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QTL mapping for leaf rust resistance in a recombinant inbred line population from the cross of wheat cultivars Zhongmai 578/Jimai 22

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Abstract

Leaf rust is a highly destructive disease that poses a significant threat to both the yield and quality of wheat. Identification of genetic loci can aid in enhancing leaf rust resistance in wheat breeding. In the present study, 262 recombinant inbred lines derived from a cross between Zhongmai 578 and Jimai 22 were used to map leaf rust resistance loci using the Wheat 50K single-nucleotide polymorphism (SNP) array across four environments. Four quantitative trait loci (QTL) on chromosomes 2B (2), 5B, and 7B were identified through composite interval mapping, designated *QLr.caas-2B.1*, *QLr.caas-2B.2*, *QLr.caas-5B*, and *QLr.caas-7B*, respectively, explaining 3.7%–19.6% of the phenotypic variances. The resistance alleles at *QLr.caas-2B.1*, *QLr.caas-5B*, and *QLr.caas-7B* originated from Zhongmai 578, while that at *QLr.caas-2B.2* came from Jimai 22. Both *QLr.caas-2B.2* and *QLr.caas-5B* overlapped with loci previously reported, whereas *QLr.caas-2B.1* and *QLr.caas-7B* are likely to be new loci. Two kompetitive allele-specific PCR (KASP) markers, *KASP-QLr.caas-2B.2* and *KASP-QLr.caas-5B*, were proven to be significantly associated with leaf rust resistance in a diverse panel of 119 wheat varieties mainly from China. Four candidate genes encoding a lectin-receptor kinase protein, F-box family protein, ankyrin repeat domain protein, and putative ABC transporter, respectively, were identified in genetic regions of the four QTL. These findings provide valuable QTL and breeding available KASP markers, facilitating the improvement of leaf rust resistance in wheat through marker-assisted breeding.

Keywords Common wheat, Kompetitive allele-specific PCR (KASP), Leaf rust resistance, Quantitative trait locus (QTL)

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Background

Leaf rust, caused by *Puccinia triticina* (*Pt*), is a devastating disease that significantly threatens wheat production, and can result in yield losses exceeding 50% (Huerta-Espino et al. 2011). With global climate warming, there has been an increasing trend in the epidemics of leaf rust in many major wheat-growing regions recently, including the Southern United States, South America, Canada, Eastern Europe, Egypt, and most wheat-growing regions in China. During the past two decades, severe wheat yield losses occurred frequently in China (Zhou et al. 2023).

Leaf rust resistance is classified into race-specific and race non-specific groups (Sapkota et al. 2020). Race-specific resistance, also referred to as all-stage resistance (ASR), is typically controlled by one or a few major genes, and arises from the interaction between resistance genes of the host and avirulence genes of the pathogen. This interaction is effective only for the specific pathogen race with corresponding avirulence genes. The resistance conferred by major genes can be overcome by virulence variation and diversification of the *P. triticina* races. Race non-specific resistance, referred to as adult-plant resistance (APR) or slow rusting resistance, is conditioned by numerous minor genes. This kind of resistance typically manifests by reducing disease severity during the later stages of plant growth. Additionally, wheat cultivars exhibiting APR demonstrate reduced infection frequencies, smaller spore sizes, and decreased spore production.

