Mapping of Stripe Rust and Leaf Rust Resistance Quantitative Trait Loci in the Chinese Spring Wheat Line Mianyang351-15

Takele Weldu Gebrewahid,^{1,2} Yue Zhou,³ Peipei Zhang,¹ Yong Ren,⁴ Pu Gao,¹ Xianchun Xia,⁵ Zhonghu He,⁶ Zaifeng Li,^{1,†} and Daqun Liu^{1,†}

¹ College of Plant Protection, Hebei Agricultural University, 289 Lingyusi Street, Baoding, Hebei 071001, China

² College of Agriculture, Aksum University, Shire-Indaslassie, Tigray 314, Ethiopia

³ Baoding University, 3027 Qiyi Donglu Street, Baoding 071001, Hebei, China

⁴ Mianyang Institute of Agricultural Science/Mianyang Branch of National Wheat Improvement Center, Mianyang 621023, Sichuan Province, China

⁵ Institute of Crop Science, National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), 12 Zhongguancun South Street, Beijing 100081, China

⁶ Institute of Crop Science, National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS) and International Maize and Wheat Improvement Center (CIMMYT) China Office, 12 Zhongguancun South Street, Beijing 100081, China

Accepted for publication 26 February 2020.

ABSTRACT

Stripe rust and leaf rust cause wheat yield losses of up to 70% worldwide. The employment of resistant cultivars is the major method to reduce losses from these diseases. The objective of this study was to detect quantitative trait loci (QTL) for stripe rust and leaf rust resistance in 150 F₆ recombinant inbred lines (RIL) derived from a cross between Mianyang351-15 and Zhengzhou 5389. Both parents and the RIL population were genotyped with the Wheat55K single nucleotide polymorphism (SNP) array and simple sequence repeat markers, and phenotyped for stripe rust severity at Mianyang in Sichuan Province and Baoding in Hebei Province, and for leaf rust severity at Zhoukou in Henan Province and at Baoding in 2014 to 2017 cropping seasons. Seven and four QTL all contributed from Mianyang351-15 were identified for resistance to stripe rust and leaf rust, respectively. Four of these

QTL on chromosomes 1BL, 2AS, 2DS, and 7BL conferred resistance to both stripe rust and leaf rust. The QTL on 1BL, 2AS, and 7BL were identified as *Lr46/Yr29*, *Lr37/Yr17*, and *Lr68*, respectively. *QYr.hbau-2DS/QLr.hbau-2DS* was detected at similar positions to previously reported loci. *QYr.hbau-1DL*, *QYr.hbau-3AS*, and *QYr.hbau-3DL* are likely to be new. Combined effects of QTL in the RIL population indicated RIL combining all QTL had the highest resistance level compared with those of lower numbers or no QTL. These QTL, with their closely linked SNP markers, are applicable for marker-assisted breeding and candidate gene discovery.

Keywords: genetic analysis, molecular mapping, single nucleotide polymorphisms, wheat rusts

Stripe rust and leaf rust, caused by *Puccinia striiformis* f. sp. *tritici* and *P. triticea*, respectively, are important diseases of wheat in many regions of the world. Stripe rust remains a significant threat in wheat growing regions worldwide and can cause yield losses as high as 70% (Chen 2005). From the 1950s until present day, stripe rust epidemics causing losses of more than one million tonnes of yield have occurred during 15 different cropping seasons in China (Wan et al. 2007). Leaf rust epidemics, which can cause yield losses up to 40% under favorable conditions (Chen 2014), were recorded in 1969, 1973, 1975, 1979, 2012, and 2015 (Peng et al. 2016; Zhou et al. 2013). Developing resistant cultivars is the most effective, economic, and environmentally friendly approach to protect wheat from losses caused by rust (Singh et al. 2013).

Resistance to rusts is generally categorized into two nonexclusive types, race-specific and race-nonspecific. Race-specific resistance, often referred to as all-stage resistance, is often simply inherited (Chen 2013) and can lose effectiveness due to virulence in the pathogen population (Chen 1995). On the other hand, race-nonspecific

resistance, often referred to as adult plant resistance (APR), tends to be controlled by genes with lesser effects on the disease response (Das et al. 1992). Resistance genes act additively and acceptable levels of resistance from nonspecific resistance genes usually depends on combined effects. The advantage of race-nonspecific resistance is its reputed durability.

Approximately 80 stripe rust resistance and 80 leaf rust resistance genes have been cataloged in wheat (McIntosh et al. 2017). Moreover, 327 stripe rust (Wang and Chen 2017) and 249 leaf rust (Pinto da Silva et al. 2018) QTL have been mapped. Searches for rust resistance genes are ongoing due to the ever-changing nature of pathogens. With the development of DNA markers, QTL mapping has been widely used to characterize resistance quantitatively (Singh et al. 2014). However, the availability of sufficient markers to decrease the size of QTL regions has been a limiting factor. Single nucleotide polymorphism (SNP) markers, however, with virtually unlimited numbers throughout the genome, now enable high resolution mapping.

High-density SNP gene-chip technology offers higher precision and density than simple sequence repeat (SSR) markers (Yu et al. 2011). Researchers from the Chinese Academy of Agricultural Sciences designed the Wheat55K and Wheat660K arrays produced by Affymetrix. The Wheat55K (Affymetrix Axiom Wheat55) is more economic for QTL discovery since all 53,063 molecular tags were carefully chosen from the Wheat660K array. The tags are evenly dispersed across the genome such that each chromosome has about 2,600 SNPs with an average genetic distance of 0.1 cM and an average physical distance of not more than 300 kb (Ren et al. 2018).

Mianyang351-15 is a spring wheat line that is susceptible to some *P. triticea* and *P. striiformis* f. sp. *tritici* pathotypes at the seedling

[†]Corresponding authors: Z. Li; lzf7551@aliyun.com, and D. Liu; ldq@hebau.edu.cn

Funding: This study was supported by the National 399 Natural Science Foundation of China (31571662 and 31601299).

