QTL Mapping of Adult-Plant Resistance to Leaf and Stripe Rust in Wheat Cross SW 8588/Thatcher using the Wheat 55K SNP Array

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Abstract

Wheat leaf rust (caused by *Puccinia triticina*) and stripe rust (caused by *Puccinia striiformis* f. sp. *tritici*) cause large production losses in many regions of the world. The objective of this study was to identify quantitative trait loci (QTL) for resistance to leaf rust and stripe rust in a recombinant inbred line population derived from a cross between wheat cultivars SW 8588 and Thatcher. The population and parents were genotyped with the Wheat 55K SNP Array and SSR markers and phenotyped for leaf rust severity at Zhoukou in Henan Province and Baoding in Hebei Province. Stripe rust responses were also evaluated at Chengdu in Sichuan Province, and at Baoding. Seven and six QTL were detected for resistance to leaf rust and stripe rust, respectively. Four QTL on chromosomes 1BL, 2AS, 5AL, and 7BL conferred resistance to both rusts. The QTL on 1BL and 2AS were identified as *Lr46/Yr29* and *Lr37/Yr17*, respectively. *QLr.hebau-2DS* from Thatcher, identified as *Lr22b*

that was previously thought to be ineffective in China, contributed a large effect for leaf rust resistance. *QLr.hebau-5AL/QYr.hebau-5AL*, *QLr.hebau-3BL*, *QLr.hebau-6DS*, *QYr.hebau-4BS*, and *QYr.hebau-6DS* are likely to be new QTL, but require further validation. Kompetitive allele-specific PCR (KASP) markers for *QLr.hebau-2DS* and *QLr.hebau-5AL/QYr.hebau-5AL* were successfully developed and validated in a diverse wheat panel from Sichuan Province, indicating their usefulness under different genetic backgrounds. These QTL and their closely linked SNP and SSR markers will be useful for fine mapping, candidate gene discovery, and marker-assisted selection in breeding for durable resistance to both leaf and stripe rusts.

Keyword: disease management, cultivar/resistance, field crops, cereals and grains, fungi

Leaf rust (or brown rust) caused by *Puccinia triticina* Erikss. (*Pt*) and stripe rust (or yellow rust) caused by *Puccinia striiformis* Westend. f. sp. *tritici* Erikss. (*Pst*) are important diseases of wheat worldwide. Leaf rust can cause yield losses of up to 40% under favorable conditions (Knott 1989). Leaf rust epidemics in China in 1969, 1973, 1975, 1979, and 2012 caused significant yield losses (Zhou et al. 2013). Stripe rust remains a significant threat in the majority of wheat growing regions throughout the world with the potential to inflict regular regional crop losses ranging from 0.1 to 5%, with rare events giving losses of 5 to 25% or more (Wellings 2011). Since the 1950s, stripe rust epidemics in China occurred at least in 15 crop seasons, and losses exceeding one million tons were recorded in four of those years (Wan et al. 2007). Although wheat rusts can be managed by using fungicides, growing resistant cultivars is a more efficient, economic, and environmentally friendly way to minimize losses (Singh et al. 2013).

Resistance to wheat rusts is generally classified into two types, race specific and race nonspecific. Race specific resistance is often controlled by a single gene that starts expressing at the seedling stage

but remains effective throughout all growth stages (all-stage resistance). However, race specific resistance may lose effectiveness when virulent races arise. For example, significant yield losses occurred in China in the 1980s and early 1990s when *Pst* races virulent to resistance gene *Yr9* that was in many widely grown wheat cultivars became predominant (Xie et al. 1990). Race nonspecific resistance tends to be controlled by polygenes and usually expresses quantitatively at postseedling stages, generally described as adult plant resistance (APR). The advantage of this type of resistance is its reputed durability (Johnson 1988).

So far, 79 *Lr* genes for leaf rust resistance and 81 *Yr* genes for stripe rust resistance have been permanently named in wheat (McIntosh et al. 2017; Qureshi et al. 2018). Three loci, namely *Lr34/Yr18/Pm38/Sr57* (Lagudah 2011), *Lr46/Yr29/Pm39/Sr58* (Lagudah 2011), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2014), have been reported to be durable, race nonspecific APR to leaf rust, stripe rust, stem rust, and powdery mildew. The individual effects of these pleiotropic APR genes are low or moderate, but when they are combined with other genes, high levels of resistance can be achieved (Singh et al. 2000). In addition to the formally named genes, more than 80 leaf rust and 140 stripe rust resistance genes or QTL have been reported (Li et al. 2014; Rosewarne et al. 2013). However, some of the genes or QTL may be redundant.

Molecular markers are widely used to map resistance genes. For many years, SSR markers were widely used for linkage mapping due to their advantages of codominance, high accuracy, high repeatability, high polymorphism, chromosome specificity, and ease of manipulation (Röder et al. 1998). However, high-density single nucleotide polymorphism (SNP) markers using gene-chip technology now provide a superior approach for QTL mapping due to reduced errors in evaluation, greater accuracy, and particularly greater density than previous systems (Yu et al. 2011). Several SNP arrays for wheat are available, such as 9K, 90K, 35K, 660K, and 820K (Allen et al. 2017; Cavanagh et al. 2013; Cui et al. 2017; Wang et al. 2014; Winfield et al. 2016). The wheat 660K SNP that is efficient for constructing high-density wheat genetic

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maps has the advantage of greater marker density and greater resolution as well as better distribution across chromosomes compared with the earlier 90K SNP chip (Cui et al. 2017). The wheat 55K SNP array consists of SNPs carefully selected from the 660K SNP chip with the features of lower cost, high accuracy, and medium but acceptable density (<http://www.capitalbiotech.com/>). These SNPs distribute uniformly in all 21 wheat chromosomes with about 2,600 SNP on each chromosome at an average physical distance smaller than 300 kb.

