QTL mapping of adult plant resistance to stripe rust in the Fundulea 900 × Thatcher RIL population

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Abstract: Wheat stripe rust, caused by Puccinia striiformis Westend. f.sp. tritici Eriks (Pst), is one of the most important diseases of bread wheat worldwide. Breeding resistant wheat cultivars is the most economical, effective and environmentally friendly way for controlling wheat stripe rust in China. The Romanian wheat line Fundulea 900 showed good resistance to wheat stripe rust at the adult stage. The present study aimed to map the quantitative trait loci (QTLs) for stripe rust resistance in 176 F2:6 recombinant inbred lines (RIL) derived from the cross of Fundulea 900 × Thatcher. The RIL population was phenotyped for stripe rust (YR) severity at Mianyang in the Sichuan province and Baoding in the Hebei province in the 2016/2017 and 2017/2018 cropping seasons. SSR markers combined with a preferred screened group (PSG) analysis were used to identify the QTLs for stripe rust in the population. Three QTLs for stripe rust resistance were mapped on chromosomes 1AL, 7BL and 7DS, respectively. All three QTLs originated from Fundulea 900 and were detected in all the environments. The QTL on 7DS was provided by the known resistance gene Yr18/Lr34. The two QTLs on chromosomes 1AL and 7BL were explained by 9.2 to 21.5% and 5.1 to 10.1% of the phenotypic variance, respectively and might be new QTLs. The QTLs identified in the study and their closely linked markers can be used for marker-assisted selection (MAS) in wheat breeding programmes.

Keywords: APR (adult-plant resistance); molecular mapping; Puccinia striiformis Westend. f.sp. tritici Eriks (Pst); QTLs; SNP array; SSR marker

Common wheat (Triticum aestivum L.) is widely cultivated worldwide and is one of the most important staple food crops for humankind (Haile et al. 2013). Stripe rust (YR), caused by Puccinia striiformis Westend. f.sp. tritici Eriks (Pst) (Westendorp 1854), is an important wheat disease worldwide (Chen 2005). Stripe rust is a significant threat in most of the wheat growing regions in the world with the potential to inflict regular regional crop losses of 5 to 25% (Wellings 2011). From the 1950s, stripe rust has occurred in at least 15 crop seasons in China, and has caused yield losses of up to 1 million tonnes in four crop seasons (Wan et al. 2007). The most effective and environmentally sound method to control this disease is widely deploying resistant cultivars with different genes. Resistance to rust is generally classified into two types, race specific and race non-specific (Johnson 1988). Race specific resistance is controlled by a single gene, and often expresses high resistance at the seedling stage. This resistance often loses effectiveness when virulent races arise. The race non-specific resistance also termed as slow...
rusting resistance is effective at later plant growth stages conferring resistance by multiple genes and is more durable (Singh et al. 2005).

To date, 83 YR resistance genes have been identified and catalogued in wheat, and 327 YR quantitative trait loci (QTL) have been reported so far (Wang & Chen 2017; Li et al. 2020). Only four genes Lr34/Yr18/Sr57/Pm38 (Singh et al. 2012), Lr46/Yr29/Sr58/Pm39 (Singh et al. 2013), Lr67/Yr46/Sr55/Pm46 (Herrera-Foessel et al. 2011), and Lr68 (Herrera-Foessel et al. 2012) were reported to be slow rusting resistance genes. Lr34 confers a moderate level of leaf rust resistance when present alone; however, combinations with additional slow rusting genes generally result in higher resistance levels (Singh et al. 2000). Therefore, mapping more, new slow-rusting genes is important for breeding cultivars with durable resistance.

Molecular markers, including restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR), resistance gene analogue polymorphisms (RGAP) and single nucleotide polymorphisms (SNP), have been widely used in the genetic mapping of wheat disease resistance genes. Among them, high-density SNP arrays provide a superior approach for QTL mapping due to their advantages of higher accuracy and, particularly, higher density than other markers (Botstein et al. 1980; Leal et al. 2010; Grativol et al. 2011).