*The e-Xtra logo stands for “electronic extra” and indicates that supplementary material are published online.

The author(s) declare no conflict of interest.

stage but shows high levels of resistance to leaf rust and stripe rust in the field (Li et al. 2010, 2006). Zhengzhou 5389, by contrast, is susceptible to both diseases at all growth stages. The objective of the present study was to map QTL for APR to stripe rust and leaf rust using 150 recombinant inbred lines (RIL) derived from a Mianyang351-15/Zhengzhou 5389 cross.

MATERIALS AND METHODS

Plant materials and pathotypes. One hundred and fifty F₆ RIL derived from the cross Mianyang351-15/Zhengzhou 5389 were used to map QTL for resistance to stripe rust and leaf rust. Mianyang351-15 is a resistant line at the Mianyang Institute of Agricultural Science in Sichuan Province obtained from the cross Mianyang99-310/GansuM180. Susceptible lines Zhengzhou 5389 and Mingxian 169 were used as checks in the respective disease nurseries. *P. striiformis* f. sp. *tritici* pathotypes CYR32 and CYR33 and *P. triticea* pathotypes THTT and TCGT, all of which were virulent to both parents at seedling stage in the greenhouse, were used to test the RIL population in field nurseries.

Field experiments. For evaluation of stripe rust severity, all 150 RIL and both parents were grown at Mianyang and Baoding (Hebei Province) in the 2014 to 2017 cropping seasons (hereafter referred as 2015MYr, 2016MYr, and 2017MYr for Mianyang, and 2017BDYr for Baoding). Leaf rust evaluations were similarly conducted at Baoding in 2016 and 2017 and Zhoukou (Henan) in 2016 (hereafter referred to as 2016BDLr, 2017BDLr, and 2016ZKLr). Inoculation of stripe rust was carried out in January at Mianyang and in April at Baoding, with leaf rust inoculation conducted in April at both Zhoukou and Baoding. Stripe rust and leaf rust epidemics were initiated by spraying aqueous suspensions of urediniospores in equal proportions (50% each pathotype) plus a few drops of Tween 20 (0.03%) onto the spreader rows at tillering. Field trials were conducted in randomized complete blocks with two replicates. Each plot consisted of a single 1.5 m row with 25 cm between rows. Approximately 70 seeds were sown in each row. Every tenth row was planted with susceptible Mingxian 169 for stripe rust trials and with Zhengzhou 5389 for leaf rust trials to encourage the spread of urediniospores. Spreader rows of Mingxian 169 and Zhengzhou 5389, respectively, were also planted perpendicular and adjacent to the test rows. Disease severities as percentages of leaf area covered with uredinia were scored twice in each environment according to the modified Cobb scale (Peterson et al. 1948). Maximum disease severity (MDS) data were used for QTL analysis.

Statistical analyses of phenotypic data. Analyses of variance (ANOVAs) were performed on MDS data using the PROC GLM program in SAS Statistical Software v9.1 (SAS Institute Inc., Cary, NC) to determine genetic, environment, and genetic × environment interaction effects. Pearson correlation coefficients among the MDS data in different environments were calculated using MDS to compare pairwise RIL responses in across different environments.

Genotyping. Genomic DNA of the RIL and parents were extracted from uninfected seedling leaves by the CTAB method (Sharp et al. 1988). DNA concentration was measured by spectrophotometry using a Thermo Scientific NanoDrop 2000. All RIL and the parents were genotyped with the Affymetrix Wheat55K iSelect SNP Array (53,064 markers) at CapitalBio Technology Corporation, Beijing (<http://www.capitalbio.com>). Monomorphic and SNP loci with

more than 10% missing values, vague SNP calling, and minor allele frequencies below 5% were excluded from further analysis (Wu et al. 2018). Five SSR markers were also used to genotype the entire population.

Redundant SNP markers giving identical segregation patterns were reduced using the BIN tool in inclusive composite interval mapping (IciMapping) V4.1 (Meng et al. 2015), and a single marker was selected to denote each bin on the basis of the minimum amount of missing data (arbitrary choice of one when frequencies of missing data were equal). The filtered SNP markers were used to construct linkage maps with the MAP tool in IciMapping V4.1. The algorithm of groups ordered was nnTwoOpt using the Kosambi map function to calculate the map distance from recombination frequencies (Kosambi 1943). The criterion and window size for Rippling were SARF and 5, respectively. Linkage maps were graphically visualized with MapChart 2.3 (Voorrips 2002).

PCR markers *csLv46g22* (E. S. Lagudah, *personal communication*), VENTRIUP/LN2 (Helguera et al. 2003), and *csGS* (Herrera-Foessel et al. 2012) for *Lr46*, *Lr37/Yr17*, and *Lr68*, respectively, were used to genotype both parents and the RIL population.

QTL detection. QTL mapping was performed with the ICIM tools in IciMapping software (Meng et al. 2015). A logarithm of odds (LOD) threshold of 3.0 was adopted for considering a significant QTL. Stepwise regression was used to detect the percentages of phenotypic variance explained (PVE) by individual QTL and determination of additive effects at LOD peaks. The identified flanking sequences of all SNP and SSR probes were subjected to BLAST against the Chinese spring wheat reference sequence (IWGSC RefSeq v1.0, https://urgi.versailles.inra.fr/blast_iwgs/bblast.php) to locate their physical positions.

RESULTS

Phenotypic evaluation of stripe rust and leaf rust responses. The MDS of susceptible controls Mingxian 169 and Zhengzhou 5389 ranged from 75 to 100% across all environments (Table 1). The mean stripe rust MDS of Mianyang351-15 and Zhengzhou 5389 were 2.75 and 82.5%, respectively, across four environments. The mean leaf rust MDS of Mianyang351-15 and Zhengzhou 5389 was 2.3 and 80%, respectively, across three environments. The average MDS of the RILs across environments ranged from 22.7 to 50.8% for stripe rust and 18.1 to 46.4% for leaf rust (Table 1). The frequency distribution for stripe rust and leaf rust MDS in each test environment showed continuous distribution skewed toward resistance (Supplementary Fig. S1).