Breeding resistant wheat cultivars is the most important, economical, and effective approach to control leaf rust (Zhou et al. 2023). To date, more than 100 leaf rust resistance genes have been identified in wheat and its relative species, of which 91 genes have been formally catalogued (Bariana et al. 2022). Among the genes conferring ASR with race-specific resistance, only *Lr9* (Sears 1956), *Lr19* (Sharma and Knott 1966), *Lr24* (McIntosh et al. 1977), *Lr28* (McIntosh et al. 1982), *Lr29* (Procunier et al. 1995), *Lr38* (Friebe et al. 1993), *Lr47* (Dubcovsky et al. 1998), *Lr51* (Helguera et al. 2005), and *Lr53* (Marais et al. 2005) remain effective in wheat production in China (Li et al. 2010). In addition, *Lr12* (Singh et al. 1999), *Lr13* (Harjit Singh et al. 1992), *Lr22a* (Dyck and Kerber 1970), *Lr22b* (Plotnikova and Stubei 2013), *Lr34* (Krattinger et al. 2009), *Lr46* (Singh et al. 1998), and *Lr67* (Moore et al. 2015) are typical APR genes. It is worth mentioning that *Lr34/Yr18/Pm38/Sr57* (Krattinger et al. 2009), *Lr46/Yr29/Pm39/Sr58* (Lagudah 2011), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2014) have demonstrated durability and exhibited pleiotropic APR against leaf rust, stripe rust, stem rust, and powdery mildew. Currently, 10 wheat leaf rust resistance genes, *Lr1* (Cloutier et al. 2007), *Lr9/Lr58* (Wang et al. 2023), *Lr10* (Feuillet et al. 2003), *Lr13* (Yan et al. 2021), *Lr14a* (Kolodziej et al. 2021), *Lr21* (Huang et al. 2003), *Lr22a* (Thind et al. 2017), *Lr42* (Lin et al. 2022), *Lr34/Yr18/Sr57/Pm38* (Krattinger et al. 2009), and *Lr67/Yr46/Pm46/Sr55* (Herrera-Foessel et al. 2014) have been successfully cloned. Additionally, more than 200 leaf rust resistance quantitative trait loci (QTL) have been mapped in various wheat populations, distributing on all 21 wheat chromosomes (Hou et al. 2023).

The Yellow and Huai River Valleys Wheat Zone (YHRVWZ) is a major wheat-producing region in China. In recent years, leaf rust has become serious in the YHRVWZ, leading to significant yield losses. It is urgent to explore new leaf rust resistance genes and breed resistant varieties. From 2006 to the present, Jimai 22 has been grown on the largest acreage in China, with an annual production area of approximately 15 million hectares during the past 13 years (Zhao et al. 2023). Moreover, Jimai 22 is widely used as an elite parent in wheat breeding. Zhongmai 578, exhibits high yield, high grain quality, and strong compatibility, and has now become an important backbone parent in wheat breeding, with a cultivated area of around 0.6 million hectares annually. Both Jimai 22 and Zhongmai 578 are susceptible to prevalent *Pt* races (THTT, PHTT, THTS) at the seedling stage but moderate to high APR resistance. The objectives of the present study were to: (1) identify stable and new QTL for APR to leaf rust, (2) develop kompetitive allele-specific PCR (KASP) markers for improvement of leaf rust resistance in wheat breeding, and (3) predict candidate genes for leaf rust resistance for future research.

Results

Phenotypic analysis

Both Zhongmai 578 (IT 4) and Jimai 22 (IT 4) were highly susceptible to the pathotypes THTT, PHTT, and THTS at the seedling stage. The maximum disease severity (MDS) of the susceptible control, Zhengzhou 5389, ranged from 90%–100% across all environments, indicating that leaf rust developed well. The averaged MDS across all environments of Zhongmai 578 and Jimai 22 were 19% and 25%, respectively, at the adult-plant stage. The MDS of 262 recombinant inbred lines (RILs) ranged from 10 to 100% in Xinxiang 2022, 10%–90% in Xinxiang 2023, 10%–80% in Zhengzhou 2023, and 3%–77% in Baoding 2023 (Additional file 1: Figure S1 and Additional file 2: Table S1). The leaf rust MDS showed continuous variation, indicating a typical quantitative nature. The correlation coefficients between environments ranged from 0.40 to 0.61 ($P < 0.01$), and the broad-sense heritability (H_b^2) of leaf rust MDS was

generally high across environments (0.68), indicating that MDS was mainly determined by genotypes (Table 1). Analysis of variance (ANOVA) showed that the effects of genotypes, G× E, and environments were all highly significant ($P < 0.001$) (Additional file 2: Table S2).

