ORIGINAL ARTICLE

Mapping of a QTL with major effect on reducing leaf rust severity at the adult plant growth stage on chromosome 2BL in wheat landrace Hongmazha

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Abstract

Key message   A major QTL (*QLr.cau-2BL*) for APR to leaf rust was detected on 2BL; an SSR marker was developed to closely link with *QLr.cau-2BL* and validated for effectiveness of MAS.

Abstract   The wheat landrace Hongmazha (HMZ) possesses adult plant resistance (APR) to leaf rust. To detect and validate quantitative trait locus (QTL) for the APR, four wheat populations were assessed for leaf rust severity in a total of eight field and greenhouse experiments. The mapping population Aquileja × HMZ (120 recombinant inbred lines, RILs) was genotyped using 90 K SNP markers. A major QTL (*QLr.cau-2BL*) was detected between the markers IWB3854 and IWB21922 on chromosome 2BL. IWB3854 and IWB21922 were positioned at approximately 531.14 Mb and 616.48 Mb, respectively, on 2BL of IWGSC RefSeq v1.0 physical map. Based on the sequences between 531.14 and 616.48 Mb on 2BL of IWGSC RefSeq v1.0, 415 simple sequence repeat (SSR) markers were developed. These markers and 28 previously published SSR makers were screened; the resulted polymorphic markers were used to genotype the relatively larger population RL6058 × HMZ (371 RILs). *QLr.cau-2BL* was mapped within a 1.5 cM interval on 2BL map of RL6058 × HMZ, and a marker (Ta2BL_ssr7) was identified to closely link with *QLr.cau-2BL*. Effectiveness of selection for *QLr.cau-2BL* based on Ta2BL_ssr7 was validated using two populations (RL6058 × HMZ $F_{2:3}$ and Jimai22 × HMZ $BC_4F_{2:3}$). In addition, polymorphism at Ta2BL_ssr7 was detected among a panel of 282 commercial wheat cultivars. We believe, therefore, that Ta2BL_ssr7 should be useful for introducing *QLr.cau-2BL* into commercial wheat cultivars and for accumulating *QLr.cau-2BL* with other APR QTL.

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Abbreviations

2BL	The long arm of chromosome 2B
ANOVA	Analysis of variance
APR	Adult plant resistance
AUDPC	The area under the disease progress curve
H^2	Broad-sense heritability
HMZ	Hongmazha, a wheat landrace
IT	Infection type
LOD	Logarithm of odds
Lr gene	Leaf rust resistance gene
MAS	Marker-assisted selection
PVE	Phenotypic variation explained
QTL	Quantitative trait locus / loci
RIL	Recombinant inbred line
SNP	Single nucleotide polymorphism

Introduction

Leaf rust (caused by *Puccinia triticina* Erikss.) is an important disease of wheat (*Triticum aestivum* L) globally; yield losses due to leaf rust can exceed 50% under favorable conditions if fungicides are not applied (Huerta-Espino et al. 2011). In China, the world's largest wheat producer, leaf rust is widespread, occurring on around 15 million ha annually (Liu and Chen 2012). Cultivation of wheats with resistance to leaf rust can reduce the need for fungicides and thus is an economical and environmentally friendly strategy to control the disease.

Currently, 79 designated leaf rust resistance (*Lr*) genes have been described (McIntosh et al. 2017; Qureshi et al. 2018). Most of these genes confer all-stage resistance that is effective throughout the life of the plant. All-stage resistance is often race-specific and vulnerable to adaptation by virulent variants in *P. triticina* population, becoming ineffective within limited years in commercial wheat fields (McDonald and Linde 2002). Compared to commercial cultivars, some landraces and wild relatives are richer sources of all-stage resistance (Qureshi et al. 2018). Another category of resistance, adult plant resistance (APR), is insignificant at the seedling growth stage, but is expressed at the post-seedling stage (Caldwell et al. 1957). Certain *Lr* genes for APR (e.g., *Lr12* and *Lr22b*) are race-specific, showing contrasting resistant/susceptible infection types (ITs) to different *P. triticina* races at the adult plant stage (Huerta-Espino et al. 2011; Park and McIntosh 1994). Quantitative trait loci (QTL) associated with APR confer primarily low disease characterized by one or more of the resistance components such as longer latent period, fewer uredinia, and smaller uredinium size (Lagudah 2011; Huerta-Espino et al. 2011). Some APR including *Lr34* and *Lr46* (Krattinger et al. 2009; Singh et al. 1998) have proven to condition durable resistance (Johnson 1981). The cloned *Lr34* and *Lr67* illustrate novel resistance mechanisms (Krattinger et al. 2009; Moore et al. 2015), which are distinct from the mechanism underlying race-specific resistance genes (Dangl et al. 2013). It is generally considered that durable resistance can be achieved by accumulating APR QTL, and such an accumulation can be facilitated by marker-assisted selection (MAS) (Lagudah 2011; Singh et al. 2000).

According to a recent review by Silva et al. (2018), 249 QTL for APR to leaf rust were previously reported. Such numerous QTL should provide a convenience for a breeding program to choose appropriate APR resources, although some of them may be redundant. These QTL differ in effect size; percentage of phenotypic variance explained (PVE) by a single QTL ranges from 1 to 73%. Some QTL could be significantly effective in only one of

the test environments; some, such as *Lr34*, are quite stable with consistent effectiveness across multiple environments and across different genetic backgrounds. From the viewpoint of practical breeding, it is easier to use major QTL (e.g., PVE > 15%) with high stability than minor QTL. It is essential to find new APR QTL for enhancing the genetic diversity of leaf rust resistance deployed over the vast and agro-ecologically diverse wheat-planting regions in the world. The recently published IWGSC RefSeq v1.0 sequences (IWGSC 2018) combined with the developed high-throughput genotyping platforms such as 90 K SNP array (Wang et al. 2014) constitute a powerful tool for detecting new QTL and for developing markers closely linked with QTL.

The wheat landrace HMZ was ever commercially cultivated on substantial scale in Northwest China before 1965. Although showing susceptible IT at both the seedling and adult plant growth stages, HMZ has consistently displayed lower leaf rust severities (< 35%) at the adult plant stage since 2002 in our field screening nurseries located in different provinces. The objectives of this study were to detect QTL associated with the APR in HMZ, to develop SSR markers closely linked with the detected major QTL, and to validate the developed marker.

Materials and methods

Wheat materials

Four wheat populations were used to detect and/or validate QTL, namely, Aquileja × Hongmazha (HMZ) with 120 RILs, RL6058 × HMZ with 371 RILs, 40 $F_{2:3}$ families from Aquileja × HMZ, and 40 $BC_4F_{2:3}$ families from Jimai22 × HMZ with Jimai22 as recurrent parent. HMZ is a Chinese winter wheat landrace with the accession number K-61293 at http://wheatpedigree.net/. The winter wheat cultivar Aquileja (PI 393993; Tevere/Giuliari//Gallini) originates from Italy. The spring wheats Thatcher (CItr 10003; Marquis/Iumillo//Marquis/Kanred; Clark 1935) and RL6058 (Thatcher*6/PI 58548) came from North America. The Chinese winter wheat Jimai22 (935024 /935106) is a commercial cultivar. Aquileja × HMZ and RL6058 × HMZ were developed from F_2 to F_6 by single seed descent and the subsequent generations (F_8, F_9 and F_{10}) were used in this study. HMZ possesses APR to leaf rust, RL6058 carries *Lr34*, and the other three wheats (Aquileja, Jimai22 and Thatcher) are susceptible to the *P. triticina* race THTT used in this study. Thatcher was used as a susceptible control throughout the experiments. A set of 282 commercial wheat cultivars (Online Resource 1 Table S1), collected from some important wheat production provinces of China, were used

to test polymorphism of an SSR marker developed in this study as detailed below.