Wheat line SW 8588 (Milan'S/SW 5193) developed by the Sichuan Academy of Agricultural Science has high levels of resistance to leaf and stripe rust in the field. Thatcher, bred by the Minnesota Agricultural Experiment Station and the Division of Cereal Crops and Diseases of U.S. Department of Agriculture (Hayes et al. 1936), is commonly used as a susceptible check for leaf rust research. Almost all widely distributed near-isogenic lines for leaf rust resistance genes were developed in the Thatcher background by scientists in Canada (McIntosh et al. 1995). The objective of this study was to determine the genetic basis of resistance to leaf rust and stripe rust in SW 8588 using a recombinant inbred line (RIL) population of SW 8588 crossed with Thatcher.

Materials and Methods

Plant materials and races. A total of 144 F₆ RILs derived from cross SW 8588/Thatcher were used to map genes/QTL for resistance to leaf and stripe rust. Thirty-six lines with known leaf rust resistance genes and 14 *Pt* races were used in multirace comparisons for leaf rust in seedling stage tests in a greenhouse. Twenty wheat lines for differentiating *Pst* races in China and six *Pst* races were used in seedling tests for stripe rust reactions. Four *Pt* races (THJS, PGTS, THND, and PHTT) and three *Pst* races (CYR32, CYR33, and CYR34) were used to test the RILs in the fields. The *Pt* races and 36 wheat lines with known *Lr* genes were from the Biological Control Center for Plant Diseases and Plant Pests of Hebei, Hebei Agricultural University. The *Pst* races and 20 wheat differential lines were kindly provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing. In addition, a panel consisting of 60 diverse wheat cultivars (lines) from Sichuan Province was used for QTL validation (Supplementary Table S1).

Evaluation of seedling responses to leaf and stripe rust in the greenhouse. SW 8588, Thatcher, Zhengzhou 5389, and the 36 lines with known *Lr* genes were tested using 14 *Pt* races for comparison of leaf rust infection types (IT). SW 8588, Thatcher, Mingxian 169, and the 20 Chinese differentials were tested for stripe rust ITs using six *Pst* races. Eight to ten seeds of each genotype were planted in a 12 × 6 multicell plastic tray (3.8 × 28.4 × 53.8 cm). Inoculations were performed at the two-leaf stage by spraying urediniospores suspended in light-weight mineral oil at a concentration of 2 to 3 mg/ml using an atomizer (64 × 51 × 33 cm, 1.5 L, PezinYL Co. Ltd., Yiwu, China) onto the seedlings. Inoculated plants were placed in plastic-covered cages at 18°C for leaf rust and 10°C for stripe rust and 100% relative humidity (RH) for approximately 24 h in darkness and then transferred to a greenhouse maintained with 12 h light and 12 h dark at 22 to 25°C for leaf rust and 18 to 22°C for stripe rust. Infection types were recorded 2 weeks after inoculation. Leaf rust was evaluated according to the Stakman 0 to 4 scale as modified by Roelfs et al. (1992), where 0 = immune, ; = hypersensitive flecks with necrosis, 1 = small uredinia surrounded by necrosis, 2 = small to medium uredinia surrounded by necrosis, X = heterogeneous, similarly distributed over the leaves, 3 = moderate size uredinia that may be associated with chlorosis, 4 = large uredinia without chlorosis, + = uredinia somewhat larger than normal, - = uredinia somewhat smaller than normal, and C = more chlorosis than normal. Numbers 0 to 2 and X were designated as resistant and 3 to 4 as susceptible. For stripe rust, ITs were recorded according to the 0 to 9 scale as modified by McNeal et al. (1971), where 0 = no visible infection; 1 = necrotic/chlorotic flecks without sporulation; 2 = necrotic/chlorotic blotches/stripes without sporulation; 3 = necrotic/chlorotic blotches/stripes with trace sporulation; 4 = necrotic/chlorotic blotches/stripes with light sporulation; 5 = necrotic/chlorotic blotches/stripes with

intermediate sporulation; 6 = chlorotic stripes with moderate sporulation; 7 = stripes without chlorosis/necrosis and with moderate sporulation; 8 = stripes without chlorosis/necrosis and with sufficient sporulation; and 9 = stripes without chlorosis/necrosis and abundant sporulation. Lines with IT 0 to 4, 5 to 6, and 7 to 9 are categorized as resistant, intermediate, and susceptible, respectively. Tests were conducted in two replications, but IT data from only one test is reported because of the lack of significant variation in both tests.