The Romanian wheat line Fundulea 900 introduced to China in 1999 was highly resistant to leaf rust and stripe rust under field conditions. In previous studies, leaf rust resistance genes were identified from the Fundulea 900 × Thatcher F2,3 population using SSR markers (Xing et al. 2014; Zhang et al. 2017). In the present study, the RIL population from the cross of Fundulea 900 × Thatcher were used to identify the QTLs to stripe rust using SSR markers and the 55K SNP array.

**MATERIAL AND METHODS**

**Plant materials and *Pst* pathotypes.** The resistant parent Fundulea 900, the susceptible parent Thatcher, and 176 F2,6 recombinant inbred line (RIL) populations were used to map the Adult-Plant Resistance (APR) QTL to the YR. A urediniospore mixture in equal proportion of two *Pst* pathotypes, CYR32 and CYR33, was used in the field trial. All the wheat accessions were maintained at the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University. The *Pst* pathotypes were kindly provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing.

**Phenotypic characterization of stripe rust resistance in the field.** A total of 176 RILs and two parents were grown at Baoding in the Hebei Province in 2016–2017 cropping season (here after referred as 2017BD) and Mianyang in the Sichuan Province in 2016–2017 and 2017–2018 cropping seasons (here after referred as 2017SC and 2018SC, respectively) for evaluating the YR severities. The field trials were conducted in randomised complete blocks with two replicates. The field plots were single 1.5 m rows sown with approximately 50 seeds and 30 cm apart between the rows. The highly susceptible line Mingxian 169 was planted every tenth row to aid the spread of the urediniospores within the trial. Spreader rows of Mingxian 169 were planted perpendicular and adjacent to the test rows. Stripe rust epidemics were initiated by spraying aqueous suspensions of urediniospores in equal proportions (50% of each pathotype) plus a few drops of Tween 20 (0.03%) onto the spreader rows at tillering. The disease severities as percentage of the leaf area covered with uredinia were scored two or three times at about one-week intervals with the first scoring 4 weeks after inoculation in each place according to the modified Cobb scale (Peterson et al. 1948). The final disease severity (FDS) was recorded for each line in each environment when the susceptible check Mingxian 169 were fully rusted. The FDS data were used for the QTL analysis. The phenotypic correlation coefficients between the FDS in the different environments were calculated by the Microsoft Excel analytical tool.

**Genotyping.** The genomic DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method (Sharp et al. 1988) from non-infected seedling leaves of the 176 RILs and two parents. The DNA concentration was quantified with a Thermo Scientific NanoDrop 2000 (Thermo Scientific, USA) and diluted to a final concentration of 50 ng/μL prior to further analysis. Based on the FDS value, equal amounts of DNA from five typical resistant RILs (FDS < 5%) and five typical susceptible RILs (FDS > 90%), respectively, were selected to form the preferred screened group (PSG) (Hao et al. 2008). At the same time, the Affymetrix Wheat 55K SNP Array (The Chinese Academy of Agricultural Sciences, China) was used to scan the whole genome of the PSG for rapid identifying the QTLs related to the stripe rust resistance.

The DNA samples of the two parents and PSG were screened for polymorphism with SSR markers...
and SNP markers were used to genotype the entire population. PCR (polymerase chain reaction) for SSR markers was performed following Helguera et al. (2003). The PCRs were performed in volumes of 10 μL containing 5 μL 2 × TapPlusMasterMix (Vazyme, Nanjing, China), 1 μL ddH₂O, 2 μL (20 ng/μL) of primer, and 2 μL (20 ng/μL) of the template DNA. The PCR products for the SSR primers were separated in 6 or 8% polyacrylamide denaturing gels that were silver-stained for fragment detection.

**QTL detection.** The software map manager QTXb17 was used to calculate the linkage distance between the markers, and QTLs ICMapping 4.0 was used to construct the genetic map and analyse the QTLs (http://www.isbreeding.net/software/?type=detail&id=18) (Li et al. 2007). The logarithm of odds (LOD = 2.5) threshold to declare a QTL for each trait was based on 1,000 permutation tests. A walk speed of 1.0 cM was chosen for the QTL detections. The quantitative trait loci effects were estimated as the proportion of the phenotypic variance (R²) explained (PVE) by the QTL. The flanking sequences of all the molecular markers were subjected to BLAST against the Chinese Spring reference sequence (https://urgi.versailles.inra.fr/blast_iwgsc/blast.php, IWGSC 2018) in order to determine the physical positions. The MapChart 2.3 (https://www.wur.nl/en/show/Mapchart.htm) was used to draw the linkage maps (Voorrips 2002).