Assessment on disease severities of RIL populations

Leaf rust severities of wheat RIL populations were evaluated in seven field experiments involving three locations (Gansu province, Wushan county, 34°42′15″N, 104°40′08″E, elevation 1650 m; Henan province, Suiping county, 33°6′30″N, 113°54′48″E, 150 m; and Shandong province, Tai'an district, 36°18′09″N, 117°13′05″E, 90 m) and three autumn-sown wheat crop seasons (2016–2017, 2017–2018 and 2018–2019; abbreviated as 2017, 2018 and 2019, respectively). The experiments were conducted for Aquileja×HMZ in Gansu and Shandong in 2017 and 2018, and for RL6058×HMZ in Gansu, Henan and Shandong in 2019. Each of the seven location×year combinations was considered as an individual "environment." Sowing was done in mid-September in Gansu and in early October in Henan and Shandong. In each environment, experiment was in a randomized complete block design with three replicates. An individual plot consisted of a single 1-m-long row with 25 cm between adjacent rows. Each plot was sown with approximately 40 seeds of a RIL. A set of parents and susceptible control wheat Thatcher were included after every 60 RIL rows. Two rows of Thatcher perpendicular and adjacent to the plot rows were sown along each field block to serve as spreader plants of inoculums. The test plants were covered with plastic films during winter months for facilitating the plants to overwinter.

Although leaf rust epidemic can be induced by naturally occurring *P. triticina* urediniospores in all of the seven test environments, artificial inoculation was applied to create higher and more homogeneous disease pressure. The *P. triticina* race THTT was used as inoculum, which has been prevalent in the *P. triticina* population in China (Liu and Chen 2012). THTT has the avirulence/virulence spectrum *Lr9, Lr19, Lr24, Lr28, Lr38, Lr39, Lr42, Lr45/Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr3ka, Lr10, Lr11, Lr14a, Lr14b, Lr15, Lr16, Lr17a, Lr18, Lr20, Lr21, Lr23, Lr25, Lr26, Lr29, Lr30, Lr32, Lr33, Lr36, Lr50, LrB* (Li et al. 2010). At the stem elongation stage of the test plants, inoculation was done on clear afternoons when there was a high likelihood of overnight dew. The spreader plants were sprayed with fresh THTT urediniospores suspended in water containing 0.04% Tween 20. The inoculated plants were then covered with plastic films to ensure long-lasting dew, and 18 h later the films were removed. About 2 weeks after inoculation when some urediniospores were obviously released from the spreader plants, the test plants were frequently misted after sunset to facilitate re-infection before disease recording was completed.

Leaf rust severity in the form of percentage of infected leaf area was scored using the modified Cobb scale (Peterson et al. 1948) on 5 to 12 flag leaves each plot entry. Recording was done three times when the leaf rust severities on Thatcher flag leaves reached $10-30\%$, $50-60\%$, and $80-90\%$, respectively. The area under the disease progress curve (AUDPC) was calculated based the three time recordings for each plot entry with the formula AUDPC = $\Sigma[(x_i + x_{(i+1)})/2] \times (t_{(i+1)} - t_i)$, where x_i and $x_{(i+1)}$ are scores for a plot entry on date t_i and date $t_{(i+1)}$, respectively, and $t_{(i+1)} - t_i$ = the number of days between date $t_{(i+1)}$ and date t_i. QTL mapping was done on AUDPC means calculated for each RIL by averaging over the three replicates within each experiment.

Identification of *P. triticina* races

To obtain some information on number and virulence of *P. triticina* races in the experimental plots, race identification was done on samples from the experiment in Shandong in 2018 following the procedure in Long and Kolmer (1989). Briefly, Thatcher leaves bearing uredinia were collected from the experimental plots. Single uredinia were isolated, and then the spores were increased. The resulted urediniospores were used to inoculate the wheat host differentials that consisted of 16 near-isogenic lines of Thatcher with respective single resistance genes *Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a,* and *Lr18*. Ten to 12 days after incubation, IT was recorded as either high (IT 3–4) or low (IT 0 to 2+). Races were designated following the three letter code system of Long and Kolmer (1989), with an additional fourth letter that described the high or low IT to Thatcher wheat lines with genes *LrB, Lr10, Lr14a*, and *Lr18* (Long et al. 2002).

Assessment on disease severities of F₂:₃ populations

To examine effectiveness of selection based on an SSR marker developed in this study, the selected 40 Aquileja×HMZ F₂:₃ families (12 plants each family; 480 plants in total) and 40 Jimai22×HMZ BC₄F₂:₃ families (18 plants each family; 720 plants in total) were assessed for leaf rust severity in a greenhouse. Sowing was done in early October 2019 in 1.2-m-long rows with 25 cm spacing between adjacent rows. Each row had six seeds 20 cm apart. The families within each cross were arranged in a randomized complete block design with two (for Aquileja×HMZ) or three (for Jimai22×HMZ) blocks. Two sets of parents and Thatcher were included in every block. From late November 2019 to mid-February 2020, heaters of the greenhouse were turned off. This resulted in the low temperatures ranging from -2 °C (the lowest, night) to 9 °C (the highest, daytime) for vernalizing the seedlings. Afterwards,

the greenhouse was running at 18–25 °C daily under natural daylight supplemented with high-pressure sodium lamps (8000–10,000 lx at top leaves) in a 16 h photoperiod. In early March, the plants were inoculated with THTT as mentioned above. The plants were frequently misted after sunset to facilitate re-infection. Leaf rust severity was scored on flag leaves when Thatcher flag leaves showed a severity around 90%. For each $F_{2:3}$ or $BC_4F_{2:3}$ family, mean disease severity was calculated by averaging over the 12 or 18 plants within the family.

Statistical analysis of disease data

All analyses were performed in SAS/STAT version 9.3 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was conducted based on AUDPC values across the four experiments for Aquileja × HMZ and across the three experiments for RL6058 × HMZ. A linear model was fitted using PROC GLM: $P_{ijk} = \mu + G_i + E_j + E_j(R_k) + G_i \times E_j + e_{ijk}$, where P_{ijk} is the phenotypic value, μ the population mean, G_i the effect of the ith genotype (RIL), E_j the effect of the jth environment, $E_j(R_k)$ the effect of the kth replicate within the jth environment, $G_i \times E_j$ the ijth effect of the genotype-by-environment interaction, and e_{ijk} designated the residual. Broad-sense heritability (H^2) on a RIL mean basis was estimated applying the variance component analysis of PROC VARCOMP with the restricted maximum likelihood method setting all effects as random; the calculation was based on the equation $H^2 = \sigma^2_G/[\sigma^2_G + \sigma^2_{G\times E}/m + \sigma^2_{error}/(m \times r)]$, where σ^2_G denotes the genotypic (i.e., RIL) variance, $\sigma^2_{G\times E}$ the genotype-by-environment interaction variance, σ^2_{error} the error variance, m the number of environments, and r the number of replicate within each environment (Holland et al. 2003). The correlation coefficients for AUDPC among different experiments were estimated applying PROC CORR (Pearson).

Genotyping, map construction and QTL analysis

The mapping population Aquileja × HMZ (120 RILs) was genotyped using the 90 K Illumina® iSelect wheat SNP array (Wang et al. 2014). One seed from F_9 generation of each RIL was used to grow plant from which DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) method (Saghai-Maroof et al. 1984). The 90 K SNP assays were performed by CapitalBio Technology (Beijing, China; http://www.capitalbiotech.com). SNP allele clustering and genotype calling were performed following the procedure of Wang et al. (2014). Data points of heterozygosity were treated as missing values. Genotypic data were filtered to retain only markers with missing values < 5% and allele frequency between 0.35 and 0.65. RILs with missing values > 5% were filtered out. After removing redundant

markers identified applying the BIN function in QTL IciMapping V4.0 software (Li et al. 2007), the retained high-quality markers (each marker representing a unique locus) were grouped and ordered using JoinMap 4.0 (Stam 1993). Genetic distances (in cM) were estimated based on the Kosambi mapping function. A linkage group was split if there was a distance ≥ 20 cM between two adjacent markers. Linkage groups were then orientated and assigned to chromosomes through alignments of the sequences flanking SNP with chromosomal survey sequence map (IWGSC 2014).