Field experiments. The RIL population and parents were grown at Baoding in Hebei Province in the 2015/2016 and 2016/2017 cropping seasons, and at Zhoukou in Henan Province in the 2015/2016 cropping season for evaluation of leaf rust response (environments hereinafter referred to as 16BDLR, 17BDLR, and 16ZKLR). Both Baoding and Zhoukou are leaf rust hotspot regions. Stripe rust phenotyping was made at Chengdu (Sichuan Province) in the 2014/2015 and 2015/2016 growth seasons, and at Baoding in 2015/2016 and 2016/2017 (15CDYR, 16CDYR, 16BDYR, and 17BDYR). Chengdu is a hotspot region for stripe rust, while Baoding is not a major stripe rust region as the temperatures at the late growth stages are usually not favorable for stripe rust development. The field trials were conducted in randomized complete blocks with two replicates. Each plot consisted of a single 1.5 m row with 30 cm between rows. Approximately 60 seeds were sown in each plot. Every tenth row was followed by rows of the highly susceptible line Zhengzhou 5389 as a control and spreader for leaf rust or Mingxian 169 for stripe rust. Spreader rows were also planted surrounding the plots. Leaf rust epidemics were initiated by inoculating the spreader rows with a water suspension of urediniospores of equal amounts of *Pt* races THJS, PGTS, THND, and PHTT with a concentration 2 to 3 mg/ml added with a few drops of Tween 20 (0.03%) at the early jointing stage (Zadoks growth stage 30; Zadoks et al. 1974) using an atomizer. These four *Pt* races were virulent on seedlings of SW 8588. Similarly, spore suspensions prepared by equal quantities of urediniospores of *Pst* races CYR32, CYR33, and CYR34 (Zhang et al. 2012) were sprayed onto plants in the spreaders for stripe rust evaluation. Disease severity was recorded two or three times at weekly intervals with the first scoring 4 weeks after inoculation. Disease severity data (0 to 100%) were recorded as percentage of leaf area covered with uredinia or necrotic stripes according to the modified Cobb scale (Peterson et al. 1948) where 0% = immune, and 100% = fully susceptible. Maximum disease severity (MDS, %) was used for QTL analysis. Correlation analysis between MDS in different environments was conducted using bivariate two-tailed Pearson's correlation coefficients by IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY). Analysis of variance (ANOVA) was performed using the generalized linear model of SPSS. Cultivars, environments (the combination of location and year), and their interaction were treated as fixed effects, and replications nested in environments were set as random.

Linkage map construction. Genomic DNA was extracted from noninfected seedlings of the parents and RILs using the Cetyltrimethylammonium Bromide (CTAB) method (Sharp et al. 1988). DNA concentration was measured using a Thermo Scientific NanoDrop 2000. DNA samples of the 144 RILs and parents were genotyped with the Affymetrix 55K SNP Array (53,064 markers) by CapitalBio Technology Company (Beijing, China). SNPs with >20% missing data were removed. Twelve SSR markers were also used to genotype the entire population. Linkage maps were constructed using the MAP function in IciMapping 4.1 (<http://www.isbreeding.net/software/?type=detail&id=18>, Li et al. 2007), and algorithm of groups ordered "nnTwoOpt" using the Kosambi map function to obtain map distances 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).

QTL detection. QTL analysis was conducted with the ICIM-ADD function using software IciMapping 4.1 (Li et al. 2007). Through 1,000 permutations at $P = 0.01$, a logarithm of odds (LOD) threshold of 2.5 was set to declare significant QTL. Stepwise regression was used to detect the percentages of phenotypic variance explained (PVE, R^2) by individual QTL and additive effects at the

LOD peaks. QTL identified in individual environments within overlapping 20 cM (centimorgan) intervals were considered to be the same.

KASP assays and QTL validation using selected RILs and a diverse bread wheat panel. SNP markers highly associated with a specific QTL were converted to KASP markers using PolyMarker (Ramirez-Gonzalez et al. 2015). KASP assays were performed in a S1000 Thermal Cycler (Bio-Rad, Hercules, CA, U.S.A.). Reaction mixtures consisted of final volumes of 5 µl containing 2.5 µl of 2× KASP V4.0 Mastermix (LGC Genomics), 0.056 µl of assay primer mix (12 mM of each allele-specific primer and 30 mM of common primer), and 50 to 100 ng of genomic DNA. The cycling conditions were as follows: 94°C for 15 min, 9 cycles of 94°C for 20 s, touchdown starting at 65°C for 60 s (decreasing 0.8°C per cycle), 32 cycles of 94°C for 20 s, and 57°C for 60 s. The end-point fluorescence data were visualized with a microplate reader (FLUOstar Omega, BMG LABTECH, Germany) and analyzed by Klustering Caller software (LGC Genomics). Only the KASP markers showing the same segregation as the corresponding SNP markers in the parents and the RIL population were used to screen the diverse bread wheat genotypes. Independent-sample *t* test was carried out using SPSS 21.0 to compare the positive and negative alleles' effect of the stable QTL on leaf rust and stripe rust based on the phenotypic data using the diverse bread wheat panel.