QTL for leaf rust resistance

Four QTL for leaf rust resistance were identified on chromosomes 2B (2), 5B, and 7B in the Zhongmai 578/Jimai 22 population, designated *QLr.caas-2B.1*, *QLr.caas-2B.2*, *QLr.caas-5B*, and *QLr.caas-7B*, respectively, explaining 3.7%–19.6% of the phenotypic variances (Table 2 and Additional file 1: Figure S2). The resistance alleles of *QLr.caas-2B.1*, *QLr.caas-5B*, and *QLr.caas-7B* were derived from Zhongmai 578, whereas that of *QLr.caas-2B.2* was contributed by Jimai 22. *QLr.caas-2B.1* was detected in Xinxiang 2022, Xinxiang 2023, and Zhengzhou 2023, with 6.1% to 16.4% of the phenotypic variances explained (PVE). *QLr.caas-2B.2* identified in Zhengzhou 2023, Baoding 2023, and Xinxiang 2022 explained 3.7% to 19.6% of the phenotypic variances. *QLr.caas-5B* was detected in Xinxiang 2022, Xinxiang 2023, and Zhengzhou 2023, with 4.9% to 7.8% of the PVE. *QLr.caas-7B* was identified in Xinxiang 2022 and best linear unbiased estimation (BLUE), accounting for 4.1%–7.8% of the phenotypic variances.

Table 1 Correlation analysis of maximum disease severities in the Zhongmai 578/Jimai RIL population among four environments

Environment	Baoding 2023	Zhengzhou 2023	Xinxiang 2023
Zhengzhou 2023	0.61**		
Xinxiang 2023	0.42**	0.45**	
Xinxiang 2022	0.40**	0.51**	0.60**

** indicates significant at $P < 0.01$

Additive effect of QTL for leaf rust resistance

To further understand the combined effects of alleles on leaf rust resistance, we examined the number of favorable alleles in each RIL. The number of favorable alleles in individual lines ranged from 0 to 4 in the Zhongmai 578/Jimai 22 population (Fig. 1). There were significant additive effects for leaf rust resistance among five groups. Lines with one, two, three, and four resistance alleles had mean MDS values of 41.0%, 34.4%, 29.7%, and 23.0%, respectively, whereas those with no favorable alleles had mean MDS values of 48.17%, significantly higher than the other four groups ($P < 0.05$).

Validation of KASP markers

To better utilize the QTL with stable and consistent effects for leaf rust resistance through molecular marker-assisted selection (MAS), we attempted to convert the closely linked single-nucleotide polymorphism (SNP) markers for *QLr.caas-2B.1* (*AX-95247569*), *QLr.caas-2B.2* (*AX-94760588*), *QLr.caas-5B* (*AX-95660479*), and *QLr.caas-7B* (*AX-109322846*) to KASP markers (Additional file 2: Table S3). Among these, *KASP-QLr.caas-2B.2* (*AX-94760588* for *QLr.caas-2B.2*) and *KASP-QLr.caas-5B* (*AX-95660479* for *QLr.caas-5B*) could distinguish two genotypes both in the Zhongmai 578/Jimai 22 RIL population and the natural population, while *KASP-QLr.caas-2B.1* (*AX-95247569* for *QLr.caas-2B.1*) and

Table 2 QTL for leaf rust resistance in the Zhongmai 578/Jimai 22 RIL population across four environments

QTL[a]	Marker interval	Nearest marker	Confidence interval (cM)	Genetic position (cM)	Physical position (Mb)	Xinxiang 2022		
						LOD[b]	R^2[c]	Add[d]
QLr.caas-2B.1	*AX-95652477*~*AX-95247569*	*AX-95659867*	0.6–29.8	13.0–22.2	10.7–12.8	10.2	13.1	−8.8
QLr.caas-2B.2	*AX-94760588*~*AX-86173872*	*AX-112286677*	83.8–121.4	95.5–101.2	110.4–189.6	2.7	3.7	5.1
QLr.caas-5B	*AX-110529435*~*AX-95660479*	*AX-108823934*	6.4–62.8	17.7–55.7	669.1–702.9	6.2	7.8	−6.9
QLr.caas-7B	*AX-109322846*~*AX-94460646*	*AX-109322846*	0–2.4	0–2.3	0.1–1.1	3.2	4.1	−5.4

QTL[a]	Xinxiang 2023			Zhengzhou 2023			Baoding 2023			BLUE		
	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add	LOD	R^2	Add
QLr.caas-2B.1	11.4	16.4	−8.7	4.3	6.1	−4.2				7.2	8.9	−2.6
QLr.caas-2B.2				5.6	8.6	5.4	15.5	19.6	3.4			
QLr.caas-5B	2.5	3.4	−4.4	3.0	3.2	−4.5				3.4	4.9	−1.9
QLr.caas-7B										5.8	7.8	−4.3