The second mapping population RL6058 × HMZ (371 RILs) was genotyped using 12 SSR markers that were developed in the present study based on the 2BL sequences of IWGSC RefSeq v1.0 (IWGSC 2018) or selected from the previously documented resources (http://wheat.pw.usda.gov/GG2/index.shtml). PCR reaction system consisted of 1.0 µl 10×PCR buffer (Mg²⁺plus), 0.1 µl 5×*Taq* DNA polymerase, 1.0 µl forward primer (1 µM), 1.0 µl reverse primer (1 µM), 2.0 µl DNA template (20–60 ng/µl), 0.25 µl dNTPs (10 mM), and 4.65 µl ddH₂O. While the normal PCR cycling (Röder et al. 1998) was performed for most of the markers, for certain markers, a touchdown program was used, which started with 94 °C for 5 min; then 10 cycles of 94 °C denaturing for 30 s, 60 °C annealing for 30 s (touchdown with − 0.5 °C each cycle), and 72 °C extending for 30 s; followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and ended with 72 °C for 10 min. Amplicons were separated in 6% denaturing polyacrylamide gels and visualized with the silver staining method (Bassam et al. 1991). Linkage map was constructed using MAPMAKER/EXP 3.0 (Lander et al. 1987).

QTL detection was performed using the composite interval mapping (CIM) method in Windows QTL Cartographer 2.5 (Wang et al. 2010). The threshold LOD score was calculated by running the permutation program set with 3000 replicates at a type I error rate of $\alpha = 0.05$. Permutation analysis was conducted for each of the seven experiments, resulting in threshold LOD values varying from 2.8 to 3.2; for simplicity, the highest threshold LOD value (3.2) was chosen as a uniform threshold for all experiments. Effect size of QTL was measured with determination coefficient R^2 (i.e., PVE).

Results

Leaf rust severity of wheat RILs

In each of the seven field experiments, leaf rust severities were approximately 90% on the susceptible control wheat Thatcher and less than 35% on the resistant landrace HMZ as exemplified by the typical symptoms shown in Fig. 1, indicating that disease pressure was high adequately for revealing resistance and susceptibility. To improve power
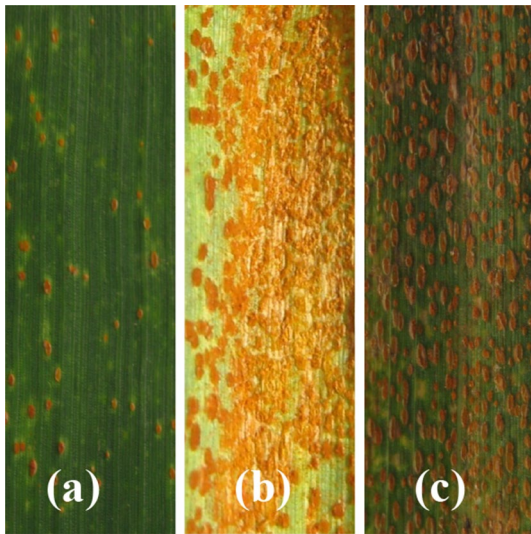
Fig. 1 Typical leaf rust symptoms of wheat lines Hongmazha (**a**), Aquileja (**b**) and Thatcher (**c**) at the adult plant growth stage

of QTL detection, disease severities were measured in AUDPC. AUDPC values of RILs varied between 93.8 and 1133.8 in the population Aquileja × HMZ and from 23.3 to 1148.7 in RL6058 × HMZ. Mean AUDPC over RILs varied from 361 to 585 dependent on populations and environments (location × year combinations) (Table 1; Online Resource 1 Tables S2 and S3). Correlation coefficients for AUDPC among the experiments were all significant with $P < 0.0001$, ranging from 0.77 to 0.93 in Aquileja × HMZ and from 0.87 to 0.96 in RL6058 × HMZ. In both populations, variances due to RIL and environment were significant at $\alpha = 0.0001$ and predominated over the variances due to RIL × environment, though the interaction variances were also significant (Table 2). Broad-sense heritability was high (0.92 in Aquileja × HMZ and 0.94 in RL6058 × HMZ). RILs

continuously distributed over AUDPC scales (Fig. 2). These results indicate that the APR in HMZ was segregating in a quantitative nature in both RIL populations.

P. triticina races in the experimental plots

Eleven races were identified from 40 uredinial isolates collected from the field experimental plots of Shandong in 2018 (Table 3). Of the 40 samples, 18 were differentiated as the artificially inoculated race THTT, and 22 were grouped into 10 naturally occurring races. In light of the limited number of samples analyzed (22), the number of races (10) should not be understood as a comprehensive survey of all naturally occurring races in the plots; the actual race number might be more than 10. It can be inferred that multiple naturally occurring races might also exist in the other six experiments of this study.

QTL mapping using Aquileja × HMZ population

After filtering out the genotypic data that could not meet the quality control criteria as described above and removing redundant markers, 1517 high-quality unique marker loci were mapped on 27 linkage groups covering all 21 chromosomes. Six chromosomes (1D, 2D, 3A, 6B, 6D and 7D) were split into two groups. The total length of the genetic map was 3656.8 cM with an average distance of 2.4 cM (min = 0.1 and max = 19.8 cM) between two adjacent markers (Online Resource 1 Table S4).

The 120 Aquileja × HMZ RILs were scanned genome-wide with the 1517 markers to detect chromosome regions associated with the AUDPC values (Table 1; Online Resource 1 Table S2) for each of the four field experiments. A major QTL for APR to leaf rust was found on chromosome arm 2BL (designated as *QLr.cau-2BL*; Fig. 3a). For reading ease, only selected markers at about 10 cM distances

Table 1 Leaf rust severities (measured in AUDPC) of the recombinant inbred line (RIL) populations Aquileja × Hongmazha (HMZ) and RL6058 × HMZ in field trials

Wheat cross/Test environment	Means of parents and susceptible check (CK)			RIL		
				Mean	Min	Max
Aquileja × HMZ/	HMZ	Aquileja	Thatcher (CK)			
Gansu 2017	185.8	953.7	1282.7	472.9	134.0	809.2
Gansu 2018	166.5	934.2	1258.6	417.5	139.8	821.9
Shandong 2017	194.7	975.8	1275.5	488.9	107.3	815.5
Shandong 2018	235.3	1066.4	1318.2	585.0	93.8	1133.8
RL6058 × HMZ/	HMZ	RL6058	Thatcher (CK)			
Gansu 2019	178.3	418.7	1286.3	463.2	53.9	1148.7
Henan 2019	185.8	422.3	1302.5	483.4	34.9	1121.5
Shandong 2019	163.5	384.6	1277.8	361.0	23.3	943.7

Table 2 Analysis of variance of leaf rust severity (measured in AUDPC) for the RIL populations Aquileja × Hongmazha (HMZ) and RL6058 × HMZ

Population/source	df	Mean square	F value	P value	H²
Aquileja × HMZ/					0.92
Environments	3	1,747,816.1	261.1	< 0.0001	
RILs	119	313,320.7	46.8	< 0.0001	
RILs × Environments	357	15,901.4	2.4	< 0.0001	
Replicates (environment)	8	18,123.4	2.7	0.006	
Error	952	6695.1			
RL6058 × HMZ/					0.94
Environments	2	4,725,816.7	483.3	< 0.0001	
RILs	370	460,731.8	47.1	< 0.0001	
RILs × Environments	740	17,958.4	1.8	< 0.0001	
Replicates (environment)	6	25,722.5	2.6	0.015	
Error	2204	9777.6			

are displayed in Fig. 3a, while more detailed information including all mapped unique markers with their positions can be found in Online Resource 1 Table S4. LOD peaks of *QLr.cau-2BL* were located between the markers IWB3854 and IWB21922. LOD values, ranging from 18.5 to 23.6 (Table 4), were significantly larger than the LOD threshold (3.2) in each of the four experiments (Fig. 3a). The parent HMZ contributed to the resistance (i.e., lower AUDPC) at *QLr.cau-2BL*, which explained from 47 to 57% of AUDPC variance.