Results

Seedling response to leaf and stripe rust in the greenhouse. Reactions to the 14 *Pt* races in the seedling tests are presented in Supplementary Table S2. SW 8588 showed resistance to nine *Pt* races (KHSS, FHJS, FGHQ, FHTS, HHTS, FHST, FHKs, KHKQ, and FHSS). Eight of nine races were avirulent to *Lr1*, indicating that SW 8588 contained *Lr1*. *Lr1* in SW 8588 was confirmed by the presence of diagnostic molecular marker WR003. The seedling reactions to stripe rust are presented in Supplementary Table S3. SW 8588 was susceptible to four *Pst* races, Gn22-1, CYR32, CYR33, and CYR34, and resistant to *Pst* race Sull. SW 8588 showed a similar IT to stripe rust with the *Yr17* line, indicating that SW 8588 might contain *Yr17*. Both SW 8588 and Thatcher showed susceptible ITs to the four *Pt* races (THJS, PGTS, THND, and PHTT) and three *Pst* races (CYR32, CYR33, and CYR34) used in the field tests at the seedling stage.

Field testing. The mean leaf rust MDS scores of SW 8588 and Thatcher were 3.7 and 76.7%, respectively, and mean leaf rust severity scores on the RILs ranged from 23.0 to 44.9% in the three environments (Table 1). The distribution of mean leaf rust MDS frequencies for the population was continuous, indicating quantitative inheritance (Fig. 1). The mean MDS scores for response to stripe rust in SW 8588 and Thatcher were 3 and 95%, respectively. The mean MDS scores for the RILs were 38.0 and 43.3% in 15CDYR and 16CDYR, respectively. However, in Baoding the mean MDS scores for SW 8588 and Thatcher were only 1 and 35%, respectively. The mean MDS scores for the entire population were 11.9% in 16BDYR and 9.9% in 17BDYR. The stripe rust MDS scores for the 144 RILs across all environments showed continuous distributions, also indicating quantitative inheritance.

Maximum disease severity scores for leaf rust across the three environments were significantly correlated with coefficients ranging from 0.71 to 0.74 ($P < 0.001$) (Table 2). For stripe rust, 15CDYR and 16CDYR were significantly correlated with a coefficient of 0.74 ($P < 0.001$). The coefficient for 16BDYR and 17BDYR was 0.64 ($P < 0.001$). However, the coefficient of correlation for 15CDYR and 17BDYR was low at 0.21 ($P < 0.05$) (Table 2). The coefficients of correlation between stripe rust and leaf rust disease scores were 0.37 to 0.54 across experiments. ANOVA confirmed significant variation among the genotypes, environments, and genotype × environment for both leaf rust and stripe rust (Supplementary Tables S4 and S5).

Linkage map construction. After removing monomorphic markers and those with missing data >20% or distorted segregation, 8,755 (16.5%) SNP markers remained, with 2,883 for the A genome, 4,330 for the B genome, and 1,542 for the D genome. These SNP markers and 12 SSR markers were used to construct genetic linkage maps that covered 2,152.23 cM, 1,614.11 cM, and 1,721.99 cM for genomes A, B, and D, with average marker densities of 0.75, 0.37, and 1.12 cM/marker, respectively (Supplementary Table S6).

QTL mapping of rust resistance. Four possible pleiotropic rust resistance QTL from SW 8588 were detected in the SW 8588/Thatcher RIL population (Table 3). The first QTL, *QLr.hebau-1BL/QYr.hebau-1BL*, detected in two leaf rust environments and

Fig. 1. Frequency distributions of SW 8588 × Thatcher recombinant inbred lines for final severity of leaf rust (A) and stripe rust (B) in field trials. Mean values for parents SW 8588 and Thatcher are indicated by arrows.

Table 1. Summary of maximum disease severity in the SW 8588 × Thatcher recombinant inbred lines population phenotyped for leaf and stripe rust responses

Parameter	Leaf rust maximum disease severity ^y			Stripe rust maximum disease severity ^z			
	16BDLR	16ZKLR	17BDLR	15CDYR	16CDYR	16BDYR	17BDYR
SW 8588	5.0	1.0	5.0	1.0	5.0	1.0	1.0
Thatcher	80.0	80.0	70.0	100.0	90.0	30.0	40.0
Population mean	44.9	33.4	23.0	38.0	43.3	11.9	9.9
Lowest MDS	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Highest MDS	100.0	100.0	100.0	100.0	100.0	70.0	60.0

^y 16BDLR, 17BDLR, and 16ZKLR: the MDS for leaf rust in 2015/2016 and 2016/2017 cropping seasons at Baoding in Hebei Province, and Zhoukou in Henan Province during the 2015/2016 cropping season.

^z 15CDYR, 16CDYR, 16BDYR, and 17BDYR: the MDS for stripe rust in 2014/2015 and 2015/2016 cropping seasons at Chengdu in Sichuan Province, and Baoding in Hebei during the 2015/2016 and 2016/2017 cropping seasons.

two stripe rust environments, explained 12.6–14.1% and 13.7–17.5% of the phenotypic variances for leaf rust and stripe rust, respectively (Table 3; Fig. 2). The second QTL, located on chromosome 2AS, was identified as *Lr17/Yr37* based on the presence of marker VENTRIUP-LN2. *Lr17/Yr37* explained 8.9–10.9% and 9.2–16.8% of the phenotypic variances for leaf rust and stripe rust, respectively, and it was stable across environments for both diseases (Table 3; Fig. 2). The third potentially pleiotropic QTL was *QLr.hebau-5AL/QYr.hebau-5AL* detected in 2016ZKLR, 2017BDLR, and 2016CDYR. This QTL had a relatively minor effect on disease response and explained 6.6–7.1% and 9.1% of the variation for leaf rust and stripe rust, respectively (Table 3; Fig. 2). The fourth potentially pleiotropic rust resistance QTL, *QLr.hebau-7BL/QYr.hebau-7BL*, again with a minor effect, was linked with *Xcfa2040*, and explained 18.4 and 5.9% of the phenotypic variances in 17BDLR and 16CDYR, respectively (Table 3; Fig. 2).

