Genome-Wide Association Mapping Reveals Novel QTL for Seedling Leaf Rust Resistance in a Worldwide Collection of Winter Wheat

Genqiao Li, Xiangyang Xu,* Guihua Bai, Brett F. Carver, Robert Hunger, J. Michael Bonman, James Kolmer, and Hongxu Dong

Abstract

Leaf rust of wheat (Triticum aestivum L.) is a major disease that causes significant yield losses worldwide. The short-lived nature of leaf rust resistance (Lr) genes necessitates a continuous search for novel sources of resistance. We performed a genome-wide association study (GWAS) on a panel of 1596 wheat accessions. The panel was evaluated for leaf rust reaction by testing with a bulk of Puccinia triticina Eriks. (Pt) isolates collected from multiple fields of Oklahoma in 2013 and two predominant races in the fields of Oklahoma in 2015. The panel was genotyped with a set of 501 single-nucleotide polymorphism (SNP) markers. A total of 14 quantitative trait loci (QTL) for leaf rust resistance were identified at a false discovery rate (FDR) of 0.01 using the mixed linear model (MLM). Of these, eight QTL reside in the vicinity of known genes or QTL, and more studies are needed to determine their relationship with known loci. QTLr.stars-7AL1 is a new QTL to bread wheat but is close to a locus previously identified in durum wheat (Triticum turgidum L. subsp. durum [Dest.] Husn.). The other five QTL, including QTLr.stars-1BL3, QTLr.stars-1DC1, QTLr.stars-2BL1, QTLr.stars-5BL1, and QTLr.stars-7AS1, are likely novel loci for leaf rust resistance. The uneven distribution of the 14 QTL in the six subpopulations of the panel suggests that wheat breeders can enhance leaf rust resistance by selectively introgressing some of these QTL into their breeding materials. In addition, another 31 QTL were significantly associated with leaf rust resistance at a FDR of 0.05.

Core Ideas

- The association panel used in this study may represent the largest in wheat GWAS
- Three pathotypes, including a bulked races collected in the field, were used
- A total of 14 QTL for leaf rust resistance were identified at a FDR of 0.01.
- Six QTL identified in this study are novel loci in bread wheat
- Thirty-one additional QTL were significant at a FDR of 0.05.

Leaf rust, caused by Puccinia, is a major foliar disease that has afflicted wheat for thousands of years (Bolton et al., 2008). Leaf rust occurs nearly everywhere wheat is grown and typically reduces yield by 5 to 15% or more depending on crop developmental stage when the initial rust infection occurs (Samborski, 1995). Yield loss is

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Abbreviations: FDR, false discovery rate; GWAS, genome-wide association study; HR, highly resistant; HS, highly susceptible; iCore, informative core; IT, infection type; LD, linkage disequilibrium; LnP(D), log probability of data; MLM, mixed linear model; MR, moderately resistant; MS, moderately susceptible; NSGC, National Small Grains Collection; PCR, polymerase chain reaction; Pt, Puccinia triticina Eriks.; QTL, quantitative trait loci; SNP, single-nucleotide polymorphism.
mainly through reduction of kernel weight and kernel number per spike (Hershman, 1985).

Genetic resistance is vital to wheat leaf rust control. Much effort has been directed to identifying Lr genes. To date, 71 Lr genes have been officially designated (Lr1–Lr74; Lr21 = Lr40; Lr39 = Lr41; Lr43 deleted) and 20 more temporarily named. These Lr genes are distributed on almost every chromosome. Of these genes, 52 originated from bread wheat and the others were derived from alien species, including Aegilops speltoides Tausch, Ae. tauschii Coss., rye (Secale cereale L.), T. timopheevii (Zhuk.), Zhuk., Agropyron elongatum (Host) P. Beauv., einkorn (T. monococcum L.), wild emmer wheat [Triticum dicoccoides (Korn. ex Asch. & Graebn.) Schweinf.], spelt [T. aestivum L. subsp. spelta (L.) Thell.], Ae. ventricosa Tausch, Ae. geniculata Roth, Ae. kotchyii Boiss., Ae. neglecta Req. ex Bertol., Ae. peregrine (Hack.) Maire & Weiller, Ae. sharonensis Eig, Ae. umbellulata Zhuk., slender wheatgrass [Elymus trachycaulus (Link) Gould ex Shinners], T. armeniacum (Stolet.) Nevski, and Thinopyrum intermedium (Host) Barkworth & D. R. Dewey (McIntosh et al., 2013, 2014; http://wheat.plw.usda.gov/GG2/Triticum/wgc/2013-2014_Supplement.pdf). The majority of these genes confer race-specific resistance in a gene-for-gene manner, and only a few of them, such as Lr34, Lr46, and Lr68, confer race-nonspecific resistance. Race-specific Lr genes provide high levels of resistance to specific races and have been widely used in wheat breeding. However, 40 to 60 Pt races are detected each year in North America, and growing large acreages of susceptible varieties provides a reservoir for new races in the southern Great Plains of the United States (Kolmer and Hughes, 2015). The large genetic variation in pathogen populations typically makes race-specific Lr genes ineffective in a few years after their deployment in the region (Kolmer, 2005), which forces wheat breeders to find new resistance sources.