**RESULTS**

**Phenotyping of stripe rust responses.** The FDS of the susceptible controls Mingxian 169 ranged from 80 to 100% across three environments, indicating stripe rust epidemics for the Fundulea 900 × Thatcher population for the YR developed well in each environment. The mean FDS of Fundulea 900 and Thatcher were 1 and 80%, respectively, across the three environments. The mean YR severities of the RILs ranged from 15.7 to 19.8% across all the environments and the FDS of the RIL population across all the environments ranged from 1% to 100%, respectively (Table 1). The frequency distribution of FDS in each environment showed a continuous distribution skewed towards resistance (Figure 1), indicating polygenic inheritance. The minority lines showed a higher FDS than Thatcher, indicating transgressive segregation. The FDS in the three environments were significantly correlated with Pearson’s correlation coefficients (r) ranging from 0.88 to 0.89 (P < 0.001) (Table 2).

**Screening of molecular markers.** The results based on the scanning with the Affymetrix 55K SNP Array (53,064 markers) showed that the QTLs for the stripe rust were mainly distributed on chromosomes 1A, 4A, 7B and 7D with 0–1 exchange in preferred screened group (PSG) (Figure 2). Therefore, the SSR markers, mainly from these four chromosomes, were used to test for polymorphism in the parents and the PSG. A total of 42 polymorphic SSR markers (Table S1 in the Electronic Supplementary Material (ESM)) were used to genotype the 176 RIL lines and construct the linkage map.

**QTL mapping of stripe rust resistance.** Three APR QTLs for the YR were detected in the population and designated as QYr.hebau-1AL, QYr.hebau-7BL and QYr.hebau-7DS, respectively (Table 3, Figure 3). All the resistance alleles were contributed by the resistant parent Fundulea 900.

### Table 1. Final disease severity (FDS) for stripe rust of 176 recombinant inbred lines (RILs) from Fundulea 900 × Thatcher in three environments

<table>
<thead>
<tr>
<th>Year and locality</th>
<th>Fundulea 900</th>
<th>Thatcher</th>
<th>RIL population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>minimum</td>
<td>maximum</td>
</tr>
<tr>
<td>YR2017BD</td>
<td>1</td>
<td>80</td>
<td>15.7</td>
</tr>
<tr>
<td>YR2017SC</td>
<td>1</td>
<td>80</td>
<td>19.0</td>
</tr>
<tr>
<td>YR2018SC</td>
<td>1</td>
<td>80</td>
<td>19.8</td>
</tr>
</tbody>
</table>

### Table 2. Pearson’s correlation coefficients (r) for the two-way comparisons of the stripe rust severity data from the different environments

<table>
<thead>
<tr>
<th>Environment</th>
<th>2017BD</th>
<th>2017SC</th>
</tr>
</thead>
<tbody>
<tr>
<td>2017SC</td>
<td>0.89**</td>
<td></td>
</tr>
<tr>
<td>2018SC</td>
<td>0.89**</td>
<td>0.88**</td>
</tr>
</tbody>
</table>

**Significant at P = 0.01; a 2017BD, 2017SC, and 2018SC – the final disease severity (FDS) in the 2016/2017 cropping season in Baoding, Hebei province, and during the 2016/2017 and 2017/2018 cropping seasons in Mianyang, Sichuan province, respectively**
All three QTLs were stably detected in all three environments (Figure 3). The first stable QTL QYr.hebau-1AL flanked by markers Xgwm164 and Xwmc611 explained 9.2, 11.2 and 21.5% of the phenotypic variances in 2017BD, 2017SC and 2018SC, respectively. The second QTL QYr.hebau-7BL with a flanking marker Xcfa2040 and Xgwm344 accounted for 5.1, 10.1 and 6.4% of the phenotypic variance in 2017BD, 2017SC and 2018SC, respectively. QLr.hebau-7DS explained 9.3, 9.1 and 13.7% of the phenotypic variances in 2017BD, 2017SC and 2018SC, respectively (Table 3). The QTL was Yr18/Lr34 based on the marker detection with csLV34. The results showed at least three main QTLs with a stable effect were present in resistant cultivar Fundulea 900, and, in fact, Fundulea 900 also carried the slow rusting gene Lr46/Yr29 based on a previous report (Zhang et al. 2017), as a result, most of the RILs showed high resistance. The lines with all the identified QTLs could be used as a resistant parent for breeding cultivars with durable resistance to stripe rust.