[a] QTL were detected with a LOD threshold of 2.5 for declaring significance based on 2000 permutations at $P < 0.05$

[b] Logarithm of odds score

[c] Percentage of phenotypic variances explained by the QTL

[d] Additive effect of resistance allele

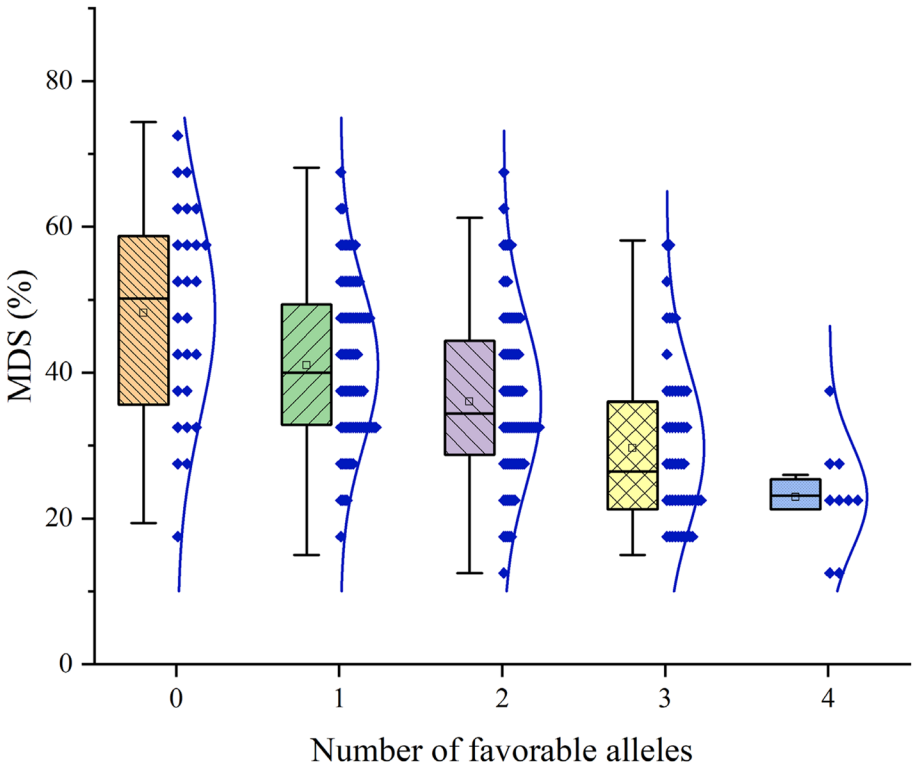

Fig. 1 The effects of favorable allele combinations on maximum disease severities (MDS) of leaf rust among different classes in the Zhongmai 578/Jimai 22 RIL population. The number of favorable alleles combined in each subset of RIL

KASP-QLr.caas-7B (*AX-109322846* for *QLr.caas-7B*) could not discriminate two genotypes. The results of the KASP markers and SNP chip were consistent, indicating the reliability of KASP markers.

Furthermore, using the KASP markers *KASP-QLr.caas-2B.2* and *KASP-QLr.caas-5B*, 262 RILs were divided into four groups: 2BR5BR, 2BS5BR, 2BR5BS, and 2BS5BS (Fig. 2). The results indicated that the genotypes with resistance alleles (2BR5BR) both at *QLr.caas-2B.2* and *QLr.caas-5B* exhibited a significantly lower MDS in all environments, as well as a lower BLUE value than the 2BS5BR, 2BR5BS, and 2BS5BS groups. Conversely,

