QTL Mapping for Adult-Plant Leaf Rust Resistance Genes in Chinese Wheat Cultivar Weimai 8

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Abstract


The wheat cultivar Weimai 8 is a good source of adult-plant resistance to leaf rust in China. In order to understand the genetic background of the resistance genes, 179 F2:3 families derived from the cross Weimai 8 and Zhengzhou 5389 (susceptible to leaf rust) were used to construct a linkage map. Results showed that there was a major QTL on chromosome 2AS, temporarily named QLr.hbau-2AS, responsible for the resistance. QLr.hbau-2AS from the resistant parent Weimai 8 was between the SSR markers Xcfd36 and Xbarc1138, with an interval length of 2.58 cM. In the 2010–2011, 2011–2012 and 2012–2013 crop seasons, it explained 25.79, 71.55 and 60.72% of the phenotypic variation, respectively. The location of QLr.hbau-2AS was close to the leaf rust resistance gene Lr37. However, they may not be the same since QLr.hbau-2AS has a different virulence test response from Lr37. This study identified two closely linked flanking molecular markers QLr.hbau-2AS and these molecular markers will help marker-assisted selection in breeding resistant cultivars in the future.

Keywords: gene postulation; molecular marker; QTL loci; wheat leaf rust

Leaf rust caused by Puccinia triticina Eriks. is an airborne foliar fungal disease of wheat that can seriously disrupt plant photosynthesis and ultimately result in a significant loss of production. Leaf rust occurs in wheat growing areas all over the world. In China, the southwest and the northwest are the two most vulnerable regions where it causes serious yield losses (HUERTA-ESPION et al. 2011). Selection and deployment of resistant cultivars is the most economic and effective measure used for the wheat leaf rust control. Study of resistance genes in wheat cultivars is a prerequisite for disease resistance breeding and crop improvement. Furthermore, exploring disease resistance genes in a wheat cultivar is of great significance to understand genetic diversity.

There are two types of resistance to leaf rust in wheat; one is known as race-specific resistance which is conferred by a single gene. This resistance is very unstable, often lost due to a change in P. triticina pathotype. The other is known as non-race-specific resistance, usually expressed during adult-plant stage and is also called adult-plant resistance (APR) or slow rusting. This resistance shows characteristics of quantitative character unlike race-specific resistance and is also known as “field resistance” (RIBEIRO et al. 2001). These genes controlling quantitative characters are called quantitative trait loci (QTL). Cultivars with slow rusting non-hypersensitive APR show slow development of rust symptoms and the ultimate disease level is low. It does not lead to sig-
significant losses, or losses that are much lower than in the susceptible controls. Slow rusting resistance reduces selection pressure greatly in *P. triticina* pathotypes and the pathogen population remains stable. Under such circumstances, both the host and the pathogen can coexist for a long time. Resistance in cultivars with slow rusting is more durable than the race-specific resistance. Epidemiologically, slow rusting resistance has a lower infection rate, long incubation period and small size of uredinia and lower numbers of spores/uredinia (Caldwell 1968).

Genetic studies have shown that slow rusting resistance is a quantitative and durable character and is controlled by several genes (Johnson & Law 1973; Bjarko & Line 1988; Das et al. 1992).

Currently, *Lr34* (Dyck 1977), *Lr46* (Singh et al. 1998), *Lr67* (Mcintosh et al. 2011) and *Lr68* (Herrera-Foessel et al. 2012) are the only known genes of slow rusting resistance to leaf rust and their field performance is good. *Lr34* gene was identified in a Canadian cultivar PI58548 (Dyck 1977) and was located on chromosome 7D (Dyck 1987). Further research has shown that this locus also carries several genes including yellow rust resistance gene *Yr18* and powdery mildew resistance gene *Pm38* (Schnurbusch et al. 2004; Lillemo et al. 2008). This gene was cloned in 2009 (Krattinger et al. 2009). Singh et al. (1998) found *Lr46* on chromosome 1B. William et al. (2003) found and reported an AFLP marker PstAAGMseCTA-1 linked with *Lr46* and the precise position of *Lr46* was at the end of the chromosome 1B long arm. It was tightly linked with yellow rust low rusting resistance gene *Yr29*. *Lr46* is widespread in Chinese wheat cultivars with different genetic backgrounds. The leaf rust slow-rusting resistance gene *Lr67* has a tight linkage with the yellow rust resistance gene *Yr46* (Herrera-Foessel et al. 2011). The recently discovered leaf rust slow-rusting resistance gene *Lr68* was located on the long arm of chromosome 7D. Compared to the number of qualitative traits of resistance genes, the number of leaf rust resistance QTLs is limited. Considering the important role in disease resistance breeding and broad application prospect, it is necessary to develop more effective QTLs and associated molecular markers.