ANOVA for MDS to both stripe rust and leaf rust of the RIL population showed a significant difference among the RILs, environments, and RILs × environment interactions (Table 2). This indicated that MDS was affected by the genotypes, environment, and their interaction. MDS scores for stripe rust in 2015MYr, 2016MYr, 2017MYr, and 2017BDYr were significantly correlated with coefficients ranging from 0.41 to 0.76 ($P < 0.001$) (Table 3). The MDS scores for leaf rust in 2016BDLr, 2017BDLr, and 2016ZKLr were also significantly correlated with coefficients of 0.24 to 0.71 ($P < 0.001$). The coefficients of correlation between stripe rust and leaf rust disease scores were 0.24 to 0.76 across trials, indicating the likelihood of pleiotropic genes conferring resistance to both rusts.

Linkage map construction. After removing SNPs with monomorphism, markers with >10% missing rate, and sites with

TABLE 1. Summary of maximum disease severities (%) in the Mianyang351-15/Zhengzhou 5389 recombinant inbred line population for stripe rust (2015MYr, 2016MYr, 2017MYr, and 2017BDYr) and leaf rust (2016BDLr, 2017BDLr, and 2016ZKLr)

Parent/parameter	2015MYr	2016MYr	2017MYr	2017BDYr	2016BDLr	2017BDLr	2016ZKLr
Mianyang351-15	5	0	1	5	1	1	5
Zhengzhou 5389	85	80	75	90	80	80	80
Population mean	34.8	39.7	22.7	50.8	26.6	18.1	46.4
Minimum range	1	0	0	1	1	1	5
Maximum range	90	100	90	100	85	90	100

distortion ratios ($P < 1E^{-5}$), 2,464 polymorphic markers remained with 826 for the A genome, 980 for the B genome, and 658 for the D genome. These were used to construct genomic linkage maps. The total linkage map comprised 670.0, 809.4, and 554.2 cM with

marker densities of 0.81, 0.83, and 0.84 cM/marker for the A, B, and D genomes, respectively (Supplementary Table S1).

Detected QTL with flanking markers, additive effect of resistance allele, and PVE values are listed in Table 4. Negative values

TABLE 2. Analyses of variance of maximum disease severities (MDS, %) for stripe rust and leaf rust in the Mianyang351-15/Zhengzhou 5389 recombinant inbred line (RIL) population^a

Source of variation	MDS to stripe rust				MDS to leaf rust			
	df	MS	F	P	df	MS	F	P
RILs	149	1,936.2	2.64*	0.0001	149	1,481.4	2.27*	0.0001
Environments	3	32.5	56.33*	0.0001	2	30,155.1	46.11*	0.0001
Replications	1	41,232.8	0.04	1.0300	1	29,954.1	0.33	0.8200
RILs × environment	149	237.6	32.46*	0.0001	148	217.8	45.8*	0.0001
RILs × replication	149	276.9	0.38	0.62	148	216.1	2.38	0.963
Error	148	732.1			149	654.1		

^a * indicates significant at 0.05 level of probability. MS = mean squares.

TABLE 3. Pearson correlation coefficients (r) for two-way comparisons of stripe rust (2015MYr, 2016MYr, 2017MYr, and 2017BDYr) and leaf rust (2016BDLr, 2017BDLr, and 2016ZKLr) severity data from all tested environments^a

Environment	2015MYr	2016MYr	2017MYr	2017BDYr	2016BDLr	2017BDLr
2016MYr	0.44*	...	...	...	...	...
2017MYr	0.53*	0.61*	...	...	...	...
2017BDYr	0.76*	0.41*	0.44*	...	...	...
2016BDLr	0.52*	0.71*	0.54*	0.41*	...	...
2017BDLr	0.56*	0.37*	0.40*	0.48*	0.33*	...
2016ZKLr	0.56*	0.39*	0.24*	0.58*	0.36*	0.34*

^a Field experiments for stripe rust at Mianyang and Baoding (2015MYr, 2016MYr, 2017MYr, and 2017BDYr) and for leaf rust at Baoding and Zhoukou (2016BDLr, 2017BDLr, and 2016ZKLr). * indicates significant at $P = 0.001$.

TABLE 4. Quantitative trait locus/loci for final disease severity to stripe rust and leaf rust by IciMapping in the recombinant inbred line population from Mianyang351-15/Zhengzhou 5389