Knowing what Lr genes are present in germplasm allows for full use and effective deployment of these genes in wheat breeding. Most known Lr genes have been mapped using the biparental population mapping approach. More recently, advances in genomics have made it feasible to genotype a large collection of germplasm lines with thousands of SNP markers. As a result, the GWAS approach, based on the principle of linkage disequilibrium (LD), has been effectively used to exploit existing allelic diversity for traits of agronomic importance. Compared with biparental population mapping, GWAS may provide a higher mapping resolution because all historical meiotic events that have occurred in ancestors of a diverse panel of germplasm can be used. Moreover, GWAS enables detection of genes or QTL present in multiple germplasm lines rather than one or two parental lines of a biparental population. Thus, GWAS is an effective alternative to traditional linkage mapping. In crops with reference genome sequences available, such as rice (Oryza sativa L.) (Huang et al., 2010; 2012a,b; Chen et al., 2014), maize (Zea mays L.) (Gore et al., 2009; Buckler et al., 2009; Tian et al., 2011; Peiffer et al., 2013, 2014; Poland et al., 2011; Chia et al., 2012), and foxtail millet [Setaria italica (L.) P. Beauv. subsp. italica] (Jia et al., 2013), high-density HapMaps have been constructed. These HapMaps greatly facilitate GWAS and have the potential to rapidly resolve complex traits to gene-level resolution (Xu and Bai, 2015).

Genome-wide association study has been widely used in wheat (Breseghello and Sorrels, 2006; Juliana et al., 2015; Maccaferri et al., 2010, 2015; Zhang et al., 2011). The self-pollination nature of wheat results in a high level of LD, which reduces the minimum number of markers needed to detect marker–trait association. Several studies have examined the degree of LD in the wheat genome and found that LD, represented by squared allele frequency correlation coefficient ($r^2$) value, decayed to <0.1 at about 10 to 20 cM (Chao et al., 2007; Zhang et al., 2010a; Juliana et al., 2015), <0.2 at 2 to 3 cM (Somers et al., 2007), and <0.3 at 1.6 cM (Maccaferri et al., 2015). These results suggested that GWAS can be performed with a limited number of molecular markers in wheat. Recently, GWAS has been successfully used to map gene and QTL for yield ( Sukumaran et al., 2015), quality traits (Breseghello and Sorrels, 2006), leaf rust resistance ( Maccaferri et al., 2010; Kertho et al., 2015; Gao et al., 2016), stem rust resistance (Maccaferri et al., 2015; Kertho et al., 2015), stem rust resistance (Yu et al., 2012; Zhang et al., 2014), and soil-borne mosaic virus resistance in wheat (Zhang et al., 2011).

The successful application of GWAS in wheat has prompted the development of genomic resources. A wheat HapMap has been constructed to facilitate GWAS in wheat (Jordan et al., 2015). Moreover, the USDA–ARS National Small Grains Collection (NSGC) has assembled a core subset of wheat accessions to maintain a high degree of genetic diversity with a manageable number of samples (Bonman et al., 2015). The NSGC wheat core subset consists of 4007 accessions, accounting for ~10% of total common wheat germplasm in the NSGC. The core was assembled using the stratified random sampling approach based on the country of origin and may reserve rare genetic variations associated with traits of agronomic importance. Thus, the core subset of wheat accessions is a valuable resource for wheat improvement. The objective of this study was to identify novel leaf rust resistance genes in the core subset of wheat germplasm using GWAS and provide resistance sources for wheat breeding.

Materials and Methods

Plant Materials

The NSGC core set germplasm consists of 4007 accessions, and 3836 of them were genotyped with Illumina iSelect 9K SNP array and Diversity Arrays Technology (DArT) markers. Based on marker data, duplicate accessions were excluded from the core to form an informative core (iCore) with 3230 accessions including 1674 winter–facultative accessions and 1556 spring wheat accessions (Bonman et al., 2015). Of the 1674 winter–facultative accessions, 1596 were available at NSGC and used in this
study. The details of these accessions, including name,
origin, and improvement status, etc., are available at the
TriticeaeCAP project website (http://www.triticeaecap.
org). This panel consists of 217 breeding lines, 589 cul-
tivars, 773 landraces, and 17 genetic stocks and was col-
lected from 85 countries with 594 from Asia, 794 from
Europe, 15 from Africa, 114 from North America, 57
from South America, four from Central America, six
from Australia, 10 from New Zealand, and two from the
Caribbean islands. Experimental seeds were provided by
the USDA–ARS NSGC. In addition, a set of 45 differen-
tial lines carrying known leaf rust resistance genes was
also used in this study (Supplemental Table S1).

Evaluation of Leaf Rust Resistance
Three Pt pathotypes were used as inocula in this
study including Pt2013, Pt52-2 (MMPSD), and Pt54-1
(TNBJG). Pt2013 was a bulked Pt race collected in
multiple fields of Oklahoma in 2013. Previous studies
suggested that a bulk field collection of Pt pathotypes
provided the broadest spectrum of virulence (Dr. Robert
Hunger, personal communication, 2016), and leaf rust
reaction to a mixture of races in the seedling test best
resembled field reaction (Gao et al., 2016). Pt52-2 and
Pt54-1 were two predominant races in Oklahoma fields
and were collected and isolated by USDA–ARS Cereal
Disease Laboratory at St. Paul, MN, in 2015.

All 1596 accessions were evaluated for Pt infection
type (IT) in a greenhouse at the USDA–ARS Wheat, Pea-
ut, and Other Field Crops Research Unit in Stillwater,
OK. A randomized complete block design was used with
two replicates. Eight seeds per entry were planted in each
cell of a 73-cell growing tray (Growing Systems, Inc.)
containing Sunshine Redi-earth Growing Mix (SunGro
Horticulture Cabada Ltd.). Each tray had 70 cells for the
test entries and three for the susceptible check, TAM110.

