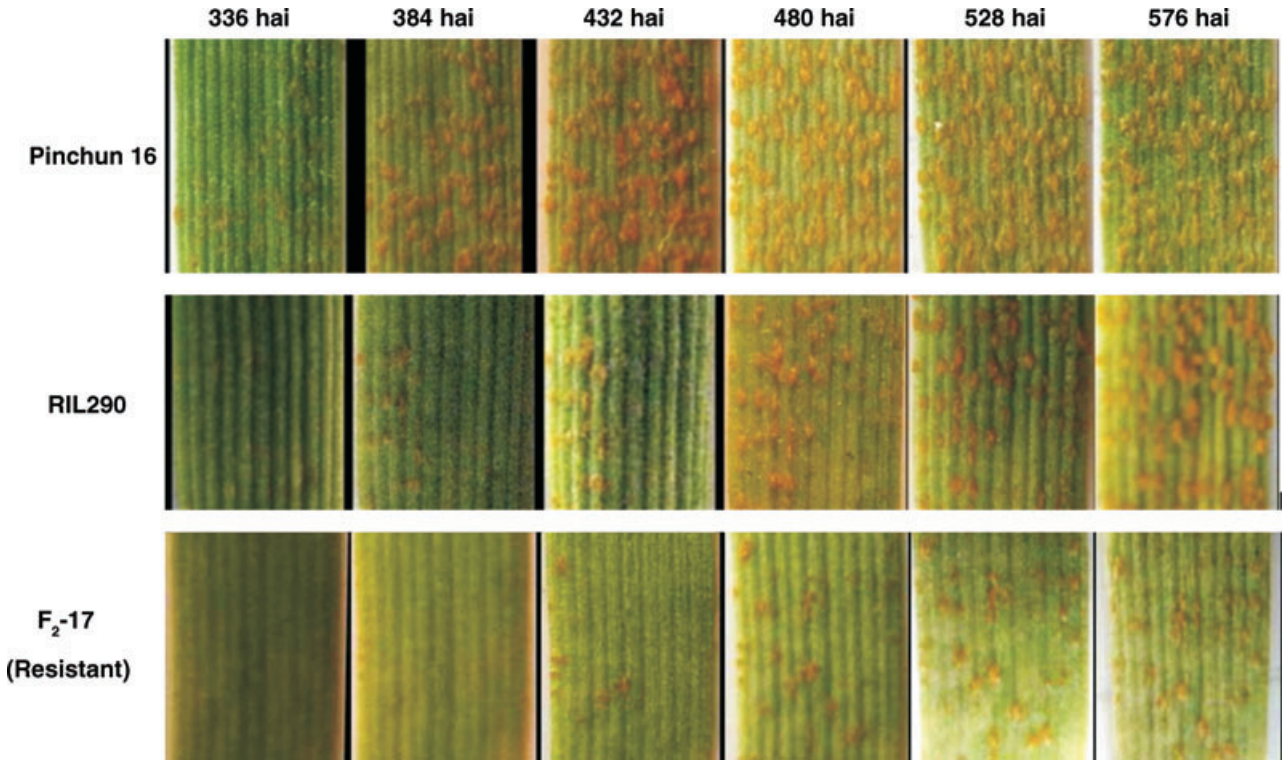

Figure 6. Typical disease reactions of the susceptible cultivar Pinchun 16, and the RIL290 and one F_2 plant that carried *Yrq1* resistance allele.

hai, hours after inoculation (pictures taken in 2010).

Table 3. Validation of *Yrq1* in an independent F_2 population derived from Pinchun 16 and RIL290

Line	Genotypes	Number	LP50 mean ± *SD*
Pinchun 16	AA		342
RIL290	BB		440
F_2 plants	AA	47	$370.7^a \pm 26.3$
	HH	60	$390.1^b \pm 21.2$
	BB	32	$402.3^c \pm 24.1$

Means marked by the different superscripts (a, b, c) differ significantly ($P < 0.05$). Validation of *Xgwm455* and *Xgdm5* as peak and proximal markers for *Yrq1*, respectively. AA, homozygous Pinchun 16 genotype; BB, homozygous RIL290 genotype; HH, heterozygous genotype.

in the syntenic region near *Yrq1* in the homeologous group 2 chromosomes in wheat, indicating the genomic sequences of the diploid D-genome of *A. tauschii* Coss. are also very important for genomic studies and cloning of genes in the hexaploid wheat.

