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

Xiaohua Cao1,2, Jianghong Zhou1, Xiaoping Gong1, Guanyao Zhao3, Jizeng Jia3,4 and Xiaoquan Qi1,4∗

1Key Laboratory of Plant Molecular Physiology, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
2Graduate University of Chinese Academy of Sciences, Beijing 100049, China
3Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China
4These authors contributed equally to this article.

∗Corresponding author
Tel: +86 10 6283 6671; Fax: +86 10 8259 6691; E-mail: xqi@ibcas.ac.cn
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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.

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,
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 $\chi^2$ 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 h.ai (hours after inoculation), whereas Xichang 76-9 was slow-rusting resistance to CYR32 with a latency period ranging from 410 to 470 h.ai 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 h.ai and from 0 to 9 scales, respectively, indicating the polycigenic 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). 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
A Major QTL for Resistance to Wheat Stripe Rust

Figure 1. The phenotypes of 118 recombinant inbred lines (RILs) (F₈) 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 becomes 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 F₂ 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 F₂ 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

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
<th>Trait F value</th>
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<td>26.8***</td>
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<tr>
<td></td>
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<td>2</td>
<td>13 787</td>
<td>28.8***</td>
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<tr>
<td></td>
<td>Line × Year</td>
<td>230</td>
<td>1 733.1</td>
<td>3.6***</td>
</tr>
<tr>
<td></td>
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<tr>
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<td>26.9***</td>
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<tr>
<td></td>
<td>Year</td>
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<td>658</td>
<td>77.1***</td>
</tr>
<tr>
<td></td>
<td>Genotype × Year</td>
<td>230</td>
<td>1 710.2</td>
<td>3.7***</td>
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<tr>
<td></td>
<td>Error</td>
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<tr>
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<td>15.2***</td>
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<td></td>
<td>Genotype × Year</td>
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<td></td>
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<td>0.985</td>
<td>103</td>
</tr>
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</table>

***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.

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 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 F2 plants were inoculated with CYR32 spores and scored with LP50. The average LP50 value of the F2 plants with the resistant genotype (BB) is significantly longer than the average LP50 values of heterozygous F2 plants (HH) and the susceptible genotypes (AA) (P < 0.01) (Table 3), indicating the presence and effectiveness of Yrq1 in Pinchun genetic background.
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
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.

Figure 4. Map positions of the 19 polymorphic simple sequence repeat (SSR) markers in the mapping population containing 118 recombinant inbred lines (RILs) (F8) from the cross of Yanzhan 1 × Xichang 76-9.

...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 Yr1, 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 was more than 18 cM away from Yrq1. QYr.caas-2DS was recently identified as a QTL for reduced...
A Major QTL for Resistance to Wheat Stripe Rust

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 Xctd51 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 macrocollinearity 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

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sequence source</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing temperature (°C)</th>
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</table>

Figure 6. Typical disease reactions of the susceptible cultivar Pinchun 16, and the RIL290 and one F₂ plant that carried Yrq1 resistance allele.

hai, hours after inoculation (pictures taken in 2010).
in the syntenic region near Yrql 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 Yrql. 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 F2 population with 139 individuals derived from a cross between Pinchun 16 and a resistance line RIL290 was used to detect the effect of the Yrql. Analysis of a dataset from the disease evaluation at the seedling stage and genotyping indicates that Yrql was successfully transferred into Pinchun 16 background and effective with slow-rusting resistance. In addition, near-isogenic lines (NILs) for Yrql are under development. These results will facilitate our efforts toward map-based cloning of Yrql 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 F2 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 uredinospores 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 cm² 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 (Neervoor and Parlevliet 1978) at the seedling stage. When the first urediospore was

#### Table 3. Validation of Yrql in an independent F2 population derived from Pinchun 16 and RIL290

<table>
<thead>
<tr>
<th>Line</th>
<th>Genotypes</th>
<th>Number</th>
<th>LP50 mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinchun 16</td>
<td>AA</td>
<td>342</td>
<td></td>
</tr>
<tr>
<td>RIL290</td>
<td>BB</td>
<td>440</td>
<td></td>
</tr>
<tr>
<td>F2 plants</td>
<td>AA</td>
<td>47</td>
<td>370.7 ± 26.3</td>
</tr>
<tr>
<td></td>
<td>HH</td>
<td>60</td>
<td>390.1 ± 21.2</td>
</tr>
<tr>
<td></td>
<td>BB</td>
<td>32</td>
<td>402.3 ± 24.1</td>
</tr>
</tbody>
</table>

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 Yrql, respectively. AA, homozygous Pinchun 16 genotype; BB, homozygous RIL290 genotype; HH, heterozygous genotype.
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: \[ LP50 = t_1 + \frac{(F/2 - n t_1)(t_2 - t_1)}{(n t_2 - n t_1)} \] where \( F \) = final number of uredinia, \( t_1 \) = hours before 50\% uredinia erupted, \( t_2 \) = hours after 50\% uredinia erupted, \( n t_1 \) = number of uredinia erupted at \( t_1 \), \( n t_2 \) = 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 cm² leaf area) by t alc 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 MgCl₂, 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 \( \chi^2 \) test. A segregation distortion region was defined by at least three adjacent marker loci showing a significant segregation distortion (\( P < 0.05 \)). 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 (h²) for stripe rust reaction was calculated using the formula h² = Vg/Vp × 100%, where Vg and Vp 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.afrc.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. tauschii 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/maia.faem/) (Da Maia et al. 2008). The cutoff for a SSR is more than 9 di-, 5 tri-, 4 tetra- or 3 pentanucleotide 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 F2 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).

Acknowledgements

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References


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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 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.

**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 genetic map 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>
<thead>
<tr>
<th>Trait 1</th>
<th>Trait 2</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield</td>
<td>Yield</td>
<td>1.0</td>
</tr>
<tr>
<td>Yield</td>
<td>Yrq1</td>
<td>-0.9</td>
</tr>
<tr>
<td>Yrq1</td>
<td>Yrq2</td>
<td>0.8</td>
</tr>
<tr>
<td>Yrq2</td>
<td>Yrq3</td>
<td>-0.5</td>
</tr>
</tbody>
</table>

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