Two QTL (*QLr.cau-5DL1* and *QLr.cau-5DL2*) were detected on 5DL (Fig. 3b). Resistance at both QTL was contributed by HMZ. Their effects on reducing AUDPC were significant at the LOD threshold (3.2) in only one or two of the four experiments (Fig. 3b) with small effect sizes (PVE = 2 to 7%; Table 4). These minor QTL need to be further tested. Only the major QTL *QLr.cau-2BL* is addressed in the remainder of the present paper.

Mapping of *QLr.cau-2BL* using RL6058 × HMZ population

The Aquileja × HMZ population was small (120 RILs). To improve mapping resolution and to examine *QLr.cau-2BL* in different genetic backgrounds, the relatively large population RL6058 × HMZ (371 RILs) was used for further mapping *QLr.cau-2BL*. Sequences of the flanking markers IWB3854 and IWB21922 on Aquileja × HMZ map (Fig. 3a) were aligned to the 2BL sequences of IWGSC RefSeq v1.0 (IWGSC 2018) at approximately 531.14 Mb and 616.48 Mb, respectively (Table 4). On the basis of the
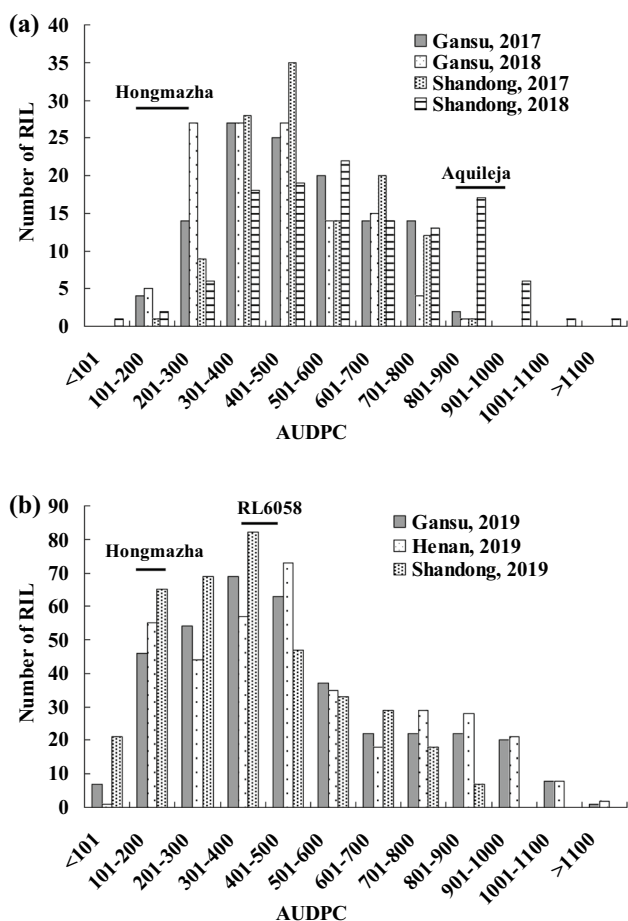
Fig. 2 Frequency distribution of leaf rust severities (measured in AUDPC) recorded in different field experiments for the wheat mapping populations Aquileja × Hongmazha (**a**) and RL6058 × Hongmazha (**b**). Horizontal bars denote the AUDPC ranges of the parental wheat lines

2BL sequences in this interval in IWGSC RefSeq v1.0, 415 SSR markers were developed, which were named with the prefix "Ta2BL_ssr" (SSR marker of common wheat 2BL) followed by a consecutive number. Information about the 415 markers and 28 other SSR markers of 2BL selected from public domain (http://wheat.pw.usda.gov/GG2/index.shtml) was presented in Online Resource 1 Table S5. After screening, 12 polymorphic markers (Table 5) were used to genotype the 371 RL6058 × HMZ RILs and a map of *QLr.cau-2BL* region was constructed (Fig. 4). QTL analysis based on the AUDPC data (Table 1; Online Resource 1 Table S3) recorded in the three field experiments mapped *QLr.cau-2BL* within a 1.5 cM interval delimited by wmc499 and Ta2BL_ssr16 (Fig. 4). Ta2BL_ssr7 was 1.0 cM distal to wmc499 and 0.5 cM proximal to Ta2BL_ssr16. LOD values ranged from 31.6 to 35.6 (Table 4; Fig. 4) and PVE values ranged from 34 to 36%. As expected, APR was associated with the amplicon from HMZ (146 bp; so-called R-allele) at

Table 3 *Puccinia triticina* races identified from the field trial plots of Shandong in 2018

| Race	Virulence (ineffective gene)[a]	Number of isolates
THTT	1, 2c, 3, 16, 26, 3 ka, 11, 17, 30, B, 10, 14a, 18	18
PHTT	1, 2a, 2c, 3, 16, 26, 3 ka, 11, 17, 30, B, 10, 14a, 18	4
THJT	1, 2a, 2c, 3, 16, 26, 11, 17, B, 10, 14a, 18	3
THTS	1, 2a, 2c, 3, 16, 26, 3 ka, 11, 17, 30, B, 10, 14a	3
THKT	1, 2a, 2c, 3, 16, 26, 3 ka, 11, 17, B, 10, 14a, 18	3
PHJT	1, 2c, 3, 16, 26, 11, 17, B, 10, 14a, 18	2
THSS	1, 2a, 2c, 3, 16, 26, 3 ka, 11, 17, B, 10, 14a	2
PHRT	1, 2c, 3, 16, 26, 3 ka, 11, 30, B, 10, 14a, 18	2
PHTS	1, 2c, 3, 16, 26, 11, 17, 30, B, 10, 14a, 18	1
PHSS	1, 2c, 3, 16, 26, 3 ka, 11, 17, B, 10, 14a	1
FHTR	2c, 3, 3 ka, 16, 26, 3 ka, 11, 17, 30, B, 10, 18	1
Total		40

[a]Lines tested were Thatcher lines with genes *Lr1, Lr2a, Lr2c, Lr3, Lr9, Lr16, Lr24, Lr26, Lr3ka, Lr11, Lr17, Lr30, LrB, Lr10, Lr14a,* and *Lr18*

Ta2BL_ssr7 and RL6058 produced an alternative amplicon (131 bp; S-allele). The sequence of wmc499 was aligned to the 2BL sequences of IWGSC RefSeq v1.0 physical map at about 594.44 Mb, while Ta2BL_ssr7 and Ta2BL_ssr16 were located at approximately 597.88 Mb and 600.14 Mb, respectively (Tables 4 and 5).