Quantitative trait loci (QTL)	Environment ^a	Trait	Genetic position (cM)	Physical position (Mb) ^b	Marker interval	LOD ^c	PVE (%) ^d	Add ^e
<i>QYr.hbau-1BL</i>	2016MYr	Stripe rust	29–30.1	662.7–670.2	<i>AX-110946149–Lr46/Yr29</i>	9.8	14.1	–10.2
	2017MYr				<i>AX-110946149–Lr46/Yr29</i>	8.4	15.0	–8.3
	2017BDYr				<i>AX-110946149–Lr46/Yr29</i>	20.6	16.7	–17.1
<i>QYr.hbau-1DL</i>	2016MYr		22.5–23.6	462.1–462.5	<i>AX-111135128–AX-111085909</i>	6.1	7.7	–7.6
	2017BDYr				<i>AX-111135128–AX-111085909</i>	10.0	12.7	–11.0
<i>QYr.hbau-2AS</i>	2015MYr		4.5–6.7	4.9–10.7	<i>Lr37/Yr17–AX-111661031</i>	7.9	23.1	–9.7
	2016MYr				<i>Lr37/Yr17–AX-111661031</i>	11.4	15.1	–10.9
	2017MYr				<i>Lr37/Yr17–AX-111661031</i>	7.8	12.7	–7.9
<i>QYr.hbau-2DS</i>	2017BDYr		11.4–12.5	17.5–21.6	<i>Lr37/Yr17–AX-111661031</i>	21.2	17.2	–17.2
	2016MYr				<i>AX-109486828–AX-109403444</i>	7.5	9.9	–8.6
	2017MYr				<i>AX-109486828–AX-109403444</i>	4.5	7.2	–5.7
<i>QYr.hbau-3AS</i>	2016MYr		13.6–14.5	45.8–54.8	<i>AX-111491666–AX-110551014</i>	3.3	4.0	–5.5
	2017MYr				<i>AX-111491666–AX-110551014</i>	4.5	7.1	–5.7
<i>QYr.hbau-3DL</i>	2016MYr		15.4–16.2	454.4–477.8	<i>AX-109333872–AX-108884636</i>	3.7	4.4	–5.8
	2017BDYr				<i>AX-109333872–AX-108884636</i>	3.6	2.3	–6.3
<i>QYr.hbau-7BL</i>	2015MYr		26.9–27.3	734.2–740.1	<i>AX-108959083–Lr68</i>	4.6	6.1	–6.1
	2016MYr				<i>AX-108959083–Lr68</i>	4.0	5.3	–7.3
	2017MYr				<i>AX-108959083–Lr68</i>	10.5	18.2	–9.2
<i>QLr.hbau-1BL</i>	2017BDYr	Leaf rust	29–30.1	662.7–670.2	<i>AX-108959083–Lr68</i>	3.0	5.5	–5.0
	2016BDLr				<i>AX-110946149–Lr46/Yr29</i>	3.7	12.5	–7.4
	2017BDLr				<i>AX-110946149–Lr46/Yr29</i>	6.4	12.7	–5.8
<i>QLr.hbau-2AS</i>	2016ZKLr		4.5–6.7	4.9–10.7	<i>AX-110946149–Lr46/Yr29</i>	3.6	11.6	–9.6
	2016BDLr				<i>Lr37/Yr17–AX-111661031</i>	6.4	21.3	–9.6
<i>QLr.hbau-2DS</i>	2016ZKLr		11.4–12.5	17.5–21.6	<i>Lr37/Yr17–AX-111661031</i>	3.8	12.4	–9.9
	2017BDLr				<i>AX-109486828–AX-109403444</i>	3.8	9.2	–5.2
<i>QLr.hbau-7BL</i>	2016BDLr		26.9–27.3	734.2–740.1	<i>AX-108959083–Lr68</i>	5.7	15.0	–16.9
	2017BDLr				<i>AX-108959083–Lr68</i>	9.0	10.1	–8.2
	2016ZKLr				<i>AX-108959083–Lr68</i>	4.8	9.2	–6.0

^a Field experiments for stripe rust at Mianyang and Baoding (2015MYr, 2016MYr, 2017MYr, 2016BDLr, and 2017BDLr) and for leaf rust at Baoding and Zhoukou (2016BDLr, 2017BDLr, and 2016ZKLr).

^b Physical position in Mega-bites (Mb) based on the Chinese Spring wheat reference sequence (IWGSC RefSeq v1.0, https://urgi.versailles.inra.fr/blast_iwgsc/blast.php).

^c Logarithm of odds (LOD) score.

^d Percentages of phenotypic variance explained by individual QTL.

^e Negative values for additive effects indicate that resistance alleles were contributed by Mianyang351-15.

for additive effects indicate that resistance alleles were contributed by Mianyang351-15 and positive additive values indicate that alleles contributing resistance were from Zhengzhou 5389. The positions of the QTL in linkage maps are shown in Figure 1.

Potential pleiotropic rust resistance QTL. Four potential pleiotropic resistance QTL were detected in the Mianyang351-15/Zhengzhou 5389 population. *QYr.hbau-1BL/QYr.hbau-1BL*, detected in 2016MYR, 2017MYR, and 2017BDYr, explained 11.6 to 16.7%

of the phenotypic variances (Table 4, Fig. 1). *QYr.hbau-2AS/QLr.hbau-2AS* identified in all stripe rust environments and in two leaf rust environments accounted for 12.4 to 23.1% of the phenotypic variances (Table 4, Fig. 1). *QYr.hbau-2DS/QLr.hbau-2DS* explained 7.2 to 9.9% of the phenotypic variances in 2016MYR, 2017MYR, and 2017BDLr (Table 4, Fig. 1). *QYr.hbau-7BL/QLr.hbau-7BL* explained 5.3 to 18.2% of phenotypic variances in all stripe rust and leaf rust environments (Table 4, Fig. 1).

Fig. 1. Simplified genetic maps showing quantitative trait loci for stripe rust and leaf rust resistance in the Mianyang351-15/Zhengzhou 5389 recombinant inbred line population.

QTL mapping of APR to stripe rust. Three additional QTL, *QYr.hbau-1DL*, *QYr.hbau-3AS*, and *QYr.hbau-3DL*, contributed by Mianyang351-15 for stripe rust resistance were detected using IciMapping V4.1 (Table 4, Fig. 1). These QTL were each detected in two stripe rust trials, and explained 7.7 to 12.7, 4.0 to 7.1, and 2.3 to 4.4% of the phenotypic variances, respectively (Table 4, Fig. 1). QTL detected in a single environment were excluded in the present study.

Effects of QTL combinations. In order to show the combined effects of QTL for stripe rust resistance, the RIL were classified into four, eight, six, and seven groups in the 2015MYYr, 2016MYYr, 2017MYYr, and 2017BDYr, respectively (Fig. 2A, B, C, and D). Based on the MDS data, RIL with more QTL generally had lower MDS. RIL combining seven QTL for stripe rust resistance showed the highest resistance level, similar to Mianyang351-15 (Fig. 2B), followed by RILs with six (Fig. 2B and D) and five (Fig. 2C) QTL showed better resistance than those with lower or no QTL. Similarly, for leaf rust resistance, RIL were classified into four,

five, and four groups in 2016BDLr, 2017BDLr, and 2016ZKLr, respectively, containing different number of QTL to determine the QTL combination effect (Fig. 2E, F, and G). RIL with a combination of four QTL had highest resistance level to leaf rust compared with those with lower numbers or no QTL (Fig. 2F).