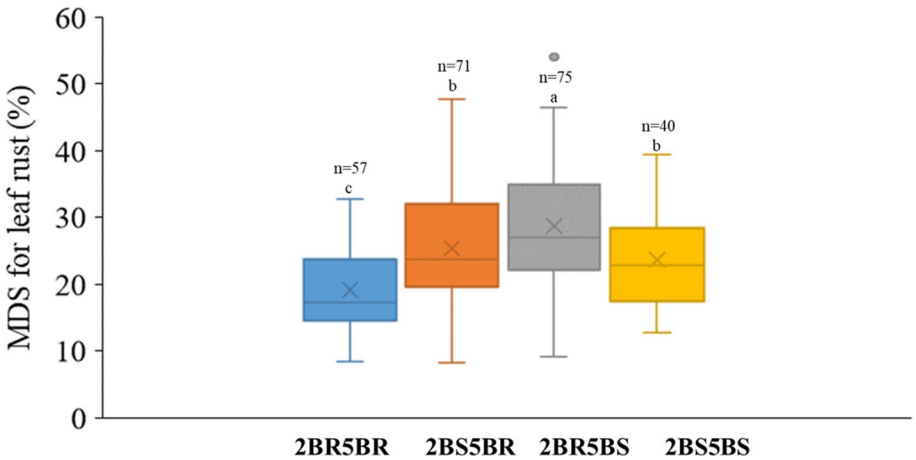

Fig. 2 Validation of KASP markers *QLr.caas-2B.2* and *QLr.caas-5B* in the Zhongmai578/Jimai22 RIL population. n means the number of lines. Different alphabets indicate significant difference at $P < 0.05$

those with both susceptibility alleles (2BS5BS) had a significantly higher MDS and BLUE value in all environments than the 2BR5BR, 2BS5BR, and 2BR5BS groups. Therefore, pyramiding the resistance alleles of *QLr.caas-2B.2* and *QLr.caas-5B* can effectively improve leaf rust resistance.

In the natural population with 119 cultivars, for *KASP-QLr.caas-5B*, the cultivars with resistance alleles at *QLr.caas-5B* had significantly higher resistance (92 lines with mean MDS 53.0) than those with susceptibility alleles (23 lines with mean MDS 63.4) in the natural population ($P < 0.05$) (Additional file 2: Table S4 and Fig. 3). Similarly, obvious differences in MDS between lines carrying the resistance allele (80 lines with mean MDS 50.8) and those possessing the susceptibility allele (15 lines with mean MDS 57.8) were also observed at *QLr.caas-2B.2* in the test of *KASP-QLr.caas-2B.2*. Moreover, the resistance alleles at *QLr.caas-2B.2* and *QLr.caas-5B* had high frequencies (67.2% and 77.3%, respectively) in the natural population.

Discussion

Since the 1970 s, CIMMYT has led groundbreaking research on APR to wheat rusts and effectively utilized this strategy to develop varieties with durable resistance that has proven effective for more than half a century (He et al. 2019b). To accelerate the breeding process for disease resistance in wheat, it is necessary to explore the genetic mechanisms of leaf rust resistance in more cultivars including Jimai 22 and Zhongmai 578, providing a reference for MAS for improvement of leaf rust resistance in wheat.

Comparison of QTL with previous reports

QLr.caas-2B.1 and *QLr.caas-2B.2*

Six known Lr genes, including *Lr13* (Dyck et al. 1966), *Lr16* (McCartney et al. 2005), *Lr23* (McIntosh and Dyck 1975), *Lr35* (Seyfarth et al. 1999), *Lr48* (Bansal et al. 2008), and *Lr73* (Park et al. 2014), and three QTL, *QLr.cimmyt-2BS* (Rosewarne et al. 2012), *QLr.hebau-2BS* (Zhang et al. 2017), and *QLr.hnau-2BS* (Hou et al. 2023), were detected on chromosome 2B. Among these, *Lr16*, *Lr23*, *Lr73*, and *QLr.cimmyt-2BS* are ASR genes and different from *QLr.caas-2B.1* and *QLr.caas-2B.2*. Dyck et al. (1966) identified an SSR marker (*Xgwm630*) significantly associated with *Lr13* on chromosome 2B (442.7 Mb). Bansal et al. (2008) reported closely linked markers *Xgwm429b-Xbarc07* for *Lr48* at 73.6–117.2 Mb on chromosome 2B. *Lr35* was mapped at 714.2 Mb on chromosome 2B and closely linked to *Xwg996*. *QLr.hebau-2BS* was closely linked with SSR markers *Xbarc55* (104.2 Mb) and *Xwmc474* (159.3 Mb), explaining about 8.5% of the phenotypic variance in the Zhou 8425B/Chinese Spring population. Hou et al. (2023) identified *QLr.hnau-2BS* in the Xinmai 26/Zhoumai 22 population, flanked by markers *AX-109943612* and *AX-109327476* (140.8–157.9 Mb), with 8.3%–18.2% of the PVE. Compared with previous studies, *QLr.caas-2B.1* flanked by *AX-95652477* (10.7 Mb) and *AX-95247569* (12.8 Mb) is likely a new QTL for leaf rust resistance based on its physical position. *QLr.caas-2B.2*, flanked by *AX-94760588* (110.4 Mb) and *AX-86173872* (189.6 Mb), is overlapped with *QLr.hebau-2BS* (Zhang et al. 2017) and *QLr.hnau-2BS* (Hou et al. 2023).