The 371 RL6058 × HMZ RILs, segregating for *Lr34* as well as for *QLr.cau-2BL*, produced a 751 bp amplicon (R-allele) and an alternative 523 bp amplicon (S-allele) at the locus *cssf5* that is a marker diagnostic for *Lr34* (Lagudah et al. 2009). Selection based on Ta2BL_ssr7 and *cssf5* for *QLr.cau-2BL* and *Lr34*, respectively, divided the 371 RILs into four groups, i.e., the RILs that carried resistant allele at both *QLr.cau-2BL* and *Lr34*, at *QLr.cau-2BL* alone, at *Lr34* alone, and none at both, respectively. As shown in Fig. 5, AUDPC mean was significantly (*P* < 0.0001) higher in "*None*," lower in "*QLr.cau-2BL* alone" and "*Lr34* alone," and further lower in "*QLr.cau-2BL* + *Lr34*" in each of the three experiments.

MAS based on Ta2BL_ssr7

Effectiveness of selection for *QLr.cau-2BL* based on Ta2BL_ssr7 was examined with the tests on the plants of Aquileja × HMZ $F_{2:3}$ and Jimai22 × HMZ $BC_4F_{2:3}$ in a greenhouse. DNA sample extracted from 1st-leaf stage F_3 seedlings of each Aquileja × HMZ $F_{2:3}$ family was used as template for PCR amplification with Ta2BL_ssr7 primers (Table 5). These families produced three genotypes in total, i.e., homozygous resistant (146 bp amplicon), homozygous susceptible (131 bp amplicon), and heterozygous (146 bp
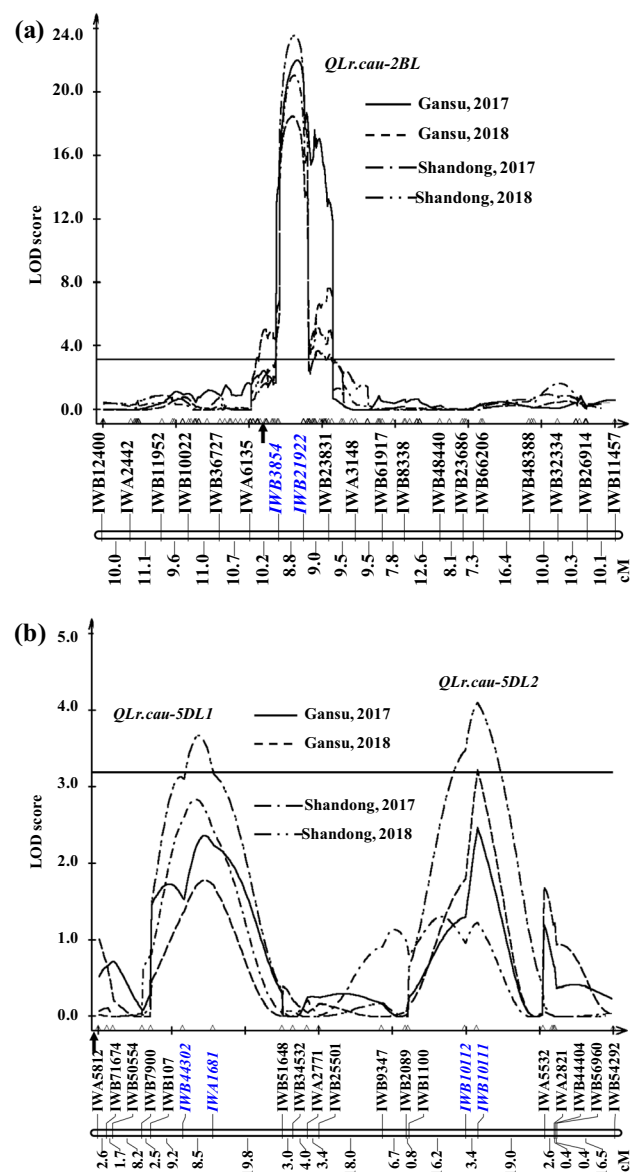
Fig. 3 Genetic maps of chromosome 2B (**a**) and 5DL (**b**) of Aquileja × Hongmazha, illustrating the logarithm of the odds (LOD) curves of *QLr.cau-2BL* (**a**) and *QLr.cau-5DL* (**b**). Marker names and intervals (in Kosambi cM) between adjacent markers are shown along the chromosome orientated with the telomere of 2BS and the centromere of 5DL to the left. *Arrows* suggest the positions of the centromeres inferred by aligning marker sequences to the chromosome survey sequence (IWGSC 2014). *Horizontal lines* indicate the threshold LOD of 3.2. Each of the small triangles along the x-axis represents a marker used for QTL mapping. For reading ease, only selected marker names at about 10 cM distances are displayed for 2B, while more detailed information including all mapped unique marker names with their positions can be found in Online Resource 1 Table S4

and 131 bp bands). Twenty of the $F_{2:3}$ families were selected for homozygous R-allele and another 20 ones were selected for homozygous S-allele. Disease tests of the selected families indicate that leaf rust severity mean value was

Table 4 QTLs for adult plant resistance to leaf rust detected on the basis of disease severity (AUDPC) in the wheat landrace Hongmazha (HMZ) using the mapping populations Aquileja×HMZ and RL6058×HMZ

QTL[a]	Population	Test environment	Chr arm	The close markers (physical position, Mb)[b]	LOD	PVE[c] %
QLr.cau-2BL	Aquileja×HMZ	Gansu 2017	2BL	IWB3854 (531.14), IWB21922 (616.48)	22.1	49
		Gansu 2018	2BL	IWB3854 (531.14), IWB21922 (616.48)	18.5	47
		Shandong 2017	2BL	IWB3854 (531.14), IWB21922 (616.48)	23.6	57
		Shandong 2018	2BL	IWB3854 (531.14), IWB21922 (616.48)	21.1	48
	RL6058×HMZ	Gansu 2019	2BL	wmc499 (594.44), Ta2BL_ssr7 (597.88), Ta2BL_ssr16 (600.14)	33.8	35
		Henan 2019	2BL	wmc499 (594.44), Ta2BL_ssr7 (597.88), Ta2BL_ssr16 (600.14)	35.6	36
		Shandong 2019	2BL	wmc499 (594.44), Ta2BL_ssr7 (597.88), Ta2BL_ssr16 (600.14)	31.6	34
QLr.cau-5DL1	Aquileja×HMZ	Gansu 2017	5DL	IWB44302, IWA1681	2.4	4
		Gansu 2018	5DL	IWB44302, IWA1681	1.8	3
		Shandong 2017	5DL	IWB44302, IWA1681	3.7	6
		Shandong 2018	5DL	IWB44302, IWA1681	2.8	5
QLr.cau-5DL2	Aquileja×HMZ	Gansu 2017	5DL	IWB10112, IWB10111	2.5	4
		Gansu 2018	5DL	IWB10112, IWB10111	3.2	5
		Shandong 2017	5DL	IWB10112, IWB10111	4.1	7
		Shandong 2018	5DL	IWB10112, IWB10111	1.2	2

[a]All of the resistance QTL in this table were contributed by HMZ. The resistance *Lr34* in RL6058 is not shown here

[b]Physical position was inferred by aligning the marker sequences to the sequences of IWGSC RefSeq v1.0 (IWGSC 2018)

[c]Percentage of phenotypic variance explained by QTL

Table 5 SSR markers developed to map QTL for leaf rust resistance in *QLr.cau-2BL* region of chromosome arm 2BL in the RIL population of RL6058×Hongmazha