Seedlings at the two-leaf stage (~12 d) were inoculated
with urediniospores of Pt2013 using a method described
previously (Xing et al., 2014) in 2015. Inoculated seed-
lings were placed in a dew chamber with no lighting at
~20°C with 100% relative humidity for 24 h and then
transferred to a greenhouse at 22 ± 2°C with supple-
mental light and a photoperiod of 16:8 (light/dark) h. A
0- to 9 scale (McNeal, 1971) was used to score leaf rust IT
of each plant when leaf rust was fully developed on sus-
ceptible checks at ~14 d after inoculation. The mean IT
of eight plants in each replicate was calculated for each
accession and used for further analysis.

Based on the leaf rust IT data from 1596 accessions,
a subset of accessions that met one of the following three
criteria were chosen for a confirmation test in 2015: (i) an
accession with a mean IT ≤ 3, (ii) plants with heteroge-
neous reactions to Pt2013 within a replicate, or (iii) the
difference in IT ≥ 2 between two replicates. The purpose
of the confirmation test was to get accurate data and
identify reliable resistance sources, and the experimen-
tal design and disease evaluation protocols described
above were employed in the confirmation test. The
confirmation test identified 59 heterogeneous accessions,
which were excluded from further analysis, leaving 1537
accessions available for association analysis. The leaf rust
IT data of two replicates were highly consistent with a
correlation coefficient of 0.947, and the mean IT over two
replicates was calculated for each accession and used for
GWAS analysis. In early 2016, the 1537 accessions were
evaluated with the races Pt52-2 and Pt54-1 using the
same experimental design.

Identification of the Lr47 Gene
using a Diagnostic Marker
A total of 61 accessions showing high resistance to
Pt2013 were tested for the presence or absence of the Lr47
gene using a T. speltoides (Tausch) Gren. ex K. Richt.–spe-
cific marker (Helguera et al., 2000). Clt 17884, a genetic
stock carrying the Lr47 gene, was used as a positive con-
trol. Genomic DNA was extracted from seeds of each
accession using a protocol reported by Dubcovsky et al.
(1994). Primer sequences and polymerase chain reaction
(PCR) conditions described previously were employed in
this study (Helguera et al., 2000). The PCR products were
separated by electrophoresis in 2% agarose gels and visu-
alized using ethidium bromide and ultraviolet light.

Data Analysis
The method for collecting genotypic data of 1537 acces-
sions was described previously (Bonman et al., 2015),
and 5011 polymorphic SNP data from the TCAP project
(http://www.triticeaecap.org) were used for the GWAS.

Among the 5011 SNPs that were analyzed with
HAPLOVIEW 4.2 (Barrett et al., 2005), 4091 were identi-
fied as nonredundant SNPs using the tagger func-
tion = 1.0. A subset of 2110 informative SNPs were selected
for structure (Q matrix) analysis using the tagger func-
tion r2 = 0.25. The Q matrix was estimated using the
program STRUCTURE 2.3.4 (Pritchard et al., 2000)
and was integrated as a covariate to correct the effects
of population structure. An admixture model was used
to test the hypotheses for two to 15 subpopulations (K
= 2–15). For each K, five independent runs were performed
with a burn-in length of 100,000, and the number of
Markov Chain Monte Carlo iterations of 50,000. The log
probability of data [LnP(D)] was estimated for each run,
and an ad hoc statistic AK that was based on the rate of
change in LnP(D) between successive K values was used
to determine the true number of subpopulations (Evanno
et al., 2005). Based on the inferred subpopulation num-
ber, a Q matrix representing population membership
coefficients of each accession was obtained.

Of the 5011 SNPs, 4716 were mapped on a consensus
reference map (Cavanagh et al., 2013; Wang et al., 2014)
and thus used for genome-wide LD analysis. An R pack-
age (R Development Core Team, 2010) was used to calcu-
late the LD for all pairwise comparisons between intra-
chromosomal SNPs and estimate the genome-wide LD
decay by plotting LD r2 from all 21 chromosomes against
the corresponding genetic distances. A trend line was
fitted by second-degree loess (Cleveland 1979) using the statistical program R (R Development Core Team, 2010).

The mixed linear model (Yu et al., 2006) was employed to identify loci governing Pt response in the 1537 accessions using TASSEL 5.0 (Bradbury et al., 2007). The SNP markers and the Q matrix were used as fixed effects. To correct the family relatedness, a kinship (K) matrix was calculated with the scaled identity-by-state method that gives a reasonable estimate of additive genetic variance (Endelman and Jannink, 2012) and used as a random effect component in the MLM model. The MLM was run with the optimum level of compression and the P3D approach (Zhang et al., 2010b) using TASSEL 5.0. The FDR of 0.01 was used as a threshold to declare significant marker–trait association with the procedure developed by Benjamini and Hochberg (1995).

Results

Linkage Disequilibrium Analysis
Among 4716 SNPs that were mapped in the 90K SNP consensus map (Cavanagh et al., 2013; Wang et al., 2014), 2220 were from the A genome, 2200 from the B genome, and 296 from the D genome. They were used to estimate genome-wide LD (Fig. 1). Based on the fitted model, genome-wide $r^2$ values declined to 0.1 at 11 cM. Thus a genomic region covering 11 cM from each side of the peak of significant associations on the same chromosome arm was defined as a QTL-harboring region, and all significant associations in this region were considered as the same QTL.