The Chinese wheat cultivar Weimai 8 was developed by Weifang Agriculture Academy of Science (Shandong, China) by crossing 88-3149 (female parent) and Aus621108 (male parent). There are a number of valuable traits in Weimai 8 including strong tillering potential, cold resistance, compact plant type, high yield, resistance to powdery mildew and leaf rust (Chen & Zhang 2005). Weimai 8 is susceptible to most leaf rusts at the seedling stage, but at the adult plant stage it shows slow rusting resistance. This paper reports QTL analysis and molecular mapping of leaf rust APR genes in Weimai 8 and provides a theoretical basis and molecular markers for leaf rust resistance in wheat.

**MATERIAL AND METHODS**

*Plant materials and *P. triticina* pathotypes*. The resistant (Weimai 8) and susceptible (Zhengzhou 5389) parents along with their 179 F$_{2:3}$ families were used for QTL mapping of leaf rust APR. There are seven Chinese *P. triticina* pathotypes used in the study. THTS, THTN, THTH and PHJN were used for seedling tests. THTT, THTS and THTQ were used for leaf rust APR tests. Leaf rust pathotypes were obtained from the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Agricultural University of Hebei, China, which were named using the Prt-coding System (Long & Kolmer 1989).

**Seedling tests in the greenhouse.** Weimai 8 and TcLr37 were tested with 4 Chinese *P. triticina* pathotypes (Table 1). TcLr37 contains only one leaf rust resistance gene that is *Lr37*. Seedlings were grown in a growth chamber. When the first leaf was fully expanded, inoculations were performed by brushing uredinospores from the fully infected susceptible genotype Zhengzhou 5389 onto the new seedlings. Inoculated seedlings were placed in plastic-covered cages and incubated at 15°C and 100% relative humidity (RH) for 24 h in darkness. They were then transferred to a growth chamber programmed with 12 h light/12 h darkness at 18–22°C and 70% RH. Infection types (IT) were scored 10–14 days after inoculation according to the Stakman scale as modified by Roelfs et al. (1992). Plants with IT 0–2+ were considered to be resistant and those with IT 3–4 were susceptible.

**Adult plant field trials.** Both resistant and susceptible parents along with their 179 F$_{2:3}$ families were crossed to develop more effective QTLs and associated molecular markers.

<table>
<thead>
<tr>
<th>Lr gene or cultivar</th>
<th>Pathotype</th>
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<tr>
<td></td>
<td>THTS</td>
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<td>Lr37</td>
<td>2+</td>
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<td>Weimai 8</td>
<td>4</td>
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Table 1. Seedling infection types of Weimai8 and TcLr37 leaf rust resistance to 4 pathotypes of *Puccinia triticina*
were tested for leaf rust resistance in field nurseries at Baoding, Hebei Agricultural University during the 2010–2011, 2011–2012 and 2012–2013 cropping seasons. Field trials were conducted in randomized complete blocks with three replications. Each plot consisted of a single 1.5 m row with 30 cm row to row distance. About 100 seeds were sown in each row. Every tenth row was occupied by the highly susceptible line Zhengzhou 5389 to serve as a control and to facilitate rust development and spread of the spores within the experiment. Additional rows of Zhengzhou 5389 were planted crosswise and adjacent to the test rows. Equal amounts of *P. triticina* pathotypes THTT, THTS and THTQ were added with a few drops of Tween 20 (0.03%) and sprayed on spreader rows at the jointing growth stage (GS 32 according to Zadoks) at dusk using a sprinkling can. The average temperature in the middle of April, Baoding, was about 15°C during the day and 10°C during the night. Following the inoculation, plants were immediately covered with plastic films aiming to trap moisture within plant canopies and the films were removed in the next morning. The experimental field was kept moist by irrigation after inoculation.