Marker name [a]	Forward primer	Reverse primer	Motif	Annealing temp. (°C)	Motif position (bp)
gwm388	ctacaattcgaaggagagggg	caccgcgtcaactacttaagc	$(tc)_5$	58	557,367,412–557,367,421
wmc499	ggaactctgtggtccacctga	cggtgtgacctagcacgaact	$(gt)_{30}$	62	594,444,189–594,444,248
Ta2BL_ssr7	gctccctctcttatcctctct	aaggattttctcctaccacct	$(tta)_{20}$	56	597,879,385–597,879,444
Ta2BL_ssr16	gacttggagtggatgtcaata	ggtgctcctggatgttatc	$(ta)_{21}$	55	600,140,778–600,140,819
Ta2BL_ssr54	acttctctcatgtcattgcag	agacaagagcgtgttcattt	$(at)_{34}$	55	614,905,247–614,905,314
Ta2BL_ssr64	catagtggaaggcaacaaat	tacattcgctcatacgatagg	$(ag)_{20}$	55	617,448,769–617,448,808
barc101	gctcctctcacgatcacgcaaag	gcgagtcgatcacactatgagccaatg	$(taa)_9$	64	621,466,441–621,466,467
Ta2BL_ssr89	cactccccgaagattttat	ttttctctagtgaggcttgtg	$(at)_{24}$	55	627,061,665–627,061,712
Ta2BL_ssr94	ccgacaatcttcaattctgt	tgacaccgacagagagagata	$(tc)_{20}$	55	629,381,238–629,381,277
Ta2BL_ssr108	accaggaggaagcaaaata	ggggatgactttacttggata	$(ta)_{22}$	55	634,015,213–634,015,256
Ta2BL_ssr247	cactcacacacgcacatc	ggagttggacaaggagga	$(ag)_{24}$	55	675,692,966–675,693,013
wmc360	tatgtgtgtgaaaaatgg	aatttcttgtagaaccga	$(at)_8$	48	718,351,668–718,351,683

[a]Markers with the prefix "Ta2BL_ssr" were developed in this study based on the 2BL sequences of IWGSC RefSeq v1.0 (WGSC 2018), and the other markers were selected from the public domain (http://wheat.pw.usda.gov/GG2/index.shtml)
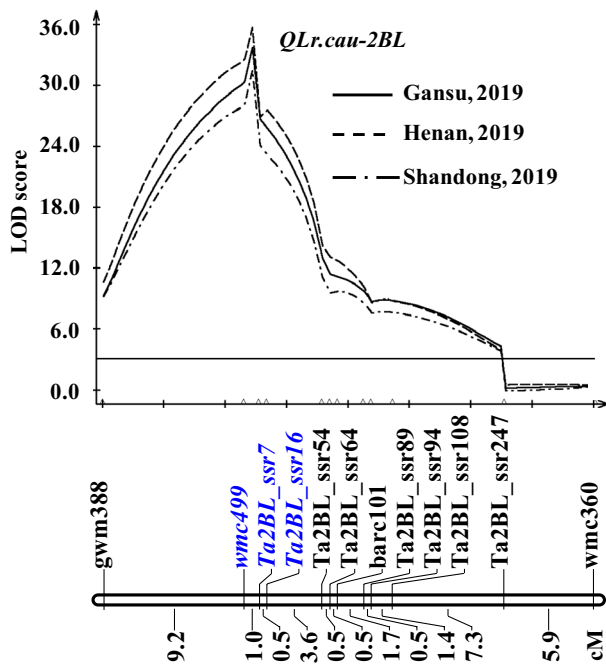
Fig. 4 Partial genetic map of 2BL of RL6058 × Hongmazha, illustrating the logarithm of the odds (LOD) value curves of *QLr.cau-2BL*. Marker names and intervals (in Kosambi cM) between adjacent markers are shown along the chromosome orientated with the centromere to the left. Horizontal line indicates the threshold LOD of 3.2

significantly ($P < 0.0001$) lower on R-group $F_{2:3}$ plants than on S-group plants (Fig. 6a; Online Resource 1 Table S6).

Likewise, Jimai22 × HMZ $BC_4F_{2:3}$ families were segregating at Ta2BL_ssr7 for homozygous resistance (146 bp amplicon) and homozygous susceptibility (131 bp amplicon). Twenty $BC_4F_{2:3}$ families were selected for homozygous R-allele and another 20 ones were selected for homozygous S-allele. Leaf rust severity mean value was significantly ($P < 0.0001$) lower on R-group $BC_4F_{2:3}$ plants than on S-group plants (Fig. 6b; Online Resource 1 Table S7). These results show that selection for *QLr.cau-2BL* based on Ta2BL_ssr7 was well effective in both cases of $F_{2:3}$ and $BCF_{2:3}$.

A set of 282 commercial wheat cultivars was detected for polymorphism at Ta2BL_ssr7 (Online Resource 1 Table S1). Of these cultivars, Tao954-1and Tao7230 produced 146 bp amplicon similar to HMZ (R-allele), 220 showed a 131 bp amplicon (S-allele), and 60 displayed a 155 bp amplicon (another S-allele; disease data not shown).

Discussion

A QTL (*QLr.cau-2BL*) for APR to leaf rust was detected and an SSR marker (Ta2BL_ssr7) closely linked with *QLr.cau-2BL* was identified using four populations. We confirmed

that Ta2BL_ssr7 is a robust marker for MAS. Our investigations during the period 2011–2017 indicated that 282 cultivars used in panel were commercially grown on large scales in China (with approximately 13.5 million ha in 2017; Online Resource 1 Table S1). Some of these cultivars were excellent in grain yield and adaptability, and these were widely grown; for instance, Jimai22 occupied more than a million ha in 2017. Of the 282 cultivars, some are still being commercially planted and the others are being used as parents for breeding new cultivars; however, 237 including Jimai22 were susceptible to leaf rust at both seedling and adult plant growth stages (Zhang's Lab., unpublished data). Only two (Tao954-1and Tao7230) of the 282 cultivars produced the same 146 bp amplicon as HMZ (R-allele; Online Resource 1 Table S1) at Ta2BL_ssr7 and the others showed alternative amplicons (131 bp or 155 bp; S-allele). Tao954-1and Tao7230 were cultivated on limited scales (< 3000 ha annually). We therefore believe that *QLr.cau-2BL* has not yet been substantially utilized in wheat breeding in China. MAS based on Ta2BL_ssr7 should facilitate the use of *QLr. cau-2BL* in practical breeding.

RL6058 carries *Lr34* and thus RL6058 × HMZ RILs were segregating for *QLr.cau-2BL* and *Lr34*. This provides us an opportunity for comparing the additive effect of these QTL. The selection based on Ta2BL_ssr7 and *cssfr5* for *QLr.cau-2BL* and *Lr34*, respectively, divided the RL6058 × HMZ RILs into different groups (Fig. 5). Comparison in disease severity between groups indicates that *QLr.cau-2BL* could reduce leaf rust severity similar to *Lr34* and acted in an additive manner (Fig. 5). Additive effect was also described for *Lr34* with *Lr68* (Herrera-Foessel et al. 2012). However, *Lr34* has been reported to work with certain rust resistance QTL in non-additive manners. For instance, Kolmer et al. (2011) indicate that *Lr34* can enhance the expression of an adult plant stem rust resistance QTL on 2BL in Thatcher, although *Lr34* by itself does not condition stem rust resistance. Rosewarne et al. (2013) address the interaction of *Yr18* with *Yr29* and further indicate that in the presence of *Lr34*, *Yr29* was less effective against stripe/yellow rust.