DISCUSSION

Resistance based on pleiotropic APR genes has been widely deployed in CIMMYT spring wheat cultivars due to its reputed durability (Singh et al. 2000). One of the parents of Mianyan351-15, GansuM180, was introduced from Mexico (Y. Ren, *personal communication*). GansuM180 is resistant to both stripe rust and leaf rust in the field whereas the other parent of Mianyan351-15, Mianyang99-310, is susceptible. Hence, the QTL mapped in this study were likely derived from GansuM180.

QYr.hbau-1BL/QLr.hbau-1BL. The pleiotropic locus *Lr46/Yr29* (Singh et al. 1998) is located on chromosome 1BL. *Lr46/Yr29*

(Continued)

Fig. 2. Maximum disease severities (%) on Mianyang351-15/Zhengzhou 5389 recombinant inbred lines containing different numbers of quantitative trait loci (QTL) recorded in the fields at Mianyang and Baoding (A, 2015MYYr, B, 2016MYYr, C, 2017MYYr, and D, 2017BDYr) tested for stripe rust and at Zhoukou and Baoding (E, 2016BDLr, F, 2017BDLr, and G, 2016ZKLr) tested for leaf rust.

has been found extensively in wheat germplasm originating from CIMMYT (Lan et al. 2014). According to Pinto da Silva et al. (2018), the *Lr46/Yr29* locus is associated to a broad-spectrum resistance level explaining 2.9 to 74.5% of the total phenotypic variance on different mapping populations under different environmental trials. In the present study, *QYr.hbau-1BL/QLr.hbau-1BL* explained 11.6 to 16.7% of the phenotypic variance conferring resistance to both stripe rust and leaf rust, mapped between *AX-110946149* and *Lr46/Yr29*. Both parents and the RIL population were tested with the gene-specific marker *csLV46g22* (E. S. Lagudah, *personal communication*) to confirm the presence of *Lr46*. The resistant parent, Mianyang351-15, and 81 RIL were genotyped as *Lr46*. Therefore, *QYr.hbau-1BL/QLr.hbau-1BL* should be *Lr46/Yr29*.

***QYr.hbau-2AS/QLr.hbau-2AS*.** According to Bariana and McIntosh (1993), the chromosome 2N segment on chromosome 2AS carries *Lr37* and *Yr17* for resistance to leaf rust and stripe rust, respectively. *QLr.inra-2Ab* (Azzimonti et al. 2014) and *QLr.hbau-2AS* (Wang et al. 2015) explained 12.2 and 25.79% phenotypic variance to leaf rust and were collocated with *Lr37*, respectively. Recent field tests of the Thatcher near-isogenic line (NIL) with *Lr37*

gave low reactions to mixtures of *P. tritici* pathotypes whereas Thatcher was susceptible. This indicated that *Lr37* was effective. *Lr37* is widely deployed in wheat cultivars from Sichuan Province (Gao et al. 2019). Tests of both parents and the RIL population with marker VENTRIUP/LN2, which detects the 2N segment (Helguera et al. 2003), showed that Mianyang351-15 and 68 RIL carried *Lr37*. Therefore, *QLr.hbau-2AS*, explaining 12.4 to 21.3% phenotypic variance to leaf rust, should be *Lr37*.

Although *Lr37* and *Yr17* are treated as pleiotropic by name, the genes are not necessarily the same, as genes in alien segments are inherited together in wheat backgrounds. Recent seedling tests showed that Mianyang351-15 provided a resistant response to an avirulent *P. striiformis* f. sp. *tritici* pathotype to *Yr17* from a foreign country in the greenhouse. As this foreign *P. striiformis* f. sp. *tritici* pathotype could not be employed in field tests, CYR32 and CYR33 *P. striiformis* f. sp. *tritici* pathotypes from China were used in the field tests and were virulent to Mianyang351-15 at the seedling test. *QYr.hbau-2AS/QLr.hbau-2AS* clearly conferred resistance in the present work despite both *P. striiformis* f. sp. *tritici* pathotypes having been documented as virulent for *Yr17*. It is possible that

Fig. 2. (Continued from previous page)

QYr.hbau-2AS (*Yr17*) conferred APR to stripe rust in the field. The associated effect of stripe rust and leaf rust resistance points to the likely effectiveness of *Yr17* in China despite reports of virulence based on seedling tests.

***QYr.hbau-2DS/QLr.hbau-2DS*.** Four QTL for stripe rust resistance, *QYr.ufs-2D* (Agenbag et al. 2012), *QYr.caas-2DS* (Lu et al. 2009), *QYr.2DS* (Suenaga et al. 2003), and *QYr.inra-2DS* (Mallard et al. 2005), were reported at the 72.6, 12.3 to 19.6, 19.6, and 72.6 Mb physical positions on chromosome 2DS, respectively (Supplementary Table S2). A partial adult plant resistance gene for stripe rust, *Yr16*, was mapped in corresponding to the flanking markers of *QYr.inra-2DS* (Worland and Law 1986). These APR genes were reported to be responsible for 5.3 to 10.3% of the observed phenotypic variance. In this study, *QYr.hbau-2DS/QLr.hbau-2DS* could be located at a similar position, detected at 17.5 to 21.6 Mb and explaining 7.2 to 9.9% of the phenotypic variance.

Eight APR genes for leaf rust resistance, *QLrid.osu-2DS* (Xu et al. 2005), *QLr.hbau-2DS* (Zhang et al. 2009), *Lr22a* (Hiebert et al. 2007), *Lr22b* (Dyck 1979), *QLr.Kat-2DS* (Bariana et al. 2001), *QLr.sfr-2DS* (Schnurbusch et al. 2004), *QLr.cim-2DS* (Lan et al. 2017), and *QLr.inra-2D* (Azzimonti et al. 2014), have been mapped on chromosome 2DS at the 19.6, 5.2 to 17.6, 17.6, 15.5, 14.3 to 24.2, 13.7 to 23, 12.3 to 15.6, and 14.4 Mb physical positions, respectively (Supplementary Table S2). These QTL were reported to explain 4 to 46.4% of the phenotypic variance. *QYr.hbau-2DS/QLr.hbau-2DS* (17.5 to 21.6 Mb) was mapped in the region of all eight APR QTL. The variation of the explained phenotypic variance in these QTL indicate the influence of genetic background and/or environmental conditions.