The ultimate goal of QTL mapping is to provide tightly-linked markers for germplasm improvement and identify the causative genes behind the QTL. Saturation of the major QTL region with high-density markers is a necessary step before positional cloning of the causative gene and for using marker-assisted selection in the breeding program. Due to the large genome size (1C = 17.33 pg, Bennett and Smith 1976), hexaploidy nature and extremely low levels of polymorphism among wheat cultivars, development of DNA markers and construction of high-density maps for the target region in hexaploid wheat is a challenging task. In this study, eight SSR markers were successfully mapped within a 2.7 cM-region of the *Yrq1*. This clearly indicates that use of *B. distachyon* genome, wheat ESTs and the draft DNA sequences (scaffolds) of the D-genome for wheat SSR development is a plausible strategy for fine-mapping and cloning of genes/QTLs in the hexaploid wheat when its genomic sequences are not available.

Ideally, the identified QTLs should be validated in other genetic backgrounds to confirm the effectiveness of the QTLs. In this study, using the closely linked markers, an F_2 population with 139 individuals derived from a cross between Pinchun 16 and a resistance line RIL290 was used to detect the effect of the *Yrq1*. Analysis of a dataset from the disease evaluation at the seedling stage and genotyping indicates that *Yrq1* was successfully transferred into Pinchun 16 background and effective with slow-rusting resistance. In addition, near-isogenic lines (NILs) for *Yrq1* are under development. These results will facilitate our efforts toward map-based cloning of *Yrq1* and use of this QTL in wheat breeding via marker-assisted selection.

Materials and Methods

Plant materials

The hexaploid wheat (*Triticum aestivum* L.) mapping population used in this study consists of 118 F_8 recombinant inbred lines (RILs) developed via single-seed descent from a cross between Yanzhan 1 and Xichang 76-9. Yanzhan 1 is susceptible to stripe rust (*Pst*) and Xichang 76-9 is slow-rusting resistant to stripe rust at the seedling stage. Both parents are resistant to stripe rust at the adult-plant stage. Yanzhan 1 and Xichang 76-9 were released in Henan and Sichuan provinces of China, respectively. Mingxian 169, a landrace from Shanxi province, is highly susceptible to all races of *Pst* at all growth stages.

Disease evaluation

A *Pst* strain CYR32, which is currently prevalent in China was used to infect the two parents, Yanzhan 1 and Xichang 76-9, the 118 RILs, and the susceptible control Mingxian 169 at both the seedling and adult-plant stages. CYR32 has a wide virulence spectrum with the avirulence/virulence formula: *Yr3b, 4b, 5, 10, 15, 16, 24, 26 / 1, 2, 3a, 4a, 6, 7, 8, 9, 11, 12, 13, 14, 18, 22, 23, 25, 27, HVII, Cle, A, G, Su, C5, SD, SpP, CV* (Yang et al. 2003; Wan et al. 2004; Cao 2008). Fresh spores were obtained by multiplying urediniospores on the susceptible control Mingxian 169 in the greenhouse.

Disease evaluation at the seedling stage

Five to seven seeds of each line were planted in a 7 × 7 × 7 cm pot filled with a potting mixture. Mingxian 169 was used as a susceptible control and was planted with an interval of eight rows of RILs. When the first leaves were fully expanded, they were fixed in horizontal position with iron weights and inoculated in a settling tower. For each inoculation, 10 mg spores that were diluted 40 times by talc to the density of about 600 spores per cm^2 were applied. After inoculation, seedlings were immediately transferred into a plastic film-covered solar greenhouse for 24 h at 10 °C, 100% humidity in the dark, and subsequently returned to normal growth conditions. Temperatures in the plastic film-covered solar greenhouse were 10–23 °C, 7–24 °C and 10–27 °C in March 2008 and 2009, and in January 2010, respectively. The photoperiod in the greenhouse was 10–11 h of natural light. Latency period (LP) for each plant was evaluated by the period at which the first pustule appeared (LP1S, in hours) (Shaner et al. 1997; Xu et al. 2005) and the period at which 50% of the final number of pustules became visible (LP50S, in hours) after the inoculation (Neervoort and Parlevliet 1978) at the seedling stage. When the first urediospore was