Population Structure
The model-based structure analysis revealed six subpopulations (S1–S6), which include 339 (22.1%), 78 (5.1%), 437 (28.6%), 379 (24.7%), 133 (8.7%), and 171 (11.1%) accessions with the $F_{st}$ values of 0.6502, 0.7943, 0.3240, 0.4588, 0.4323, and 0.4562 from S1 to S6, respectively (Fig. 2).
Iranian Landraces were predominant in S1, accounting for 87.6% in the subpopulation, and the remaining 12.4% originated from central Asia and Europe; therefore, this subpopulation was designated as Iranian landrace group 1. Iranian landraces were well represented in the iCore subset. Among 425 Iranian accessions used in this study, 297 belong to S1. Wheat cultivars from eastern Europe (Romania, Bulgaria, Russia, Croatia, and Ukraine) were predominant in S2, accounting for 87.2% (eastern European cultivar group). The others in this group were from Asia (9%), United States (2.6%), and South America (1.3%). The origins of accessions in S3 were diverse. The 437 accessions in this subpopulation, including 79 breeding lines, 207 registered cultivars, 139 landraces, and 12 genetic stocks, were collected from 60 countries in Europe, North America, Asia, Africa, South America, Australia, New Zealand, and the Caribbean islands (Cuba). European cultivars, especially western European cultivars, were predominant in S4, accounting for 77% of all accessions. The majority of cultivars released from western Europe, including Germany, England, the Netherlands, Sweden, Switzerland, Portugal, Spain, Italy, France, Finland, Denmark, and Belgium, belonged to S4 (western European cultivar group). Some cultivars and landraces from other regions, including Asia, United States, and eastern Europe, were also included in this group. Subpopulation S5 was characterized by cultivars and landraces collected from eastern Asia, including China, Japan, and South Korea, and southern Asia including Bhutan, Nepal, and India. Accessions from these countries accounted for 68% of the total accessions in S5. A few landraces from western Asia (Iran), and one or two each from other 29 countries, were also included in this group (east-Asian accession group). Most (91.4%) of group S6 were landraces with a large portion (66.3%) from Iran. The remaining accessions in this group came from Turkey, Afghanistan, Macedonia, Armenia, Azerbaijan, Georgia, United States, Lebanon, and Jordan (Iranian landrace group 2).

**Evaluation of Leaf Rust Reaction**

Among the tested accessions, only a limited number of them (4%) showed a high level of seedling resistance to *Pt2013* (highly resistant [HR]; IT = 0–3), and their mean IT distribution was significantly skewed toward susceptibility (Fig. 3). About 17.3, 36.6, and 43.1% were...
moderately resistant (MR; IT = 4–5), moderately susceptible (MS; IT = 6–7), and highly susceptible (HS; IT = 8–9), respectively. The paucity of HR germplasm has lost their effectiveness to Pt2013 (Supplemental Table S1). In the present study, we also tested a set of 45 differential lines with known Lr genes and found that only a few of them were highly resistant to Pt2013 (Supplemental Table S1) including lines with Lr19 (IT = 0), Lr25 (IT = 1), Lr29 (IT = 3), Lr45 (IT = 0), Lr47 (IT = 1), and Lr51 (IT = 3).

Compared with Pt2013, Pt52-2 and Pt54-1 were less virulent. About 8.0, 4.0, 18.6, and 69.4% accessions in the association panel was rated as HR, MR, MS, and HS, respectively, and in the S6 group, these numbers were 0.6, 9.4, 32.7, and 57.3%, respectively. The mean IT scores of S3 (6.53) and S5 (6.83) were significantly lower than those of S1 (7.61) and S6 (7.34) (p < 0.01) but significantly higher than that of S4 (5.91) (p < 0.01). Similar results were observed when the association panel was inoculated with Pt52-2 and Pt54-1.

Quantitative Trait Loci for Leaf Rust Resistance

The MLM analysis revealed a total of 14 QTL that were significantly associated with leaf rust resistance at FDR of 0.01 in three experiments (Table 1). Six, eight, and five significant QTL were detected for resistance to the pathotypes Pt2013, Pt52-2, and Pt54-1, respectively. Each QTL explained 1.36 to 8.35% of the phenotypic variance for resistance to Pt2013, 1.35 to 7.23% for resistance to Pt52-2, and 1.48 to 2.64% for resistance to Pt54-1. Of these QTL, QLr.stars-1BC1 was significant for resistance to all three pathotypes, while QLr.stars-1BS1 and QLr.stars-7A1 were significant for resistance to pathotypes Pt2013 and Pt54-1. The remaining 10 QTL were significant for resistance to only a single pathotype at FDR of 0.01 (Table 1). Among them, however, QLr.stars-1DC1, QLr.stars-1BL1, QLr.stars-4BL1, and QLr.stars-4AL1 were significant for resistance to two to three pathotypes if a FDR was set at 0.05 (Supplemental Table S1). Moreover, 31

![Fig. 4. Box plot showing distribution of wheat leaf rust infection types (ITs) in six subpopulations inoculated with Pt2013. The diamond represents mean IT value of each subpopulation.](image-url)
additional significant loci were detected at a FDR of 0.05 (Supplemental Table S1).