QLr.caas-5B

Aiay Kumar et al. (2013) identified two loci for leaf rust resistance, viz. *QLr.ccsu-5B.4* (*Xcdo1326* and *Xbarc140* at 595.4–598.2 Mb) and *QLr.ccsu-5B.5* (*Xbarc42* and *Xbarc69* at 605.6 Mb), explaining 8.4% and 7.3% of the phenotypic variances, respectively. Carpenter et al. (2017) identified two loci for leaf rust resistance on chromosome

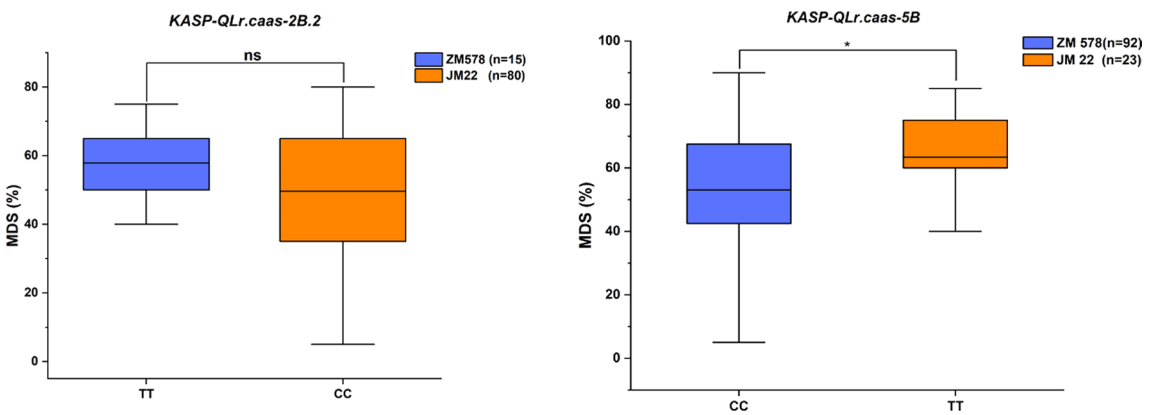

Fig. 3 Validation of QTL *QLr.caas-2B.2* and *QLr.caas-5B* in the panel of 119 wheat cultivars from the Huang-Huai River Valleys region. *, $P < 0.05$; ns, not significant

5B, i.e. *QLr.vt-5B.1* (*IWB7835* and *IWB24418* at 701.2 Mb) and *QLr.vt-5B.2* (*IWB32871* and *IWB26068*, at 656.8–671.2 Mb). In this study, *QLr.caas-5B* is flanked by *AX-110529435* and *AX-95660479*, located in the region from 669.1–702.9 Mb; it is overlapped with *QLr.vt-5B.2* (Carpenter et al. 2017). Interestingly, we found an APR locus (*QPm.caas-5BL*) for powdery mildew resistance in the Zhongmai 578/Jimai 22 RIL population, which was mapped at 662.03–670.12 Mb with flanking markers *AX_112287291* and *AX_95662216* (Zhao et al. 2023), and overlapped with *QLr.caas-5B*. This locus is likely a multiple disease resistance (MDR) QTL. There are 26 and 48 lines with no resistance alleles for leaf rust and powdery mildew, respectively, in the Zhongmai 578/Jimai 22 population, whereas 31 lines had only the resistance allele at *QLr.caas-5B* and 52 lines had only the resistance allele at *QPm.caas-5BL* in this population. The lines carrying resistance alleles at both *QLr.caas-5B* and *QPm.caas-5BL* had much lower MDS than those lacking any resistance alleles. In addition, Mu et al. (2019) reported *QYr.nwafu-5BL* for stripe rust resistance from Xinong1376, flanked by *IWB7873* (670.8 Mb) and *IWB13284* (672.6 Mb), with 10.0% of PVE. *QYr.spa-5B* in AC Cadillac was identified in the genetic interval 669.9–678.5 Mb (Bokore et al. 2017). *QYrid.ui-5B* in IDO444 was closely linked to *Xbarc59* (670.7 Mb) (Chen et al. 2012). Thus, *QLr.caas-5B* could be a pleiotropic APR gene for leaf rust, powdery mildew, and stripe rust.