Three additional leaf rust APR QTL were detected on chromosomes 2DS, 3BL, and 6DS (Table 3; Fig. 2). *QLr.hebau-2DS* with the resistance allele from Thatcher was detected in all leaf rust tests. It was flanked by markers *AX-111031419* and *AX-109507893* and explained 11.3–23.7% of the variation across the environments.

QLr.hebau-3BL with its resistance allele from SW 8588 was detected only in 16BDLR and explained 5.6% of the phenotypic variances. *QLr.hebau-6DS* from Thatcher, flanked by *AX-86175025* and *AX-108780681*, was detected only in 16ZKLR with 6.3% phenotypic variance explained (PVE).

QYr.hebau-4BS and *QYr.hebau-6DS* were also identified in single environments. *QYr.hebau-4BS* from Thatcher was detected in 16CDYR with a PVE of 5.5%. *QYr.hebau-6DS* from SW 8588 was detected in 17BDYR with a PVE value of 9.3%. The QTL detected in this study with their flanking markers and physical positions are listed in Supplementary Table S7.

Average effects of leaf rust and stripe rust resistance QTL. The average effects of QTL for resistance to each disease were estimated in +QTL RILs and -QTL RILs based on closely linked markers (Fig. 3; Table 4). *QLr.hebau-1BL/QYr.hebau-1BL* contributed to resistance by reducing leaf rust severity in three environments by 14.0–20.5% and stripe rust severity at the two Chengdu environments (16CDYR and 17CDYR) by 23.1 and 24.5%. *Lr37/Yr17* reduced mean leaf rust and stripe rust severity by 13.9–18.2% and 6.1–22.9%, respectively (Table 4). *QLr.hebau-5AL* and *QYr.hebau-5AL* gave detectable reductions only in 16HNLR and 15CDYR,

Table 2. Pearson correlation coefficients (r) for two-way comparisons of leaf and stripe rust severity data from different environments

Environment ^y	16BDLR ^z	16ZKLR	17BDLR	15CDYR	16CDYR	16BDYR
16ZKLR	0.74**					
17BDLR	0.72**	0.71**				
15CDYR	0.41**	0.39**	0.37**			
16CDYR	0.51**	0.50**	0.46**	0.74**		
16BDYR	0.37**	0.53**	0.45**	0.29**	0.54**	
17BDYR	0.48**	0.43**	0.54**	0.21*	0.42**	0.64**

^y 16BDLR, 17BDLR, and 16ZKLR: the MDS for leaf rust in 2015/2016 and 2016/2017 cropping seasons at Baoding in Hebei Province, and Zhoukou in Henan Province during the 2015/2016 cropping season; 15CDYR, 16CDYR, 16BDYR, and 17BDYR: the MDS for stripe rust in 2014/2015 and 2015/2016 cropping seasons at Chengdu in Sichuan Province, and Baoding during the 2015/2016 and 2016/2017 cropping seasons.

^z ** $P < 0.01$, * $P < 0.05$.

Table 3. Quantitative trait locus/loci for maximum disease severity to leaf and stripe rust by ICIM in the recombinant inbred lines population derived from cross SW 8588/Thatcher