**Leaf rust assessment.** Assessment of leaf rust was initiated when susceptible control Zhengzhou 5389 had more than 50% severity (the rust incidence area of the whole leaf) and disease scoring was carried out once a week until the appearance of the disease in 2011–2012 was more serious than in 2010–2011 and 2012–2013, but overall incidence trends were consistent. The correlation coefficient of the 2010–2011 and 2011–2012 FDS of 179 

**DNA extraction and molecular marker screening.** Genomic DNA was extracted from the seedlings of the 

**Phenotypic evaluation.** The FDS of the susceptible control Zhengzhou 5389 was 100%; the FDS of Weimai 8 was 10%. Statistics of the three-year field testing results indicated that data was continuously distributed and supported quantitative inheritance (Figure 1). It can be inferred that Weimai 8 may carry some QTL for leaf rust resistance. The level of the disease in 2011–2012 was more serious than in 2010–2011 and 2012–2013, but overall incidence trends were consistent. The correlation coefficient of the 2010–2011 and 2011–2012 FDS of 179 families was 67.62%, 2010–2011 and 2012–2013 it was 66.04%, 2011–2012 and 2012–2013 it was 86.36%, indicating that there is a low genotype-by-year interaction in Weimai 8.

**QTL mapping.** Overall, 13 molecular markers were found to be polymorphic by screening between the 10 resistant lines and 10 susceptible lines. They are *Xbarc124*, *Xwmc382*, *Xwmc407*, *Xbarc212*, *Xgwm512*, *Xgwm614*, *Xbarc1114*, *Xgwm210*, *Xcfd36*, *Xgwm400*,...
Xbarc1138, sc372, and sc385. These molecular markers are all located on chromosome 2A. Using the Map Manager QTXb20 and QTL Icimapping 3.2 analysis, we found that with 13 molecular markers in one linkage group, the length was 20.5 cM (Figure 2). There was a wheat leaf rust resistance QTL locus, located on chromosome 2AS, temporarily named QLr.hbau-2AS. The QTL may be between the two flanking markers Xbarc1138 (Figure 3) and Xcfd36 (Figure 4) with an interval length of 2.5 cM. In 2010–2011, the site explained 25.79% of the phenotypic variation; in 2011–2012, the site explained 71.55% of the phenotypic variation; and in 2012–2013, the site explained 60.72% of the phenotypic variation (Table 2). Another QTL was detected in the 2010–2011 crop season in addition to QLr.hbau-2AS with a genetic distance of 2 cM. Based on the position alone, the two QTLs are probably the same. However, the estimates shown in Table 2 indicate otherwise. In all three environments, the QTL in the Xbarc1138-Xcfd36 interval has a strong negative additive effect and a much smaller negative dominance effect. In contrast, the second QTL that was detected in 2010–2011 has a weak negative additive effect and a larger positive dominance effect. If in fact the plants were in the F$_{2:3}$ generation...
in 2010–2011 but were in later generations in the other years, this would explain why this QTL was detected only in the first year because the families became more and more homozygous leading to the dominance effect becoming no longer detectable.

**DISCUSSION**

QTLicimapping 3.2 analysis showed that the LOD score was very high in three years FDS, so the probability of existing QLr.hbau-2AS was very high. The correlation coefficient of FDS in three-year field study was also very high, and QLr.hbau-2AS explained 25.7%, 72% and 61% of the phenotypic variance in three years respectively and proved that the leaf rust resistance of QTL QLr.hbau-2AS was less affected by the environment and is relatively stable. The relatively low percentage explained by the QTL in 2010–2011 was probably due to the fact that scores taken from F2 plants were not highly accurate.

At present only one designated gene Lr37 is located on chromosome 2AS (BARIANA & MCINTOSH 1993). The resistance of Lr37 is very good. It is known that Lr37 was from chromosome 2N of Aegilops ventricosasa which was transferred to the wheat genome, and the 2NS/2AS translocation cultivar VPM1 was bred (MAIA 1967). Studies at Sydney University found that VPM1 harbours three rust resistance genes: the leaf rust resistance gene Lr37, stem rust resistance gene Sr38 and yellow rust resistance gene Yr17. These three genes are closely linked to the wheat chromosome 2AS (BARIANA & MCINTOSH 1993) and have been used all over the world (MCINTOSH et al. 1995). HELGUERA et al. (2003), using RFLP marker cmwg682, positioned Lr37-Yr17-Sr38 linkage group at 10 cM of chromosome 2AS from the telomere or centromere 2AS. According to the bread wheat high-density SSR marker map by SOMERS et al. (2004), QLr.hbau-2AS was positioned at the end of the short arm of chromosome 2A, close to the location of Lr37. Although the pedigree of Weimai 8 was not clear, according to the race test results, QLr.hbau-2AS may not be the same as Lr37.

**Acknowledgements.** This study was supported by National Natural Science Foundation (International/Regional Co-
operation and Exchange Program No. 31361140367), Hebei Provincial Outstanding Youth Project No. YQ2013024 and the joint Specialized Research Fund for the Doctoral Program of Higher Education (No. 20131302120004).

References


Received for publication April 17, 2015
Accepted after corrections August 11, 2015

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