visible, an area of about 2 cm long was marked in the middle part of the leaves and was photographed with a digital camera (Panasonic DMC-LX3) at 24 h intervals until the number of uredinia no longer increased. The mature spore pustules within the delimited areas were counted by eye in the photos. The latency period (LP) for each line was estimated by averaging the LP values for the five to seven inoculated seedling leaves. The LP50 was calculated according to the following formula: $LP50 = t_1 + ((F/2 - nt_1)(t_2 - t_1)/(nt_2 - nt_1))$ where F = final number of uredinia, t_1 = hours before 50% uredinia erupted, t_2 = hours after 50% uredinia erupted, nt_1 = number of uredinia erupted at t_1, nt_2 = number of uredinia erupted at t_2 (Das et al. 1993). Three experiments were conducted in the course of 3 years and each experiment consisted of three replications arranged in a randomized complete block design. Images of typical reactions of the RILs at the seedling stage are presented in **Figure 1**.

Disease evaluation at the adult-plant stage

Each line was planted in one-row plot consisting of five to seven plants spaced 20 cm apart with 20 cm apart between rows in the plastic film-covered solar greenhouse on the 26th of December in 2008 and the 12th of November in 2009. Mingxian 169 was used as a susceptible control and was planted with an interval of 20 rows of RILs. Temperatures in the greenhouse were between 7 °C (night) and 27 °C (noon) from November to February. Two to three months after planting the RILs and the two parental lines had reached the heading stage during March. When the flag leaves of all lines unfolded completely, the inoculation was conducted on the 18th of February in 2009 and the 19th of February in 2010, respectively. The flag leaves were inoculated with fresh urediospores, which were diluted 30 times (about 400 spores per cm^2 leaf area) by talc using a soft-hair brush. After inoculation, the plastic film-covered solar greenhouse was covered completely and kept in darkness for 24 h with 100% humidity and about 10 °C, and was subsequently returned to normal growth conditions. Normally, temperatures in the greenhouse were 7–24 °C and 10–27 °C in March of 2009 and 2010, respectively, and the photoperiod was 10–11 h of natural light. Infection type (IT) data were used for disease evaluation at the adult-plant stage. On the 18th–21st days after inoculation, infection type data were scored based on a 0–9 scale as follows (Line and Qayoum 1992): 0 = no visible signs or symptom, 1 = necrotic and/or chlorotic flecks; no sporulation, 2 = necrotic and/or chlorotic blotches or stripes; no sporulation, 3 = necrotic and/or chlorotic blotches or stripes; trace sporulation, 4 = necrotic and/or chlorotic blotches or stripes; light sporulation, 5 = necrotic and/or chlorotic blotches or stripes; intermediate sporulation, 6 = necrotic and/or chlorotic blotches or stripes; moderate sporulation, 7 = necrotic and/or chlorotic blotches or stripes; abundant sporulation, 8 = chlorosis behind sporulating area; abundant sporulation, 9 = no necrosis or chlorosis; abundant sporulation. Infection types 0–3, 4–6 and 7–9 were considered resistant, intermediated, and susceptible, respectively. Two experiments were conducted in March 2009 and 2010 and each experiment consisted of three replications arranged in a randomized complete block design. Images of typical reactions of the parents and the levels of '0–9' scale at adult plant stage are presented in Figure S2.

DNA preparation and genotyping

Genomic DNA was isolated from 2-week-old wheat leaves of each RIL using a modified version of the cetyltrimethylammonium bromide (CTAB) method (Threadgold and Brown 2003). DNA was resuspended in double-distilled water to a concentration of 50 ng/μL.