Figure 1. Frequency distributions of the stripe rust final disease severity (FDS) for Fundulea 900 × Thatcher recombinant inbred lines (RILs) in the three environments The FDS mean values for Fundulea 900 and Thatcher are indicated by arrows; BD – Baoding; SC – Sichuan

Figure 2. Frequency distributions of the single nucleotide polymorphism (SNP) loci with 0–1 exchange in the preferred screened group (PSG) on the different chromosomes
DISCUSSION

**QYr.hebau-1AL.** In the present study, QYr.hebau-1AL was mapped between Xgwm164 and Xwmc611 on chromosome 1AL near the centromere. So far, four QTLs, QYr.sun-1A.1 in Janz, QYr.sun-1A.1 in Renan, QYr.cim-1A.1 in Pastor and QYr.caas-1A.1 in Naxos were mapped on chromosome 1AL (Rosewarne et al. 2013). QYr.caas-1A.1 in Naxos was at the end of 1AL. Another three QTLs, QYr.sun-1A.1_Janz, QYr.sun-1A.1_Renan and QYr.cim-1A.1_Pastor, were also close to the centromere, but they had a different genetic background with QYr.hebau-1AL. Therefore, the relationship between QYr.hebau-1AL and the other reported QTLs requires further investigation.

**QYr.hebau-7BL.** Eight known YR genes or QTLs, Yr52 in PI 183527 (Ren et al. 2012), Yr59 in PI 178759 (Zhou et al. 2014), Yr79 in PI182103 (Feng et al. 2013), were also close to the centromere, but they had a different genetic background with QYr.hebau-1AL. Therefore, the relationship between QYr.hebau-1AL and the other reported QTLs requires further investigation.

**QYr.hebau-7DS.** Eight known YR genes or QTLs, Yr37 in PI 183527 (Ren et al. 2012), Yr79 in PI 178759 (Zhou et al. 2014), Yr79 in PI182103 (Feng et al. 2013), were also close to the centromere, but they had a different genetic background with QYr.hebau-1AL. Therefore, the relationship between QYr.hebau-1AL and the other reported QTLs requires further investigation.

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### Table 3. QTLs for stripe rust identified from the Fundulea 900 × Thatcher recombinant inbred line (RIL) populations by IciMapping

<table>
<thead>
<tr>
<th>QTL</th>
<th>Year and locality</th>
<th>Marker interval</th>
<th>LOD^b</th>
<th>PVE (%)^c</th>
<th>Add^d</th>
</tr>
</thead>
<tbody>
<tr>
<td>QYr.hebau-1AL</td>
<td>2017BD</td>
<td>gwm164-wmc611</td>
<td>2.8</td>
<td>9.2</td>
<td>–4.1</td>
</tr>
<tr>
<td></td>
<td>2017SC</td>
<td>gwm164-wmc611</td>
<td>3.4</td>
<td>11.2</td>
<td>–4.9</td>
</tr>
<tr>
<td></td>
<td>2018SC</td>
<td>gwm164-wmc611</td>
<td>6.1</td>
<td>21.5</td>
<td>–6</td>
</tr>
<tr>
<td></td>
<td>2017BD</td>
<td>cfa2040-gwm344</td>
<td>2.9</td>
<td>5.1</td>
<td>–3.6</td>
</tr>
<tr>
<td>QYr.hebau-7BL</td>
<td>2017SC</td>
<td>cfa2040-gwm344</td>
<td>3.9</td>
<td>10.1</td>
<td>–6.5</td>
</tr>
<tr>
<td></td>
<td>2018SC</td>
<td>cfa2040-gwm344</td>
<td>3.3</td>
<td>6.4</td>
<td>–5.8</td>
</tr>
<tr>
<td></td>
<td>2017BD</td>
<td>csLv34-barc92</td>
<td>3.7</td>
<td>9.3</td>
<td>–4.9</td>
</tr>
<tr>
<td>QYr.hebau-7DS</td>
<td>2017SC</td>
<td>csLv34-barc92</td>
<td>3.6</td>
<td>9.1</td>
<td>–6.1</td>
</tr>
<tr>
<td></td>
<td>2018SC</td>
<td>csLv34-barc92</td>
<td>5.6</td>
<td>13.7</td>
<td>–7.3</td>
</tr>
</tbody>
</table>