QLr.caas-7B

Maccaferri et al. (2008) identified a leaf rust resistance locus, *QLr.ubo-7B.1*, closely linked to *Xgwm573* (91.4 Mb) on chromosome 7B in the Colosseo/Lloyd RIL population. Messmer et al. (2000) reported *QLr.sfrs-7B.1* (158.2 Mb), closely linked to leaf rust resistance with 12.8% of the PVE. *QLr.caas-7B* was tightly linked with *AX-109322846* and *AX-94460646* and located at 0.1–1.1 Mb, different from these loci. Thus, *QLr.caas-7B* is a new locus (Table 3).

Prediction of candidate genes for leaf rust resistance

Four candidate genes potentially associated with leaf rust resistance were identified in this study (Additional file 2: Table S5 and Additional file 2: Figure S3). *TraesCS2B03G0062000* for *QLr.caas-2B.1* encodes a lectin-receptor kinase protein. Wang et al. (2019) showed that orthologous genes at the *Rphq2* locus for quantitative leaf rust resistance from cultivated barley and *Rph22* from wild bulbous barley affect the host status to leaf rust. Both genes encode lectin receptor-like kinases. Overexpression of *Hv-LecRK* in the 'Golden SusPtrit' barley variety (a variety susceptible to non-host leaf rust) can significantly improve the rust resistance of barley. The expression level of *TraesCS2B03G0366400* for *QLr.caas-2B.2*, encoding an F-box/LRR protein, was up-regulated in flag leaves. It is generally acknowledged that resistance genes harboring LRR domains play a crucial role in modulating plant resilience against pathogens and insects (Yin et al. 2018). Yin et al. (2018) hypothesized that *Traes_4BS_C868349E1* could be a pivotal candidate gene underlying *Lr39* resistance to stripe rust, potentially activating wheat defense mechanisms through the regulation of hormone signaling pathways, including jasmonate and abscisic acid. *TraesCS5B03G1331100*, associated with the ankyrin repeat domain protein, is linked to *QLr.caas-5B*. Ankyrin, as an important regulatory factor, plays a significant role in immune signaling (Li et al. 2006). *TraesCS7B03G0000200* for *QLr.caas-7B* encodes a pleiotropic drug resistance ATP-binding cassette (ABC) transporter protein. In *Arabidopsis*, the ABC transporter protein encoded by the PEN3 gene localized to the plasma membrane and is postulated to contribute to the secretion of antifungal compounds into the

Table 3 Polymorphic KASP markers used in this study

SNP marker	KASP	Physical position (Mb) [a]	Primer name [b]	Sequence (5'to 3') [c]
AX-94760588	*KASP-QLr.caas-2B.2*	110.4	*KASP-QLr.caas-2B.2 A*	**GAAGGTGACCAAGTTCATGCT**gtcaccatcGgtttTggtcG
			KASP-QLr.caas-2B.2B	**GAAGGTCGGAGTCAACGGATT**gtcaccatcGgtttTggtcA
			KASP-QLr.caas-2B.2 C	caatcaacgagaccggcaG
AX-95660479	*KASP-QLr.caas-5B*	702.9	*KASP-QLr.caas-5BA*	**GAAGGTGACCAAGTTCATGCT**acccctcaagtctgagcttG
			KASP-QLr.caas-5BB	**GAAGGTCGGAGTCAACGGATT**acccctcaagtctgagcttA
			KASP-QLr.caas-5BC	gctgacaaggacctcgacg

[a] Physical positions (Mb) of markers were obtained by blasting SNP flanking sequences against the Chinese Spring genome sequence RefSeq v2.1 (Index of/download/iwgsc/IWGSC_RefSeq_Assemblies/v2.1 (inrae.fr))

[b] A and B indicate allele-specific primers; C indicates the common reverse primer

[c] FAM and HEX tails used for KASP marker assays are bolded

apoplast (Stein et al. 2006; He et al. 2019). The pleiotropic resistance gene *Lr34/Yr18/Sr57/Pm38* also encodes an ABC transporter in wheat (Krattinger et al. 2009).