The 14 QTL significant at FDR of 0.01 were located on eight chromosomes including five loci on chromosome 1B, two each on chromosomes 2D and 7A, and one each on chromosomes 1D, 2B, 4A, 4B, and 5B (Table 1). For two of the QTL on 1B, QLr.stars-1BS1 is on the short arm, while QLr.stars-1BC1 is in the centromere region where recombination is usually suppressed. The genetic distance between them is <10 cM. However, the physical distance between them may be greater than that indicated by the genetic distance because of recombination suppression. We further calculated the LD between the two tag SNPs underlying these two QTL, IWA1566 and IWA435, and found that the LD was only 0.089, suggesting that these two QTL were likely different loci.

Three SNPs cosegregating in the association panel, IWA6110, IWA6541 and IWA6129, were significantly associated with leaf rust resistance to both pathotypes Pt2013 and Pt54-1. These SNPs have not been mapped to the 90K SNP consensus map yet. To determine the genomic locations of IWA6110, IWA6129, and IWA6541, we compared their sequences (~200 bp) with other SNP sequences from 90K SNP arrays and found that IWA6110 and IWA6541 were identical to IWB3972 and IWB63777, respectively. IWB3972 was previously mapped to the centromere region of chromosome 1B and IWB63777 on the proximal region of 1BS. IWB3792 and IWB6377

**Table 1.** Designated name, representative single-nucleotide polymorphism (SNP; TagSNP), chromosome arm, location on the Illumina SNP9K consensus map, \( p \)-value, and \( R^2 \)-value of each quantitative trait loci (QTL) that was significant at a false discovery rate of 0.01 when the association panel was evaluated using Pt pathotypes Pt2013, Pt52-2, and Pt54-1.

<table>
<thead>
<tr>
<th>Pt pathotype</th>
<th>QTL</th>
<th>TagSNP</th>
<th>SNP</th>
<th>Chromosome arm†</th>
<th>Map location</th>
<th>( p )-value</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt2013</td>
<td>QLr.stars-1BC1</td>
<td>IWA435</td>
<td>5</td>
<td>1BC</td>
<td>60.13</td>
<td>( 2.09 \times 10^{-13} )</td>
<td>4.76</td>
</tr>
<tr>
<td></td>
<td>QLr.stars-1BL1</td>
<td>IWA1563</td>
<td>1</td>
<td>1BL</td>
<td>148.23</td>
<td>( 3.31 \times 10^{-5} )</td>
<td>1.36</td>
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<td>QLr.stars-1BS1</td>
<td>IWA6566</td>
<td>7</td>
<td>1BS</td>
<td>48.08–57.72</td>
<td>( 2.31 \times 10^{-11} )</td>
<td>3.64</td>
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<td></td>
<td>QLr.stars-1BS1</td>
<td>IWA6110</td>
<td>3</td>
<td>1BS</td>
<td>–</td>
<td>( 9.06 \times 10^{-15} )</td>
<td>4.83</td>
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<td></td>
<td>QLr.stars-1DC1</td>
<td>IWA362</td>
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<td>48.81</td>
<td>( 1.73 \times 10^{-5} )</td>
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<td>QLr.stars-2DL1</td>
<td>IWA1083</td>
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<td>2DL</td>
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<td>( 1.67 \times 10^{-6} )</td>
<td>1.82</td>
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<td>IWA6736</td>
<td>3</td>
<td>7AL</td>
<td>188.95</td>
<td>( 5.71 \times 10^{-24} )</td>
<td>8.35</td>
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<td>Pt52-2</td>
<td>QLr.stars-1BC1</td>
<td>IWA435</td>
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<td>1BC</td>
<td>60.13</td>
<td>( 2.25 \times 10^{-5} )</td>
<td>1.43</td>
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<td>( 5.96 \times 10^{-6} )</td>
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<td>IWA579</td>
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<td>1BL</td>
<td>115.79</td>
<td>( 4.08 \times 10^{-5} )</td>
<td>1.35</td>
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<td>QLr.stars-2DS1</td>
<td>IWA609</td>
<td>6</td>
<td>2DS</td>
<td>79.50–92.80</td>
<td>( 2.19 \times 10^{-23} )</td>
<td>7.23</td>
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<td>QLr.stars-4DL1</td>
<td>IWA2460</td>
<td>2</td>
<td>4DL</td>
<td>73.40–83.38</td>
<td>( 2.65 \times 10^{-5} )</td>
<td>1.40</td>
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<td>IWA4571</td>
<td>4</td>
<td>5BL</td>
<td>106.17–106.94</td>
<td>( 1.85 \times 10^{-6} )</td>
<td>1.75</td>
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<td>QLr.stars-7AL1</td>
<td>IWA3760</td>
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<td>7AL</td>
<td>45.42</td>
<td>( 6.38 \times 10^{-6} )</td>
<td>1.59</td>
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<td>Pt54-1</td>
<td>QLr.stars-7AL1</td>
<td>IWA6736</td>
<td>1</td>
<td>7AL</td>
<td>188.95</td>
<td>( 6.29 \times 10^{-8} )</td>
<td>1.95</td>
</tr>
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<td></td>
<td>QLr.stars-1BC1</td>
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<td>1BC</td>
<td>42.93</td>
<td>( 3.43 \times 10^{-7} )</td>
<td>1.97</td>
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<tr>
<td></td>
<td>QLr.stars-2DL1</td>
<td>IWA4012</td>
<td>3</td>
<td>2DS</td>
<td>92.80–93.40</td>
<td>( 7.87 \times 10^{-7} )</td>
<td>2.64</td>
</tr>
<tr>
<td></td>
<td>QLr.stars-2BL1</td>
<td>IWA1488</td>
<td>1</td>
<td>2BL</td>
<td>193.90</td>
<td>( 1.31 \times 10^{-5} )</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>QLr.stars-1BS2</td>
<td>IWA6110</td>
<td>3</td>
<td>1BS</td>
<td>–</td>
<td>( 1.87 \times 10^{-10} )</td>
<td>1.79</td>
</tr>
</tbody>
</table>

† L, S, and C refer to long and short chromosome arms, and centromere, respectively.