^aQTLs overlapping within one-log support confidence interval were assigned the same symbol; ^blogarithm of odds (LOD) score; ^cpercentage of the phenotypic variance explained by the QTL; ^additive effect of the resistance allele

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![Graph 1](https://example.com/graph1.png)

![Graph 2](https://example.com/graph2.png)

Figure 3. Positions of the QTLs conferring the Adult-Plant Resistance (APR) to the stripe rust on chromosomes 1AL, 7BL and 7DS, respectively

Logarithm of odds (LOD) = 2.5, which is indicated by a black line that is perpendicular to the LOD axis in the graphs.
Yr2018), YrZH84 in Zhou 8425B (Li et al. 2006), Yr39 in Alpowa (Lin & Chen 2007), Yr59 in C591 (Li et al. 2009), QYr.caas-7BL.1 in SHA3/CBRD (Ren et al. 2012a), and QYr.hebau-7BL in Fuyu 3 (Gebrewahid et al. 2020), were mapped on chromosome 7BL. According to the position of the closely linked markers (Table 4), the eight genes were mapped at 732.4 Mb, 723.9 Mb, 750.6 Mb, 723.9-750.6 Mb, 604.7 Mb, 699.9-718.4 Mb, 557.0 Mb, and 712.3-723.2 Mb, respectively. In the present study, QYr.hebau-7BL.1 was closely linked with Xwms577-Xbarc1073 in Fuyu 3 at 718.4 Mb. This QTL was mapped at a similar position with Yr59, YrZH84, Yr67, and QYr.hebau-7BL. Yr59, YrZH84, and Yr67 were major genes, but QYr.hebau-7BL.1 was a minor gene in the study; therefore, the three genes might be different from QYr.hebau-7BL.1. In addition, QYr.hebau-7BL in Fuyu 3 were considered to be Lr68 (Gebrewahid et al. 2020), but the APR for leaf rust was not identified in Fundulea 900 (Zhang et al. 2017). In the previous study, a seedling gene LrFun from Fundulea 900 was also mapped on 7BL at a similar position as QYr.hebau-7BL (Xing et al. 2014), but the gene had lost its resistance in the field trial (Zhang et al. 2017). Therefore, it is possible that QYr.hebau-7BL.1 was also different from QYr.hebau-7BL and might be a new QTL for YR.

Yr18/Lr34 and Lr46/Yr29. In the current study, QYr.hebau-7DS is a known race non-specific APR gene Yr18/Lr34 (Table 4). In the previous study, the gene Yr18/Lr34 also conferred the APR for leaf rust using the same population (Zhang et al. 2017). In fact, the other slow rusting gene Lr46/Yr29 was also present in Fundulea 900 and conferred the APR for leaf rust with comparatively weak effects (Zhang et al. 2017). However, the stripe rust resistance of Lr46/Yr29 was not identified in the present study. Lr46/Yr29 often had a reduced effect when it was in combination with Lr34/Yr18 (Suenaga et al. 2003; Lillemo et al. 2008).

CONCLUSION

In this study, three QTLs for stripe rust resistance were detected in the Fundulea 900 × Thatcher cross. The three QTLs were mapped on the 1AL, 7BL and 7DS chromosomes, respectively. All three QTLs originated from Fundulea 900 and were detected in all the environments. The QTL on 7DS was provided by the known gene Yr18/Lr34. The two QTLs on chromosomes 1AL and 7BL might be new QTLs. The QTLs identified in the study and their closely linked markers can be used for marker-assisted selection (MAS) in wheat breeding programmes.
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