***QYr.hbau-7BL/QLr.hbau-7BL*.** *Lr68* (Herrera-Foessel et al. 2012) is located at position 734.2 Mb on chromosome 7BL (Supplementary Table S2). *QYr.hbau-7BL/QYr.hbau-7BL*, conferring resistance to stripe rust and leaf rust, was mapped between *AX-108959083* and *Lr68*, explained by 5.3 to 18.2% of phenotypic variance. The *csGS* marker for *Lr68* (Herrera-Foessel et al. 2012) was positive for Mianyang351-15 and 84 RIL. Zhang et al. (2019) identified a QTL conferring resistance to stripe rust and leaf rust and linked with *Xcfa2040* (718.5 to 732.1 Mb) at a similar position to *Lr68*, explained by 5.9 to 18.4% of the phenotypic variance. Gebrewahid et al. (*unpublished data*) also found a QTL for stripe rust resistance flanked by *wms577* and *barc1073* (712.3 to 723.2 Mb) at a similar position to *Lr68*, explained by 2.9 to 21.9% of phenotypic variance. The phenotypic variance explained by *QYr.hbau-7BL/QYr.hbau-7BL* to the rust diseases is almost in line with the reports by Zhang et al. (2019) and Gebrewahid et al. (*unpublished data*). Although *Lr68* has not been linked with stripe rust resistance in the past, the present identification of *QYr.hbau-7BL/QYr.hbau-7BL* suggests that *Lr68* might also be associated with stripe rust resistance (Supplementary Table S2).

***QYr.hbau-1DL*.** Ponce-Molina et al. (2018a) reported a pleiotropic QTL, *QLr.cim-1DL/QYr.cim-1DL*, explained 11.6 to 22.6% of the phenotypic variance to leaf rust and stripe rust resistance on chromosome 1DL. This locus is located at about 414.6 Mb (Supplementary Table S2). It appears that *QYr.hbau-1DL* (462.1 to 462.5 Mb), explained 7.7 to 12.7% of the phenotypic variance, might be different from *QLr.cim-1DL/QYr.cim-1DL* based on their physical position.

***QYr.hbau-3AS and QYr.hbau-3DL*.** Lillemo et al. (2008) mapped *QYr.3AS* contributed by the CIMMYT line Saar and explained 8.1 to 20.7% of phenotypic variance closely linked to *Xbarc310*. According to the positions of the flanking marker, *QYr.3AS* should be located at a 1.2 to 7.1 Mb physical position (Supplementary Table S2). However, the location of *QYr.hbau-3AS* at 45.8 to 54.8 Mb, explained by 4 to 7.1% of phenotypic variance, suggests a difference from *QYr.3AS*.

QYr.cim-3DL (Yang et al. 2013) linked to *Xgdm8* and explained by 3.6% of phenotypic variance was positioned at about 357.1 Mb

on chromosome 3DL. *QYr.hbau-3DL* mapping at 454.4 to 477.8 Mb, explained 2.3 to 4.4% of phenotypic variance, appears to be different from *QYr.cim-3DL* based on their physical position.

Conclusion. In this study, seven QTL for stripe rust and four QTL for leaf rust resistance were identified. Four of these QTL on chromosomes 1BL, 2AS, 2DS, and 7BL conferred a pleiotropic effect to both stripe rust and leaf rust resistance. Among these, *QYr.hbau-1BL/QLr.hbau-1BL*, *QYr.hbau-2AS/QLr.hbau-2AS*, and *QYr.hbau-7BL/QYr.hbau-7BL* were major QTL and found to be *Lr46/Yr29*, *Lr37/Yr17*, and *Lr68*, respectively. Although *Lr68* has not been linked with stripe rust resistance in the past, identification of *QYr.hbau-7BL/QYr.hbau-7BL* in this study suggests that *Lr68* might also be associated with stripe rust resistance. *QYr.hbau-2DS/QLr.hbau-2DS* was detected at similar positions to previously reported loci. Three QTL, namely *QYr.hbau-1DL*, *QYr.hbau-3AS*, and *QYr.hbau-3DL* are likely to be new. The novel QTL identified in this study will require validation in other populations and environments. Combined effects of QTL in the RIL population indicated that RIL combining all QTL at each environmental trial had the highest resistance level compared with RIL with lower number or no QTL. The closely linked SNP and SSR markers can be used in marker assisted selection and pyramiding of APR genes in wheat breeding programs to improve stripe and leaf rust resistance.

ACKNOWLEDGMENTS

We thank R. A. McIntosh, University of Sydney, for the critical review of an early draft of this manuscript.