Fig. 5. Inferred locations of single-nucleotide polymorphism (SNP) marker IWA6541, IWA6110, and IWA6129 on the SNP9K consensus map (right) and homoeologous sequence corresponding to each marker on the Brachypodium chromosome 2 physical map (left). IWA6129 is tentatively mapped in between IWA6541 and IWA6110.

were 1.54 cM apart on the 90K consensus map (Fig. 5). We further searched these three SNP sequences against Chinese Spring reference genome sequence (urgi.versailles.inra.fr/blast/blast.php). Three contigs mapped to chromosome 1B, including 1BS_3441348, 1BS_3445781,
and 1BS_3456976, contained IWA6541, IWA6129, and IWA6110 sequence, respectively. We searched these contigs against *Brachypodium* physical map using BLASTn (http://www.plantgdb.org/BdGDB/cgi-bin/blastGDB.pl) and located them to 23.8, 34.8, and 59.8 Mb of chromosome 2, respectively (Fig. 5). Considering the collinearity between wheat and the *Brachypodium* genome (Brenchley et al., 2012), we located IWA6129 in between IWA6110 and IWA6541 at the 58.37 to 59.91 cM region on the consensus map. Given that LDs between these SNPs and two tag SNPs, IWA1566 and IWA435, were estimated to be 0.2 and 0.06, respectively, we conclude that these SNPs represent the same locus as IWA1566, QLr.stars-1BS1.

Three QTL were detected on 1BL. QLr.stars-1BL1 was significant for resistance to *Pt2013*, while QLr.stars-1BL2 and QLr.stars-1BL3 were significant for resistance to *Pt52-2*. Their locations on the 90K consensus map are 148.23, 81.58, and 115.79 cM, respectively, thus they are likely different QTL. The other nine QTL were identified in different chromosome arms of seven chromosomes (Fig. 6).

**Allele Effects of the Quantitative Trait Loci Identified in This Study**

In the *Pt2013* experiment, the allelic effects of the six QTL identified in this study varied from 0.26 to 2.27 for IT score. For two of them, allele substitution resulted in IT change >1.0 (Table 2). The favorable allele number in each accession ranged from 0 to 5 with an average of 1.57 at six loci. The mean IT values decreased from 7.42 for germplasm without any favorable allele to 3.82 for those possessing five favorable alleles (Supplemental Fig. S1). All 23 accessions having five favorable alleles were rated as either HR or MR to *Pt2013*. It is of great interest to know whether the leaf rust resistance of these accessions would prove to be durable.

A significant correlation between favorable allele numbers and IT values was observed ($r = -0.401, p < 10^{-7}$). The observed phenotypic values and the predicated phenotypic values (calculated as the sum of all QTL effects for each accession) were also significantly correlated ($r = -0.53, p < 10^{-7}$), suggesting that pyramiding these QTL may enhance leaf rust resistance.

Similarly, favorable alleles were negatively correlated with the IT scores when plants were inoculated...
with $Pt52-2 (r = -0.35, p < 10^{-7})$ and $Pt54-1 (r = -0.45, p < 10^{-7})$. The favorable alleles in each accession varied from 0 to 7 with a mean allele number of 2.4 at eight QTL resistant to $Pt52-2$, and 0 to 4 with an average of 0.6 at five loci resistant to $Pt54-1$. The allele substitution effects for IT score ranged from 0.3 to 2.5 and 0.37 to 1.7 in the two experiments, respectively (Table 2). Notably, the favorable allele of $QLr.stats-1BS1$ was not detected in east-Asian cultivars or landraces (S5), while the favorable allele of $QLr.stats-2BL1$ was not present in cultivars from eastern Asia and eastern Europe, respectively.

**Discussion**

The availability of high-density consensus linkage maps makes it feasible to determine the relationship of QTL identified in this study with known $Lr$ genes or QTL. Based on 90K SNP consensus map (Wang et al., 2014), the relationship between the known loci for leaf rust resistance and the genomic regions harboring QTL identified in this study was illustrated in Fig. 6.

Two named genes, $Lr71$ and $LrZh84$, and three QTL, $QLr.pser-1BL$, $QLr.sfr-1BS$, and $QLr.sfr-1B$, for leaf rust resistance were previously mapped to the genomic regions harboring $QLr.stats-1BC1$ and $QLr.stats-1BS1$ (Zhou et al., 2013; Li and Bai, 2009; Schnurbusch et al., 2004; Messmer et al., 2000; Schnurbusch et al., 2004). Another QTL mapped in this region, $QLr.pser-1BL$, showed adult plant resistance (Li and Bai, 2009). Thus, the relationship between these known genes or QTL and the QTL identified in this study remain to be investigated.