QLr.cau-2BL showed consistence effect on reducing disease severity across all of the three wheat populations (Aquileja × HMZ, RL6058 × HMZ and Jimai22 × HMZ) and across all of the eight experiments (Table 3; Fig. 6). The wheat lines used in these crosses represent considerably diverse genetic backgrounds. Aquileja is an Italian winter wheat, and RL6058, a spring wheat, originates from North America. The Chinese winter wheat Jimai22 is morphologically and phenologically different from Aquileja, for instance, in plant height and maturity time. The eight experiments represent significantly different environments. For example, the test site in Gansu is geographically separated by more than 1100 km from the site in Shandong. In Gansu, the experimental plots were located at an elevation

Fig. 5 Boxplots showing the effects of QTL combinations on dis-▶
ease severity in RL6058×Hongmazha RIL population. The y-axis
defines the score of disease severity (AUDPC) , and the x-axis defines
four RIL groups with different QTL combinations, i.e., presence of
resistance allele at both *QLr.cau-2BL* (represented by the 146 bp
amplicon) plus *Lr34* (751 bp), *QLr.cau-2BL* alone (146 bp), *Lr34*
alone (751 bp), and *None* (131 bp at *QLr.cau-2BL* and 523 bp at
Lr34), respectively. Disease was assessed in the field of Gansu (**a**),
Henan (**b**) and Shandong (**c**). *** Indicates significant difference at
α=0.0001 based on a Fisher's least significant difference (LSD) test.
NS means no significant difference. Within each box, the small dia-
mond and the horizontal line indicate the mean and median AUDPC,
respectively. The top and bottom edges of a box illustrate the 75th
and 25th percentiles, respectively. Whiskers (vertical lines outside a
box) extend to the extreme data points, and small circles denote outli-
ers

of 1650 m and wheat plants mature in the late July. The plots
in Shandong were located at an elevation of 90 m and wheat
matures in mid-June.

The APR in HMZ, primarily conferred by *QLr.cau-2BL*,
might be effective against many *P. triticina* races than the
artificially inoculated race THTT. We have observed HMZ
for its APR in field nurseries in Gansu and Shandong since
2002. Leaf rust epidemic in the nurseries was induced by
both artificially inoculated race (THTT) and naturally occur-
ring urediniospores. We analyzed some uredinial samples
once every three years or so and always found multiple races.
For example, 11 races were identified from the experimental
plots of Shandong in 2018 (Table 3). These races were col-
lectively virulent to 27 *Lr* genes including 14 ones in the
wheat differentials (*Lr1, Lr2a, Lr2c, Lr3, Lr16, Lr26, Lr3ka,
Lr11, Lr17, Lr30, LrB, Lr10, Lr14a* and *Lr18*) and 13 pre-
sent in other lines (*Lr2b, Lr3bg, Lr14b, Lr15, Lr17a, Lr20,
Lr21, Lr23, Lr25, Lr32, Lr33, Lr36* and *Lr50*) as tested by Li
et al. (2010). In addition, THTT was virulent to *Lr12, Lr13,
Lr22b* and *Lr37* at the adult plant growth stage (Zhang's
Lab, unpublished data). Similarly, nine races were found
from the plots of Gansu and Shandong in 2014 (Du et al.
2015). More races were comprehensively surveyed in China
by other researchers. Liu and Chen (2012) reported that 79
P. triticina races were detected from 613 single-uredinial
isolates collected between 2000 and 2006 from the provinces
including Gansu, Henan, and Shandong. Kolmer (2015)
and Ma (2020) also addressed the race diversity in these
provinces. We believe that HMZ might have been subjected
to inoculations by diverse races and the APR in HMZ was
effective to these races. From 2002 to 2020, leaf rust severity
was consistently less than 35% on HMZ, in contrast around
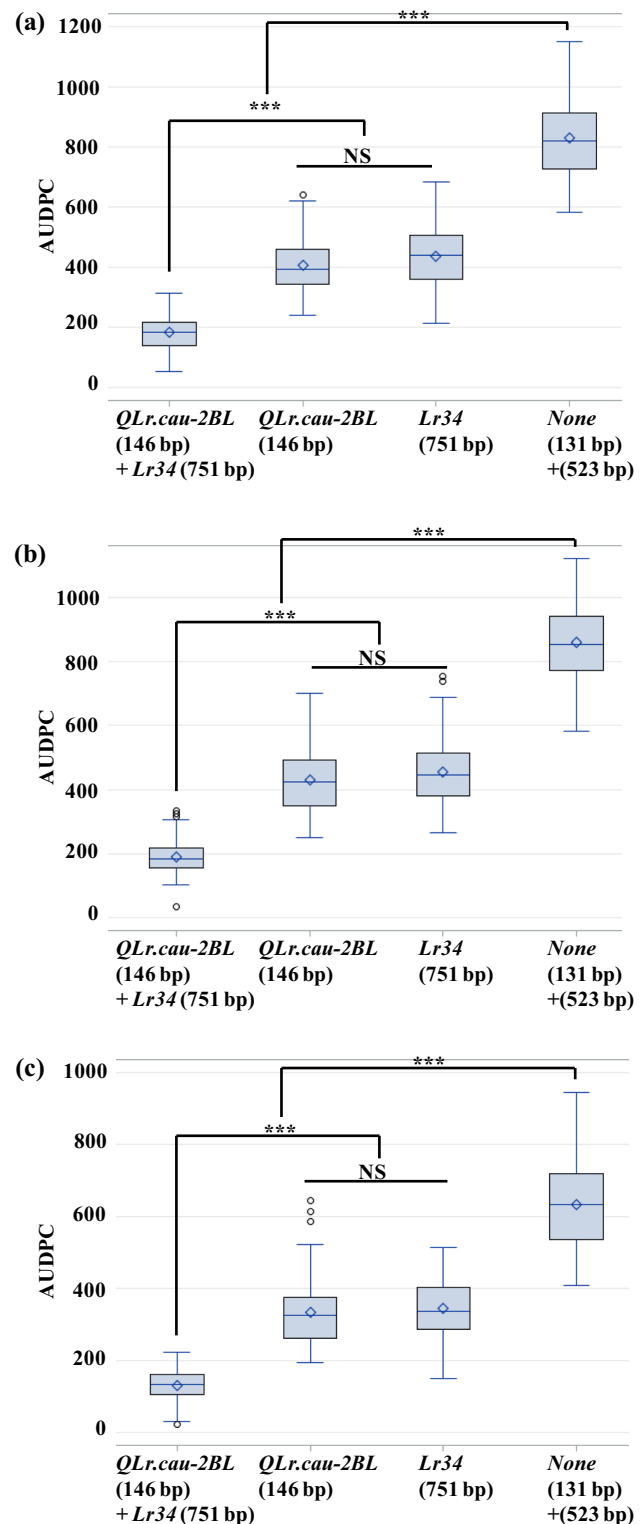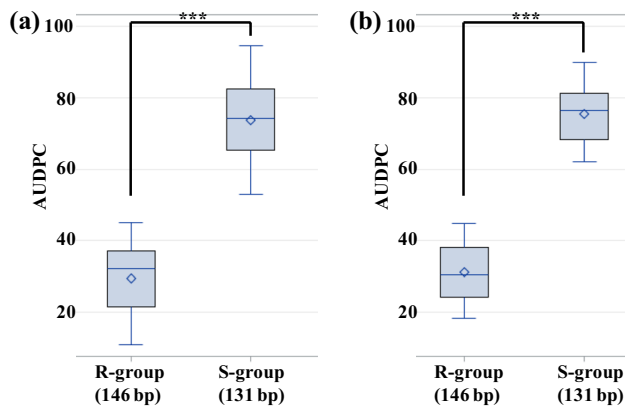
Fig. 6 Boxplots showing effects of the marker Ta2BL_ssr7 (representing *QLr.cau-2BL*) on disease severity in the $F_{2:3}$ population of Aquileja×Hongmazha (**a**) and the $BC_4F_{2:3}$ population of Jimai22×Hongmazha (**b**). The y-axis defines the score of disease severity, and the x-axis defines different groups of test plants. R-group carried the resistant allele in homozygous status at *QLr.cau-2BL*, and S-group carried the susceptible allele in homozygous status. *** Indicates a significant difference at $\alpha = 0.0001$ based on a Fisher's least significant difference (LSD) test. Refer to the caption of Fig. 5 for descriptions of box

90% on the susceptible control wheat Thatcher in our field nurseries.