LITERATURE CITED

- Agenbag, G. M., Pretorius, Z. A., Boyd, L. A., Bender, C. M., and Prins, R. 2012. Identification of adult plant resistance to stripe rust in the wheat cultivar Cappelle-Desprez. *Theor. Appl. Genet.* 125:109-120.
- Azzimonti, G., Marcel, T. C., Robert, O., Paillard, S., Lannou, C., and Goyeau, H. 2014. Diversity, specificity and impacts on field epidemics of QTLs involved in components of quantitative resistance in the wheat leaf rust pathosystem. *Mol. Breed.* 34:549-567.
- Bariana, H. S., Hayden, M. J., Ahmed, N. U., Bell, J. A., Sharp, P. J., and McIntosh, R. A. 2001. Mapping of durable adult plant and seedling resistances to stripe rust and stem rust diseases in wheat. *Aust. J. Agric. Res.* 52: 1247-1255.
- Bariana, H. S., and McIntosh, R. A. 1993. Cytogenetic studies in wheat. XV. Location of rust resistance gene in VPM1 and their genetic linkage with other disease resistance genes in chromosome 2A. *Genome* 36:476-482.
- Chen, X. M. 1995. Gene number and heritability of wheat cultivars with durable, high-temperature, adult plant (HTAP) resistance and interaction of HTAP and race-specific seedling resistance to *Puccinia striiformis*. *Phytopathology* 85:573-578.
- Chen, X. M. 2005. Epidemiology and control of stripe rust (*Puccinia striiformis* f. sp. *tritici*) on wheat. *Can. J. Plant Pathol.* 27:314-337.
- Chen, X. M. 2013. High-temperature adult-plant resistance, key for sustainable control of stripe rust. *Am. J. Plant Sci.* 4:608-627.
- Chen, X. M. 2014. Pages 52-55 in: *Stripe Rust: The Good, Bad and Ugly*. Washington Grain Commission, Pullman, DC.
- Das, M. K., Rajaram, S., Mundt, C. C., Kronstad, W. E., and Singh, R. P. 1992. Inheritance of slow rusting resistance in wheat. *Crop Sci.* 32: 1452-1456.
- Dyck, P. L. 1979. Identification of the gene for adult-plant leaf rust resistance in Thatcher. *Can. J. Plant Sci.* 59:499-501.
- Gao, P., Zhou, Y., Gebrewahid, T. W., Zhang, P., Yan, X., Li, X., Yao, Z., Li, Z., and Liu, D. 2019. Identification of known leaf rust resistance genes in common wheat cultivars from Sichuan province in China. *Crop Prot.* 115:122-129.
- Helguera, M., Khan, I. A., Kolmer, J., Lijavetzky, D., Zhong-qi, L., and Dubcovsky, J. 2003. PCR assays for the *Lr37-Yr17-Sr38* cluster of rust resistance genes and their use to develop isogenic hard red spring wheat lines. *Crop Sci.* 43:1839-1847.
- Herrera-Foessel, S. A., Singh, R. P., Huerta-Espino, J., Rosewarne, G. M., Periyannan, S. K., Vickers, L., Calvo-Salazar, V., Lan, C. X., and Lagudah, E. S. 2012. *Lr68*: A new gene conferring slow rusting resistance to leaf rust in wheat. *Theor. Appl. Genet.* 124:1475-1486.
- Hiebert, C. W., Thomas, J. B., Somers, D. J., McCallum, B. D., and Fox, S. L. 2007. Microsatellite mapping of adult-plant leaf rust resistance gene *Lr22a* in wheat. *Theor. Appl. Genet.* 115:877-884.