Similarly, $QLr.stats-1BL1$, $QLr.stats-1BL2$, and $QLr.stats-1BL3$ were in the proximity of known genes or QTL (Fig. 6). $QLr.stats-1BL1$ and $QLr.stats-1BL2$ reside in the genomic regions where $Lr46$ and $Lr33$ were located, respectively (William et al., 2003; Dyck and Sykes, 1994). $Lr46$ is an important durable leaf rust resistance gene that exhibits adult plant resistance. $Lr46$ was first identified in the wheat cultivar Pavon. The effect of $Lr46$ resembles that of $Lr34$ at the adult plant stage but is weaker than that of $Lr34$ at the seedling stage (Martínez et al., 2001).

**Table 2 Allelic effects of the tag single-nucleotide polymorphisms (SNPs) underlying quantitative trait loci (QTL) for leaf rust resistance and the distribution of favorable alleles in six subpopulations**

<table>
<thead>
<tr>
<th>QTL name</th>
<th>TagSNP</th>
<th>SNP allele</th>
<th>$Pt52-2$</th>
<th>$Pt54-1$</th>
<th>$S1$</th>
<th>$S2$</th>
<th>$S3$</th>
<th>$S4$</th>
<th>$S5$</th>
<th>$S6$</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>$QLr.stats-1BC1$</td>
<td>IWA435</td>
<td>$T/C$</td>
<td>0.72</td>
<td>0.80</td>
<td>0.37</td>
<td>0.9</td>
<td>6.4</td>
<td>17.6</td>
<td>3.4</td>
<td>29.3</td>
<td>8.2</td>
</tr>
<tr>
<td>$QLr.stats-1BL1$</td>
<td>IWA8153</td>
<td>$T/C$</td>
<td>0.52</td>
<td>−</td>
<td>−</td>
<td>2.1</td>
<td>30.8</td>
<td>29.3</td>
<td>66.0</td>
<td>15.0</td>
<td>11.7</td>
</tr>
<tr>
<td>$QLr.stats-1BS1$</td>
<td>IWA6110</td>
<td>$A/G$</td>
<td>2.27</td>
<td>−</td>
<td>1.70</td>
<td>0.0</td>
<td>25.6</td>
<td>0.5</td>
<td>5.5</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>$QLr.stats-1BL2$</td>
<td>IWA362</td>
<td>$T/C$</td>
<td>0.26</td>
<td>−</td>
<td>−</td>
<td>78.8</td>
<td>16.7</td>
<td>35.2</td>
<td>29.3</td>
<td>77.4</td>
<td>56.1</td>
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<td>$QLr.stats-2BL1$</td>
<td>IWA1488</td>
<td>$A/G$</td>
<td>−</td>
<td>0.40</td>
<td>−</td>
<td>2.1</td>
<td>6.4</td>
<td>14.9</td>
<td>10.3</td>
<td>44.4</td>
<td>15.2</td>
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<tr>
<td>$QLr.stats-2BL2$</td>
<td>IWA579</td>
<td>$T/C$</td>
<td>−</td>
<td>0.31</td>
<td>−</td>
<td>40.6</td>
<td>6.4</td>
<td>9.8</td>
<td>9.2</td>
<td>84.2</td>
<td>68.4</td>
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<tr>
<td>$QLr.stats-2BC1$</td>
<td>IWA1488</td>
<td>$A/G$</td>
<td>−</td>
<td>−</td>
<td>1.00</td>
<td>3.8</td>
<td>0.0</td>
<td>1.6</td>
<td>4.2</td>
<td>14.3</td>
<td>7.0</td>
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<tr>
<td>$QLr.stats-2CL1$</td>
<td>IWA609</td>
<td>$A/G$</td>
<td>2.50</td>
<td>−</td>
<td>−</td>
<td>3.2</td>
<td>5.1</td>
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<td>2.1</td>
<td>23.8</td>
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<td>$QLr.stats-2BS1$</td>
<td>IWA4012</td>
<td>$A/G$</td>
<td>−</td>
<td>−</td>
<td>1.02</td>
<td>5.3</td>
<td>22.3</td>
<td>4.5</td>
<td>7.7</td>
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<td>14.9</td>
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<tr>
<td>$QLr.stats-4AL1$</td>
<td>IWA2460</td>
<td>$T/C$</td>
<td>0.42</td>
<td>−</td>
<td>−</td>
<td>1.5</td>
<td>3.8</td>
<td>13.7</td>
<td>8.4</td>
<td>70.7</td>
<td>14.6</td>
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<tr>
<td>$QLr.stats-4BL1$</td>
<td>IWA6480</td>
<td>$A/G$</td>
<td>−</td>
<td>0.56</td>
<td>−</td>
<td>96.5</td>
<td>16.7</td>
<td>28.4</td>
<td>20.3</td>
<td>78.9</td>
<td>97.7</td>
</tr>
<tr>
<td>$QLr.stats-5BL1$</td>
<td>IWA4571</td>
<td>$T/C$</td>
<td>−</td>
<td>0.30</td>
<td>−</td>
<td>81.4</td>
<td>88.5</td>
<td>71.6</td>
<td>92.3</td>
<td>70.7</td>
<td>93.0</td>
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<tr>
<td>$QLr.stats-7SL1$</td>
<td>IWA3760</td>
<td>$A/C$</td>
<td>0.48</td>
<td>−</td>
<td>−</td>
<td>1.8</td>
<td>74.4</td>
<td>33.4</td>
<td>71.0</td>
<td>14.3</td>
<td>36.3</td>
</tr>
</tbody>
</table>

† The favorable alleles are in bold type.