According to a recent review on QTL for APR to leaf rust (Silva et al. 2018), 14 QTL were previously reported to be located on 2BL. Ten of these QTL were mapped within intervals delimited by flanking markers and the others were tagged with single markers. These 14 QTL appear to be quite diverse with respect to effect magnitude, consistence of effectiveness, resistance component, and pleiotropy. Six QTL (*QLr.osu-2B*, *QLrlp.osu-2B*, *QLr.inra-2B*, *QLr.cim-2B*, *QTL-2BL*, and *QLr.ifa-2BL*) were mapped using biparental populations (Online Resource 1 Table S8), and the other eight were detected using wheat panels for genome-wide association study (GWAS). The resistance alleles at *QLr.osu-2B* and *QLrlp.osu-2B* originated from the wheat line CI 13227 and were located in the vicinity of SSR marker barc167 (Xu et al. 2005a, b). These two QTL had pleiotropic relationship for decreasing infection rate and prolonging latent period with PVE values up to 16.6% (Xu et al. 2005a, b). *QLr.inra-2B* from the French cultivar Apache could lengthen latent period (PVE > 20%) without significant effect on other resistance components such as infection efficiency and lesion size (Azzimonti et al. 2014). The minor QTL *QLr.cim-2B* from the cultivar Avocet-S was associated with low disease severity (Ren et al. 2017). *QTL-2BL* from the Mexican cultivar Opata 85 reduced disease severity with a PVE value of 12.0% (Nelson et al. 1997). *QLr.ifa-2BL* from the Austrian cultivar Capo was effective in multiple

environments (overall PVE = 10.5%), and this QTL was coincided, in chromosome position, with a QTL for yellow rust resistance (Buerstmayr et al. 2014).

The eight GWAS QTL (*QLr.IWA2025*, *QLr.IWA207*, *QLr.IWA5177*, *QLr.IWA2509*, *QLr.wpt-2BL*, *qNV.Lr-2B.2*, *qNV.Lr-2B.3*, and *Lr_Ho*; Online Resource 1 Table S8) were mapped using diverse wheat panels involving landraces, core collections, registered cultivars, breeding lines, and synthetic hexaploid wheats (Gerard et al. 2018; Joukhadar et al. 2020; Riaz et al. 2018; Turner et al. 2017). All these QTL were associated with low disease severity. *QLr.IWA2025*, *QLr.IWA207*, *QLr.IWA5177* and *QLr.WA2509* showed PVE values up to 16.3% (Turner et al. 2017). *QLr.wpt-2BL*, identified based on analyses of 96 wheat accessions chosen from a larger panel (710 genotypes), expressed in all six experiments with PVE values from 7.8 to 11.7% (Gerard et al. 2018). *qNV.Lr-2B.2* and *qNV.Lr-2B.3*, detected using 288 wheat lines originating from the "N. I. Vavilov Institute of Plant Genetic Resources," were effective in multiple environments (Riaz et al. 2018). The minor QTL *Lr_Ho* was mapped with high precision applying a large wheat panel (2300 accessions), and this QTL was suggested to be a contribution to durable resistance (Joukhadar et al. 2020).

We inferred the physical positions of the 14 QTL by aligning their flanking/tagging marker sequences to the 2BL sequences of IWGSC RefSeq v1.0 (IWGSC 2018) (Fig. 7; Online Resource 1 Table S8). All of these QTL (except *Qlr.inra-2B*) are separated from *QLr.cau-2BL* by at least 70 Mb (Fig. 7). *Qlr.inra-2B* originates from the French cultivar Apache (Azzimonti et al. 2014). HMZ produced a 146 bp amplicon (R-allele) at Ta2BL_ssr7, whereas Apache produced a 131 bp amplicon (S-allele) (Online Resource 1 Table S1). On the basis of these results, we believe that *QLr.cau-2BL* is highly likely to be a previously unreported QTL for APR to leaf rust, although further tests are required for a conclusive novelty.

In addition to the 14 APR QTL, two designated *Lr* genes were previously mapped on 2BL, i.e., *Lr50* linked (6.7 cM) with the SSR marker gwm382 near the telomere of 2BL (Brown-Guerdira et al. 2003) and *Lr58* that co-segregated with the SSR marker cfd50 in the distal region of 2BL (Kuraparthy et al. 2007). Both genes confer all-stage race-specific resistance and were transferred to *T. aestivum* from *T. timopheevii* and *Aegilops triuncialis*, respectively; thus, both genes should be genetically different from *QLr.cau-2BL* that is harbored in a *T. aestivum* landrace (i.e., HMZ).

Realizing that *QLr.cau-2BL* alone may provide inadequate level of APR if disease pressures are high, we are further checking the combinability of *QLr.cau-2BL* with APR including *Lr46* and *Lr67*. The present study indicates that *QLr.cau-2BL* acted synergistically with *L34* and

Fig. 7 Physical position of *QLr. cau-2BL* compared with the previously reported QTL for adult plant resistance (ARP) to leaf rust on 2BL (10-Mb tick size map). Black bar indicates an interval harboring a QTL flanked by two markers, and dot indicates a single marker representing a QTL. *QLr.cau-2BL* was written in increased font. Refer to Online Resource 1 Table S8 for detailed information including full references
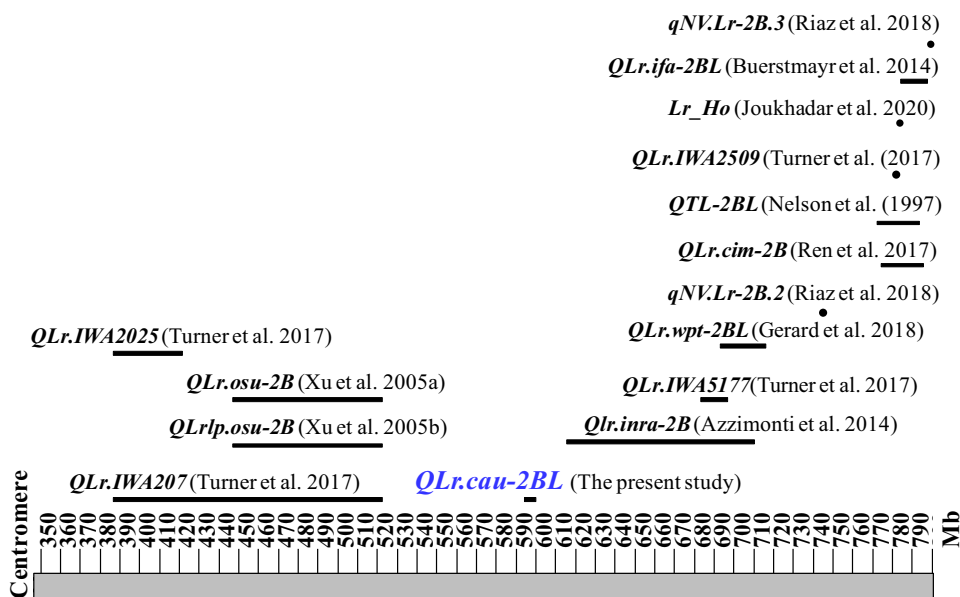
consequently reduced leaf rust to an adequate level (Fig. 5). Fine mapping of *QLr.cau-2BL* is also underway.

Author contribution statement ZZ and WQ conceived the study. YZ and ZW designed and managed the experiments, developed the SSR markers of 2BL, and constructed the genetic maps. YZ, ZW, WQ, JF, JR, ZZ and XJ performed the disease experiments and/or SSR genotyping. XZ statistically analyzed the disease data. ZZ and XZ collected the disease data from greenhouse experiment. ZZ, XZ, QW and JF developed the RILs. YZ and ZW drafted the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The authors declare that the experiments comply with the current laws of China.

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