QTL ^a	Environment	Pos ^y	Marker interval	LOD ^w	PVE (%) ^x	Add ^y	Contributing parent ^z
<i>QLr.hebau-1BL</i>	16ZKLR	26	AX-89740962-AX-108930953	6.5	12.6	-10.3	SW 8588
	17BDLR	26	AX-89740962-AX-108930953	5.3	14.1	-9.2	
<i>QYr.hebau-1BL</i>	15CDYR	29	AX-109299717-AX-109892648	7.2	17.5	-12.1	SW 8588
	16CDYR	29	AX-109299717-AX-109892648	7.1	13.7	-11.7	
<i>QLr.hebau-2AS</i>	16BDLR	38	<i>Xcfd36-Xwms636</i>	5.1	10.9	-10.8	SW 8588
	16ZKLR	32	<i>Xwmc407-Xbarc1138</i>	4.8	8.9	-8.9	
	17BDLR	32	<i>Xwmc407-Xbarc1138</i>	4.1	10.8	-8.3	
<i>QYr.hebau-2AS</i>	15CDYR	36	<i>Xwmc407-Xbarc1138</i>	5.4	12.1	-10.2	SW 8588
	16CDYR	37	<i>Xbarc1138-Xcfd36</i>	8.7	16.8	-13.0	
	16BDYR	37	<i>Xbarc1138-Xcfd36</i>	2.6	9.6	-3.0	
	17BDYR	38	<i>Xcfd36-Xwms636</i>	2.8	9.2	-3.3	
<i>QLr.hebau-5AL</i>	16ZKLR	252	AX-110679506-AX-110996595	3.6	7.1	-8.5	SW 8588
	17BDLR	252	AX-110679506-AX-110996595	3.0	6.6	-7.8	
<i>QYr.hebau-5AL</i>	16CDYR	259	AX-110909761-AX-110957925	4.9	9.1	-11.5	SW 8588
<i>QLr.hebau-7BL</i>	17BDLR	78	AX-110568331- <i>Xcfa2040</i>	7.2	18.4	-11.9	SW 8588
<i>QYr.hebau-7BL</i>	16CDYR	82	<i>Xcfa2040-Xwmc273</i>	3.2	5.9	-7.7	SW 8588
<i>QLr.hebau-2DS</i>	16BDLR	0	AX-111031419-AX-109507893	10.6	23.7	16.0	Thatcher
	16ZKLR	0	AX-111031419-AX-109507893	7.2	13.7	10.9	
	17BDLR	0	AX-111031419-AX-109507893	4.4	11.3	8.3	
<i>QLr.hebau-3BL</i>	16BDLR	10	AX-111014259-AX-111534420	2.8	5.6	-7.8	SW 8588
<i>QLr.hebau-6DS</i>	16ZKLR	82	AX-86175025-AX-108780681	2.8	6.3	8.0	Thatcher
<i>QYr.hebau-4BS</i>	16CDYR	44	AX-109867593-AX-111641369	3.2	5.5	8.5	Thatcher
<i>QYr.hebau-6DS</i>	17BDYR	112	AX-109915375-AX-95175830	2.8	9.3	-3.3	SW 8588

^a QTL overlapping in the one-log support confidence intervals were assigned the same symbol.

^y Peak position in centimorgans from the first linked marker of the relevant linkage group.

^w Logarithm of odds (LOD) score.

^x Percentages of phenotypic variance explained by individual QTL.

^y Additive effect of resistance allele.

^z Parent with the resistance allele.

respectively (Table 4). Significant reductions in disease severity of 15.7 and 25.0% were made by *QLr.hebau-7BL* in environments 16BDLR and 17BDLR. *QYr.hebau-7BL* reduced stripe rust severity by 15.0% in 16CDYR and 10.6% in 15CDYR (Table 4). The mean MDS for *QLr.hebau-2DS* ranged from 1.0 to 70.0% (except for one line rated at 90%) compared with 5.0–90.0% without it (Fig. 3G), and *QLr.hebau-2DS* conferred a 23.5–37.1% reduction in leaf rust severity (Table 4).

KASP markers development and validation of the QTL effects using KASP markers in the bread wheat panel. Four and two SNP markers associated with *QLr.hebau-2DS* and *QLr.hebau-5AL/QYr.hebau-5AL* representing the peak or flanking markers for the identified QTL on these chromosomes, respectively, were successfully converted to KASP markers (Supplementary Table S8). All these KASP markers were successfully used to genotype the wheat cultivars/lines in the diverse wheat panel, and the allelic analyses were conducted based on

Fig. 2. Simplified genetic maps of seven chromosomes showing quantitative trait loci for leaf rust and stripe rust resistance in the SW 8588/Thatcher population. Lengths of chromosomes do not represent the same genetic distance.

the field phenotyping data. The Student *t* test indicated that MDS scores for the positive and negative alleles of both *QLr.hebau-2DS* and *QLr.hebau-5AL/QYr.hebau-5AL* showed significant differences at $P < 0.05$ (Supplementary Table S9).

Discussion

Seven QTL for leaf rust resistance and six QTL for stripe rust resistance were detected in the current study. The total phenotypic variance explained by the QTL ranged from 40.2 to 61.2% across

environments for leaf rust and 9.6–51.1% across environments for stripe rust, confirming their significant effects in reducing disease severity. The relatively low correlation coefficients for stripe rust observed in Baoding and Chengdu might be due to different environments. The QTL detected in the study were compared with the known genes or QTL based on the chromosome position, molecular marker, pedigree, and resistance to rusts.

Based on the positions of closely linked markers, *QLr.hebau-1BL*/*QYr.hebau-1BL* appeared to be the pleiotropic gene *Lr46/Yr29*. The

Fig. 3. Comparison of SW 8588 × Thatcher recombinant inbred lines for mean maximum disease severity of leaf rust (A, C, E, G, and I) and stripe rust (B, D, F, and H) in the presence or absence of each quantitative trait locus in field trials.

result was also confirmed by gene-specific marker *csLV46G22* kindly provided by Dr. E. S. Lagudah, CSIRO, Plant Industry, Canberra, Australia (data not presented).