‡ S1, Iranian landrace group 1; S2, eastern European cultivar group; S4, western European cultivar group; S5, east-Asian accession group; S6, Iranian landrace group 2.
in wheat cultivar Arina, rather than *Lr54*. In addition, *QLr.stars-2DS1*, *QLr.stars-4AL1*, and *QLr.stars-4BL1* coincided with *Lr2* (*Lr15*), *Lr30*, and *Lr12* (*Lr31*) (Singh and Bowden, 2011), respectively. Further studies may facilitate determination of the relationship between known resistance genes and QTL identified in this study.

*QLr.stars-7AS1* is <4 cM away from *Lr47* (Fig. 6), *Lr47*, transferred from chromosome 7S of *T. speltoides* to chromosome 7A of common wheat (Dubcovsky et al., 1998), was highly resistant to all three pathotypes used in this study (Supplemental Table S1). To determine the relationship between *QLr.stars-7AS1* and *Lr47*, we used a diagnostic marker (Helguera et al., 2000) to detect wheat accessions carrying the *Lr47* gene from those showing high resistance to *Pt2013*. Our results suggested that no accession in this panel carries *Lr47*. Thus, *QLr.stars-7AS1* is a novel locus.

*QLr.stars-7AL1* was significantly associated with resistance to *Pt2013* and *Pt54-1* and is 15.4 cM distal to *Lr20* on the linkage map (Neu et al., 2002; Wang et al., 2014), indicating that *QLr.stars-7AL1* may be independent from *Lr20* and likely a novel locus in bread wheat. A recent GWAS study revealed that Xcfa2257, a simple-sequence repeat marker that is 2 cM distal to *QLr.stars-7AL1*, was associated with leaf rust resistance in durum wheat (Maccaferri et al., 2010), lending credence to *QLr.stars-7AL1*. The SNP allele associated with the resistant form of this QTL was present at high frequencies in the eastern European cultivar group (74.4%) and the western European cultivar group (67.8%) but at a low frequency in the east-Asian accession group (6.8%). Therefore, it is worthy to evaluate the effectiveness of this QTL against *Pt* races in eastern Asia, and it may diversify leaf rust resistance sources in this region if it turns out to be resistant.

Another four QTL, *QLr.stars-1BL3*, *QLr.stars-1DC1*, *QLr.stars-2BL1*, and *QLr.stars-5BL1*, are likely novel loci for leaf rust resistance. Based on locations of molecular markers flanking known *Lr* genes on the 90K consensus map, *QLr.stars-1BL3* is 30 cM from *QLr.stars-1BL2/Lr33* and at least 17.5 cM from *Lr46* (William et al., 2003). The genetic distance between *QLr.stars-2BL1* and the two closest leaf rust resistance genes, *Lr35* and *Lr13* (Seyfarth et al., 1999, 2000), is at least 27.2 cM (Fig. 6). Moreover, no gene has been identified in the genomic regions harboring *QLr.stars-1DC1* and *QLr.stars-5BL1*. These novel loci represent valuable additions to the leaf rust resistance gene pool available for wheat breeding.

QTL identified in this study showed obvious regional distribution. For example, 10 SNPs at the *QLr.stars-1BS1* locus were significantly associated with leaf rust resistance, and three of them, *IWA6110*, *IWA6129*, and *IWA6541*, showed the largest effect. The favorable alleles of these SNPs were mainly found in eastern and western European cultivars (*S2* and *S4*), and were completely absent in east-Asian accessions and Iranian landraces (*S1*, *S4*, and *S5*). Thus, if *QLr.stars-1BS1* is effective to *Pt* races in eastern Asia, it should be a valuable resistance source for wheat breeding programs in this region. On the contrary, the favorable allele of *IWA1488*, the tagSNP of *QLr.stars-2BL1*, is present at a relatively higher frequency in east-Asian accessions (*S3*), and no cultivars in eastern Europe (*S2*) carry this allele. Use of this QTL may diversify resistance sources if it confers resistance to *Pt* races in this region. Given that SNP markers associated with these QTL are available and high-throughput assays, such as Kompetitive Allele-Specific PCR, can be easily developed for these markers (Cabral et al., 2014), QTL identified in this study can be reliably introgressed into elite cultivars using marker-assisted selection.

**Conclusions**

Genome-wide association study is an effective gene discovery approach complementary to the traditional linkage mapping. In this study, 14 QTL were significantly associated with leaf rust resistance at a FDR of 0.01. Of these, eight QTL were mapped in the vicinity of known genes or QTL in bread wheat. Allelism tests may confirm their relationship with known genes or QTL. *QLr.stars-7AL1* was the most significant QTL identified in this study, explaining 8.4% of the total phenotypic variance. This QTL is new to bread wheat but is close to a locus previously identified in durum wheat. The other five QTL, including *QLr.stars-1BL3*, *QLr.stars-1DC1*, *QLr.stars-2BL1*, *QLr.stars-5BL1*, and *QLr.stars-7AS1*, are likely novel loci for leaf rust resistance. The distribution of these 14 QTL was uneven in the six subpopulations, suggesting that wheat breeding programs can enhance leaf rust resistance by introgressing specific QTL identified in this study into new cultivars. In addition, another 31 loci were significantly associated with leaf rust resistance at a less stringent FDR of 0.05 and will be valuable additions to the leaf rust resistance gene pool.

**Supplemental Information Available**

Supplemental information is available with the online version of this manuscript.

**Acknowledgments**

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**References**