A total of 1 000 SSR primer pairs including primer sets from Beltsville Agricultural Research Station (BARC), Wheat Microsatellite Consortium (WMC), IPK Gatersleben (GWM/GDM), INRA (CFD/CFA) and John Innes Centre (PSP) (http://wheat.pw.usda.gov) were used to screen the parents. Primer pairs that detected polymorphism between the parents were used to genotype the RILs. PCR amplification of SSRs was carried out in a 20 μL reaction mixture containing 1 × buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 250 nM primer, 1U Taq polymerase, and 100 ng template DNA. PCR reactions were conducted in a

A 9 600 thermal cycler (Bio-Rad Hercules, CA, USA) using the following program: one step of 3 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 50–65 °C, 30 s at 72 °C, and a final extension step of 5 min at 72 °C. Each 20 μL of PCR products was denatured by adding 8 μL formamide buffer (90% formamide, 10% sucrose, 0.02% bromophenol blue, and 0.02% xylene cyanol) and heating at 95 °C for 5 min. Five percent polyacrylamide gel electrophoresis (PAGE) gels containing 7 M urea were pre-run in 1 × TBE buffer (90 mM of Tris-borate, 2 mM of EDTA, pH 8.3) at 2 500 V and 85 W for 30–50 min. Five microlitres of each sample was loaded and the gels were run at 75 W for approximately 1.0–1.5 h, and visualized by silver staining (Bassam et al. 1991).

Genetic map construction and QTL analysis

Segregation of marker loci was tested for goodness-of-fit to the expected 1:1 ratio using the χ^2 test. A segregation distortion region was defined by at least three adjacent marker loci showing a significant segregation distortion ($P \leq 0.05$). A genetic linkage map was constructed with SSR markers using JoinMap version 3.0 (Van Ooijen and Voorrips 2001). Recombination values were converted to genetic distances using the Kosambi mapping function (Kosambi 1944). SSR

markers were assembled into genetic linkage groups using a log-likelihood (LOD) threshold value of 5.0. Linkage groups were assigned to the hexaploid wheat chromosomes according to the SSR markers with the known map positions (Somers et al. 2004).

For QTL analysis, CIM (Zeng 1994) was performed with WinQTL Cartographer version 2.5 (Wang et al. 2007) using Model 6, and five markers were used as controls with a window size of 10 cM. Significant thresholds for QTL detection were calculated for each dataset using 1 000 permutations and a genome-wide error rate (a) of 0.05. CIM analysis was performed on LP1S, LP50S and ITA data for each year separately.

Statistical analysis

LP1S, LP50S and ITA were used for analysis of variance (ANOVA) and QTL detection. Analyses of variances involving estimations of genotype × environment interactions were conducted using a statistical software GGEbiplot (Yan and Kang 2003). Broad-sense heritability (h^2) for stripe rust reaction was calculated using the formula $h^2 = V_G/V_P \times 100\%$, where V_G and V_P were the estimates of genotypic and phenotypic variances, respectively. Phenotypic correlation coefficients (r) among phenotypic traits in RILs were performed by use of the SPSS (Statistics Package for Social Science) Statistics 17.0.

Development of the region-specific markers for a major effect QTL

The availability of a large number of RFLP probes on the wheat genetic maps (Gale et al. 1995; Appels 2003) provides a useful resource for comparative mapping among grass species. These maps and the map constructed in this study were used to identify rice (http://rapdb.dna.affrc.go.jp/) and *B. distachyon* (L.) (http://www.brachypodium.org/) syntenic regions to *Yrq1* region on the wheat 2DS (**Figure 3**). The coding sequences of the annotated genes in the syntenic region of *Yrq1* in *B. distachyon* were used to search the wheat ESTs database (http://www.ncbi.nlm.nih.gov/) using cutoff parameters of *E*-value $< 1E^{-10}$, identity >80% and a minimum of 100 bp match length by using BLASTN. Then the identified homologous wheat ESTs was used to search a draft sequence of the 60 folds of the genome-equivalent coverage of the D-genome (*A. tauschiii* Coss.) (Jizeng Jia, 2010, unpubl. data) by using the BLASTN for the identification of the homologous scaffolds. The sequences of scaffolds were used to search for SSRs using the SSR primer design software SSR Locator (http://minerva.ufpel.edu.br/Ĩmaia.faem/) (Da Maia et al. 2008). The cutoff for a SSR is more than 9 di-, 5 tri-, 4 tetra- or 3 penta-nucleotide repeats. Primer design was based on the criteria of the 50% GC content, a minimum melting temperature of 50 °C, the absence of secondary structure, a length of 18–26 nucleotides and an amplified product range of 100–350 base-pairs.