- Kosambi, D. D. 1943. The estimation of map distance from recombination values. *Ann. Eugen.* 12:172-175.
- Lan, C., Hale, I. L., Herrera-Foessel, S. A., Basnet, B. R., Randhawa, M. S., Huerta-Espino, J., Dubcovsky, J., and Singh, R. P. 2017. Characterization and mapping of leaf rust and stripe rust resistance loci in hexaploid wheat lines UC1110 and PI610750 under Mexican environments. *Front. Plant Sci.* 8:1450.
- Lan, C., Rosewarne, G. M., Singh, R. P., Herrera-Foessel, S. A., Huerta-Espino, J., Basnet, B. R., Zhang, Y., and Yang, E. 2014. QTL characterization of resistance to leaf rust and stripe rust in the spring wheat line Francolin#1. *Mol. Breed.* 34:789-803.
- Li, Z. F., Xia, X. C., He, Z. H., Li, X., Zhang, L. J., Wang, H. Y., Meng, Q. F., Yang, W. X., Li, G. Q., and Liu, D. Q. 2010. Seedling and slow rusting resistance to leaf rust in Chinese wheat cultivars. *Plant Dis.* 94:45-53.
- Li, Z. F., Xia, X. C., Zhou, X. C., Niu, Y. C., He, Z. H., Zhang, Y., Li, G. Q., Wan, A. M., Wang, D. S., Chen, X. M., Lu, Q. L., and Singh, R. P. 2006. Seedling and slow rusting resistance to stripe rust in Chinese common wheats. *Plant Dis.* 90:1302-1312.
- Lillemo, M., Asalf, B., Singh, R. P., Huerta-Espino, J., Chen, X. M., He, Z. H., and Bjørnstad, Å. 2008. The adult plant rust resistance loci *Lr34/Yr18* and *Lr46/Yr29* are important determinants of partial resistance to powdery mildew in bread wheat line Saar. *Theor. Appl. Genet.* 116:1155-1166.
- Lu, Y. M., Lan, C. X., Liang, S. S., Zhou, X. C., Liu, D., Zhou, G., Lu, Q. L., Jing, J. X., Wang, M. N., Xia, X. C., and He, Z. H. 2009. QTL mapping for adult-plant resistance to stripe rust in Italian common wheat cultivars Libellula and Strampelli. *Theor. Appl. Genet.* 119:1349-1359.
- Mallard, S., Gaudet, D., and Aldeia, A., Abelard, C., Besnard, A. L., Sourdille, P., and Dedryver, F. 2005. Genetic analysis of durable resistance to yellow rust in bread wheat. *Theor. Appl. Genet.* 110:1401-1409.
- McIntosh, R. A., Dubcovsky, J., Rogers, W. J., Morris, C., and Xia, X. C. 2017. Catalogue of gene symbols for wheat: 2017 supplement. <https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf>
- Meng, L., Li, H., Zhang, L., and Wang, J. 2015. QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop J.* 3:269-283.
- Peng, H., Lü, G. Q., and Wang, J. R. 2016. Analysis on the characteristics and causes of main diseases of wheat in Henan Province in 2015. *China Plant Prot.* 36:29-33.
- Peterson, R. F., Campbell, A. B., and Hannah, A. E. 1948. A dia-grammatic scale for estimating rust intensity on leaves and stems of cereals. *Can. J. Res.* 26:496-500.
- Pinto da Silva, G. B., Zanella, C. M., Martinelli, J. A., Chaves, M. S., Hiebert, C. W., McCallum, B. D., and Boyd, L. A. 2018. Quantitative trait loci conferring leaf rust resistance in hexaploid wheat. *Phytopathology* 108:1344-1354.
- Ponce-Molina, L. J., Huerta-Espino, J., Singh, R. P., Basnet, B. R., Alvarado, G., Randhawa, M. S., Lan, C. X., Aguilar-Rincon, V. H., Lobato-Ortiz, R., and Garcia-Zavala, J. J. 2018a. Characterization of leaf rust and stripe rust resistance in spring wheat 'Chilero'. *Plant Dis.* 102:421-427.
- Ren, T., Hu, Y., Tang, Y., Li, C., Yan, B., Ren, Z., Tan, F., Tang, Z., Fu, S., and Li, Z. 2018. Utilization of a Wheat55K SNP Array for mapping of major QTL for temporal expression of tiller number. *Front. Plant Sci.* 9:333.
- Schnurbusch, T., Paillard, S., Schori, A., Messmer, M., Scharchermayr, G., Winzeler, M., and Keller, B. 2004. Dissection of quantitative and durable leaf rust resistance in Swiss winter wheat reveals a major resistance QTL in the *Lr34* chromosomal region. *Theor. Appl. Genet.* 108:477-484.
- Sharp, P. J., Kreis, M., Shewry, P. R., and Gale, M. D. 1988. Location of β -amylase sequences in wheat and its relatives. *Theor. Appl. Genet.* 75:286-290.
- Singh, A., Knox, R. E., DePauw, R. M., Singh, A. K., Cuthbert, R. D., Campbell, H. L., Shorter, S., and Bhavani, S. 2014. Stripe rust and leaf rust resistance QTL mapping, epistatic interactions, and colocalization with stem rust resistance loci in spring wheat evaluated over three continents. *Theor. Appl. Genet.* 127:2465-2477.
- Singh, A., Pandey, M. P., Singh, A. K., Knox, R. E., Ammar, K., Clarke, J. M., Singh, R. P., Pozniak, C. J., DePauw, R. M., McCallum, B. D., Cuthbert, R. D., Randhawa, H. S., and Fetch, T. G., Jr. 2013. Identification and mapping of leaf, stem and stripe rust resistance quantitative trait loci and their interactions in durum wheat. *Mol. Breed.* 31:405-418.
- Singh, R. P., Mujeeb-Kazi, A., and Huerta-Espino, J. 1998. *Lr46*: A gene conferring slow-rusting resistance to leaf rust in wheat. *Phytopathology* 88: 890-894.
- Singh, R. P., Nelson, J. C., and Sorrells, M. E. 2000. Mapping *Yr28* and other genes for resistance to stripe rust in wheat. *Crop Sci.* 40:1148-1155.
- Suenaga, K., Singh, R. P., Huerta-Espino, J., and William, H. M. 2003. Microsatellite markers for genes *Lr34/Yr18* and other quantitative trait loci for leaf rust and stripe rust resistance in bread wheat. *Phytopathology* 93: 881-890.
- Voorrips, R. E. 2002. MapChart: Software for the graphical presentation of linkage maps and QTLs. *Heredity* 93:77-78.
- Wan, A. M., Chen, X. M., and He, Z. H. 2007. Wheat stripe rust in China. *Aust. J. Agric. Res.* 58:605-619.
- Wang, J., Li, Z., Shi, L., Zhu, L., Ren, Z., Li, X., Liu, D., and Shah, S. J. A. 2015. QTL mapping for adult-plant leaf rust resistance genes in Chinese wheat cultivar Weimai 8. *Czech J. Genet. Plant Breed.* 51:79-85.
- Wang, M. N., and Chen, X. M. 2017. Chapter 5: Stripe rust resistance. Pages 353-558 in: *Stripe Rust*. X. M. Chen and Z. S. Kang, eds. Springer, Dordrecht.
- Worland, A. J., and Law, C. N. 1986. Genetic analysis of chromosome 2D of wheat. *Z. Pflanzenzücht.* 96:331-345.
- Wu, J., Huang, S., Zeng, Q., Liu, S., Wang, Q., Mu, J., and Yu, S. 2018. Comparative genome-wide mapping versus extreme pool-genotyping and development of diagnostic SNP markers linked to QTL for adult plant resistance to stripe rust in common wheat. *Theor. Appl. Genet.* 131:1777-1792.
- Xu, X. Y., Bai, G. H., Carver, B. F., Shaner, G. E., and Hunger, R. M. 2005. Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat. *Theor. Appl. Genet.* 110:244-251.
- Yang, E. N., Rosewarne, G. M., Herrera-Foessel, S. A., Huerta-Espino, J., Tang, Z. X., Sun, C. F., Ren, Z. L., and Singh, R. P. 2013. QTL analysis of the spring wheat "Chapio" identifies stable stripe rust resistance despite inter-continental genotype $\times$ environment interactions. *Theor. Appl. Genet.* 126:1721-1732.
- Yu, H. H., Xie, W. B., Wang, J., Xing, Y. Z., Xu, C. G., Li, X. H., Xiao, J. H., and Zhang, Q. F. 2011. Gains in QTL detection using an ultra-high density SNP map based on population sequencing relative to traditional RFLP/SSR markers. *PLoS One* 6:e17595.
- Zhang, L., Li, Z., Lillemo, M., Xia, X., Liu, D., Yang, W., Luo, J., and Wang, H. 2009. QTL mapping for adult-plant resistance to leaf rust in CIMMYT wheat cultivar Saar. *Sci. Agric. Sin.* 42:388-397.
- Zhang, P., Li, X., Gebrewahid, T. W., Liu, H., Xia, X., He, Z., Li, Z. F., and Liu, D. 2019. QTL mapping of adult-plant resistance to leaf and stripe rust in wheat cross SW 8588/Thatcher using the wheat 55K SNP array. *Plant Dis.* 103:3041-3049.
- Zhou, H. X., Xia, X. C., He, Z. H., Li, X., Wang, C. F., Li, Z. F., and Liu, D. Q. 2013. Molecular mapping of leaf rust resistance gene *LrNJ97* in Chinese wheat line Neijiang 977671. *Theor. Appl. Genet.* 126:2141-2147.