SW 8588 is a derivative of the CIMMYT cultivar 'Milan' that carries a 2N alien segment in chromosome 2A (Cruz et al. 2016). That segment carries genes *Lr37* and *Yr17* for resistance to leaf rust and stripe rust, respectively (Bariana and McIntosh 1993). Since genes located in alien segments behave as linkage blocks in wheat backgrounds, these genes are likely to be at different loci rather than being pleiotropic. The QTL covering *Yr17* clearly conferred resistance in the current work despite the three *Pst* races having been documented as virulent for *Yr17*. This effect could be conferred by a linked gene in the translocated *Aegilops verticosa* chromosomal segment for high-temperature adult-plant (HTAP) resistance as reported by Liu et al. (2018). The associated effect on leaf rust response indicated that *Lr37* was effective. In another field test (unpublished data), the Thatcher NIL with the 2N translocation (*Lr37*) produced a low reaction to a mixture of *Pt* races THJS, PGTS, THND, and PHTT, confirming that *Lr37* behaved as an APR gene. Whereas some *Yr17*-avirulent isolates are easily confirmed in seedling tests, others give ambivalent responses; hence reports of virulence should be checked by adult plant tests with relevant controls (Milus et al. 2015), as *Yr17* is linked to a HTAP resistance QTL (Liu et al. 2018). The finding of significant contributions to resistance to both diseases in this study indicates that both *Yr17* (and/or the HTAP resistance QTL) and *Lr37* were effective in our tests. If respective virulences were present in the mixed inoculum used to induce epidemics, then either of the virulent components were not present in adequate quantities to overcome the resistance genes. Otherwise, combinations of these genes and others conferred resistance in the lines. *Lr37/Yr17* is widely deployed in Sichuan wheat cultivars based on marker VENTRIUP-LN2 (Gao et al. 2019; Helguera et al. 2003), and as the wheat genotypes carrying these genes also have additional genes for rust resistance, especially HTAP resistance to stripe rust, these resistance sources still can be used in wheat breeding.

Three designated seedling-effective *Lr* genes, *Lr14a* (Dyck and Samborski 1970), *Lr14b* (Dyck and Samborski 1970), and APR gene *Lr68* (Herrera-Foessel et al. 2012), and four *Yr* APR genes, *Yr39* (Lin and Chen 2007), *Yr52* (Ren et al. 2012a), *Yr59* (Zhou et al. 2014), and *Yr67* (Li et al. 2009), have been reported on chromosome 7BL. *Lr14a* and *Lr14b* were not effective against the *Pt* races used in the current study, and no data are currently available for *Lr68*. *QLr.hebau-7BL* and *QYr.hebau-7BL* were detected in 17BDLR and 16CDYR, and both were linked with marker *Xcfa2040*. The position is similar to *Lr68* that is present in CIMMYT line Parula. Hence, *QLr.hebau-7BL*, present in SW 8588 (a derivative of the CIMMYT line Milan), might be *Lr68*. This QTL also conferred resistance to stripe rust in the current study, but there is no report to indicate that *Lr68* is associated with stripe rust resistance.

Yr39 is a HTAP resistance gene linked with *Xgwm131*, which is about 63 cM from *QYr.hebau-7BL* based on the consensus map

(Somers et al. 2004). The linkage between *Yr52* and *Yr39* was 36.5 ± 6.75 cM (Lin and Chen 2007). It is likely that *QYr.hebau-7BL* is different from *Yr52* and *Yr39*. *Yr59* was flanked by markers *Xwgp5175* and *Xbarc32* at a similar position to *QYr.hebau-7BL* based on the consensus map (Somers et al. 2004). *Yr67* (*YrC591*) in the Indian cultivar C591 is linked with *Xcfa2040* and *SC-P35M48* which are mapped in a similar position to *QYr.hebau-7BL*. However, both *Yr59* and *Yr67* were described as genes with large effects, whereas *QYr.hebau-7BL* has a relatively small effect. Therefore, the relationship between *QYr.hebau-7BL* and other resistance genes on chromosome 7BL needs further investigation.

The only leaf rust resistance QTL, *QLr.cimmyt-5AL*, was previously identified on chromosome 5AL in Avocet (Rosewarne et al. 2012). It was flanked by *wPT-0373* and *wPT-0837* and about 2 cM from *Vrn-A1*, which is located at 587.0 Mb on the IWGSC RefSeq v1.0 physical map. The position of *QLr.hebau-5AL* is 589.3 to 591.5 Mb according to the physical positions of the closely linked markers. Although the two QTL were mapped to the similar regions, they appear to have different origins.

Five QTL for stripe rust resistance on chromosome 5A are reported in common wheat varieties; these were present in lines Opatá 85 (Boukhatem et al. 2002), Pastor (Rosewarne et al. 2012), Pingyuan 50 (Lan et al. 2010), SHA3/CBRD (Ren et al. 2012b), and Franco-lin#1 (Lan et al. 2014). These QTL were located in three different regions of 5A (Rosewarne et al. 2012). The first region is from 558.3 to 562.9 Mb, the second from 461.9 to 537.0 Mb, and the third from 664.0 to 703.0 Mb. It is difficult to determine the relationships between *QYr.hebau-5AL* (578.2 to 580.4 Mb) and the QTL in the first region only by chromosome location. *QLr.hebau-5AL* and *QYr.hebau-5AL* were mapped at a similar region, so they might be the same gene with pleiotropic effects for resistance to both leaf rust and stripe rust. Further studies are needed to test this hypothesis.