QTL validation

The map positions of the major QTL for stripe rust resistance detected in the RI population on chromosome 2D, was validated via progeny tests in an independent F_2 population, which was developed from the cross between Pinchun 16 and RIL290. Pinchun 16 is a highly susceptible line from the Chinese Academy of Agricultural Sciences and RIL290 has only the *Yrq1* (i.e. it lacks the other three resistance QTLs).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. The genetic linkage maps constructed based on 144 simple sequence repeats (SSRs) by using 118 recombinant inbred lines (RILs) from the cross of Yanzhan 1 × Xichang 76-9.

Locus name and corresponding location are indicated on the right hand side, and genetic distances (cM) between them are indicated on the left hand side. Asterisks at the end of the markers denote the significantly distorted loci (*significant distortion at $P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ levels, respectively). The "Y" letter indicates marker exhibiting an excess of Yanzhan 1 alleles.

Figures S2. Infection types of '0–9' scales at the adult-plant stage (pictures taken in 2010).

Descriptions of levels:

0 = no visible signs or symptom.

1 = necrotic and/or clorotic flecks; no sporulation.

2 = necrotic and/or chlorotic blotches or stripes; no sporulation.

3 = necrotic and/or chlorotic blotches or stripes; trace sporulation.

4 = necrotic and/or chlorotic blotches or stripes; light sporulation.

5 = necrotic and/or chlorotic blotches or stripes; intermediate sporulation.

6 = necrotic and/or chlorotic blotches or stripes; moderate sporulation.

7 = necrotic and/or chlorotic blotches or stripes; abundant sporulation.

8 = chlorosis behind sporulating area; abundant sporulation.

9 = no necrosis or chlorosis.

Figure S3. Likelihood plots of quantitative trait locus (QTLs) for slow-rusting resistance on chromosomes 2DS, 3AS, 6A and 7BL identified by composite interval mapping in the cross of Yanzhan 1/Xichang 76-9.

(A) *Yrq1* on chromosome 2D; (B) *Yrq2* on chromosome 3A; (C) *Yrq3* on chromosome 6A; and (D) *Yrq4* on chromosome 7B. The LOD plot of each trait (LP1S, LP50S and ITA) is represented separately. The LOD score is the log base 10 of the likelihood ratio under the hypotheses of linkage and non-linkage. LOD threshold for each dataset was established by conducting a permutation test with 1 000 permutations. Marker loci are listed to the right and centiMorgan (cM) distances are shown to the left. *p,* proximal flanking marker; *pk,* QTL peak marker (i.e. the locus associated with the highest LOD score); *d,* distal flanking marker.

Figure S4. Collinearity of chromosomal region harboring the newly developed SSRs on chromosome 2DS in wheat between the corresponding genomic region of *Brachypodium* chromosome 5.

Physical locations corresponding to the SSR-derived scaffolds on the genetic map of 2DS are indicated as million pairs on the genomic region of *Brachypodium*. Marker loci are listed to the right and centiMorgan (cM) distances are shown to the left. The red shaded region indicates the chromosomal interval harboring *Yrq1*.

Table S1. Pearson's linear correlation coefficients (*r*) among traits in 118 recombinant inbred lines (RILs) (F_8) derived from the cross Yanzhan1 × Xichang 76-9.

Table S2. Summary of quantitative trait locus (QTL) for slow-rusting resistance to stripe rust in wheat detected by composite interval mapping (CIM).