The APR QTL *QLr.hebau-2DS* conferring a major resistance effect from Thatcher was detected in all leaf rust environments. Thatcher contains the race-specific APR gene *Lr22b* on chromosome 2DS (Dyck 1979), and *QLr.hebau-2DS* was mapped to the same region according to the physical position of closely linked markers *Xgwm296* and *Xgwm455*. Thatcher showed a susceptible response indicating that *Lr22b* was not effective against at least some components of the race mixture used in the field. A minor resistance effect from Thatcher to leaf rust was also observed in another study (Zhang et al. 2017). When the four *Pt* races (THJS, PGTS, THND, and FHDT) used in the field were separately inoculated on adult plants of Thatcher in the greenhouse, intermediate reactions obtained with PGTS and FHDT indicated that *Lr22b* was effective (unpublished data). The MDS for leaf rust on Thatcher in the field tests were about 80%, whereas the frequency distributions extended to 100%. The leaf rust levels on Thatcher could have been lower because part of the inoculum was avirulent.

QLr.hebau-3BL was detected in only one environment. To date, the cataloged *Lr* gene, *Lr77* (Kolmer et al. 2018), and two leaf rust

Table 4. Comparison of maximum disease severity scores for leaf and stripe rust among 144 SW 8588 × Thatcher recombinant inbred lines with and without the resistance alleles

<i>Lr</i> locus	No. ^y	Leaf rust			<i>Yr</i> locus	No.	Stripe rust			
		16BD ^z	16ZK	17BD			15CD	16CD	16BD	17BD
– <i>QLr.hebau-1BL</i>	68	55.1 a	42.9 a	29.6 a	– <i>QYr.hebau-1BL</i>	63	50.7 a	56.7 a	13.1 a	11.2 a
+ <i>QLr.hebau-1BL</i>	56	34.8 b	22.4 b	15.6 b	+ <i>QYr.hebau-1BL</i>	65	27.6 b	32.2 b	9.8 a	8.1 a
– <i>Lr37</i>	69	52.9 a	39.8 a	25.5 a	– <i>Yr17</i>	69	45.0 a	54.0 a	13.9 a	12.4 a
+ <i>Lr37</i>	50	34.7 b	25.9 b	19.9 a	+ <i>Yr17</i>	50	30.6 b	31.1 b	7.8 b	5.5 b
– <i>QLr.hebau-5AL</i>	35	49.5 a	42.4 a	30.2 a	– <i>QYr.hebau-5AL</i>	29	49.9 a	59.1 a	14.7 a	10.0 a
+ <i>QLr.hebau-5AL</i>	94	42.0 a	29.1 b	18.9 a	+ <i>QYr.hebau-5AL</i>	103	34.8 a	38.0 b	11.2 a	10.0 a
– <i>QLr.hebau-7BL</i>	65	53.2 a	37.6 a	34.7 a	– <i>QYr.hebau-7BL</i>	65	44.4 a	51.8 a	12.6 a	12.0 a
+ <i>QLr.hebau-7BL</i>	59	37.5 b	28.9 a	9.7 b	+ <i>QYr.hebau-7BL</i>	59	33.8 a	36.8 b	11.8 a	8.4 a
– <i>QLr.hebau-2DS</i>	44	63.5 a	46.3 a	36.5 a						
+ <i>QLr.hebau-2DS</i>	58	26.4 b	21.0 b	13.0 b						

^y Number of RILs in the population.

^z Different letters within columns following the mean indicate significant differences based on Student *t* tests ($P < 0.01$).

APR QTL have been mapped on chromosome 3B, including *QLr.sfrs-3B* (Messmer et al. 2000) and *QLr.fcu-3BL* (Chu et al. 2009). *Lr77* is quite effective, so the QTL on 3BL in the current study is likely not *Lr77*, since *QLr.hebau-3BL* was effective in only one test. *QLr.sfrs-3B* detected in the Forno/Oberkulmer RIL population was located at the centromere region (Li et al. 2014). However, *QLr.fcu-3BL* was mapped at 586.5 Mb on 3BL, whereas *QLr.hebau-3BL* was mapped to the 761.3 to 761.6 Mb region. Hence, *QLr.hebau-3BL* was different from the two QTL mentioned above and might be a new QTL.

QLr.hebau-6DS was detected in 16ZKLR with 6.3% PVE. No known QTL for leaf rust resistance was previously documented on chromosome 6DS, and hence *QLr.hebau-6DS* is new.

Eight APR QTL for stripe rust were previously mapped near the centromere on chromosome 4B (Rosewarne et al. 2013). However, *QYr.hebau-4BS* was located in the terminal region of 4BS according to the physical locations of the closely linked markers, indicating that the QTL is novel.

QRYr6D.1 was reported on chromosome 6DS flanked by *Xgwm325* and *Xbarc175* and located at 79.9 to 411.8 Mb (Agenbag et al. 2012). *QYr.hebau-6DS* in the present study was located at 5.8 to 8.9 Mb, thus it is different from *QRYr6D.1* and should be a novel QTL for stripe rust resistance.

While some QTL identified in the present study could be novel, they need to be validated in other populations and environments. The genetic distances between the tightly linked SNP or SSR markers and QTL contour peaks ranged from 0.0 to 2.5 cM. Some SNP markers identified here are successfully converted to KASP markers (Semagn et al. 2014). These KASP markers can be more efficiently used in marker-assisted selection for pyramiding APR genes in wheat breeding programs to improve the levels of leaf rust and stripe rust resistance.

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