Application in wheat breeding

Although traditional breeding has enhanced wheat resistance to diseases, the selection process remains time-consuming and less efficient due to the difficulties in field measurement of disease response. KASP markers provide a high-throughput, cost-effective, and flexible tool for MAS breeding. The four stable QTL identified in this study showed additive effects on leaf rust resistance when combined, suggesting the genetic complexity in regulating wheat leaf rust and the importance of pyramiding specific QTL. We succeeded in developing two KASP markers, *KASP-QLr.caas-2B.2* and *KASP-QLr.caas-5B.* The stable leaf rust resistance QTL *QLr.caas-5B* (*KASP-QLr.caas-5B*) and *QLr.caas-2B* (*KASP-QLr.caas-2B.2*) can be valuable tools for MAS in breeding programs. Additionally, several varieties with resistance alleles at *QLr.caas-2B.2* and *QLr.caas-5B* loci perform excellent agronomic traits, such as Huaimai 21 (MDS: 5), Xinmai9408 (MDS: 12.5), Xinmai 19 (MDS: 10), Zhoumai 13 (MDS: 12.5), and Lumai 15 (MDS: 35), can also be good parental lines for wheat breeding.

Conclusions

In this study, four QTL for leaf rust resistance were detected in the Zhongmai 578/Jimai 22 RIL population, explaining 3.7%–19.6% of the phenotypic variances. Among these, *QLr.caas-5B* is likely to be a multiple disease resistance QTL. Two KASP markers, *KASP-QLr.caas-2B.2* and *KASP-QLr.caas-5B*, were found to be significantly associated with leaf rust resistance in the RIL and natural populations. In addition, four candidate genes for these QTL were predicted. These findings offer valuable QTL and molecular markers for improvement of wheat leaf rust resistance in marker-assisted breeding.

Methods

Plant materials and phenotypic evaluation

Zhongmai 578 and Jimai 22 were inoculated with *Pt* pathotypes THTT, PHTT, THTS at the seeding stage following Li et al. (2010). The mapping population, derived from a cross between Zhongmai 578 and Jimai 22, consisted of 262 RILs. All RILs and parents were grown in Xinxiang (34°53′N, 113°23′E, Henan Province) during 2021–2022 and 2022–2023 cropping seasons, in Zhengzhou (34°48′N, 113°39′E, Henan Province) and Baoding (38°52′N, 115°29′E, Hebei Province) in the 2022–2023 season, hereafter designated as Xinxiang 2022, Xinxiang 2023, Zhengzhou 2023, and Baoding 2023, respectively. The field trials were conducted in randomized complete blocks with three replications in each environment. Each plot consisted of a single row, with 1.5 m in length and 0.2 m between rows, and 60 seeds were planted per row. Zhengzhou 5389, a highly susceptible cultivar to leaf rust, was planted as a control every 10 rows. Field management followed local agricultural practices as described by Liu et al. (2023).

A natural population comprising 119 cultivars, primarily from the YHRVWZ region in China, was employed to validate KASP markers. These accessions were grown in Baoding of Hebei Province, and Zhengzhou in Henan Province during 2020–2021 and 2022–2023 cropping seasons. The field trial was carried out in randomized complete blocks with three replications at each location. Each plot contained two rows, with 2 m in length and 20 cm between rows.

Xinxiang is a hotspot for leaf rust, with the prevalent physiological races of THTT, PHTT, and THTS in the field. Therefore, a natural inoculation was employed in Xinxiang 2022 and 2023. The mixture with equal amounts of *Pt* pathotypes THTT, PHTT, and THTS were used for field inoculation in Zhengzhou 2023 and Baoding 2023. At the beginning of April, wheat plants at the tillering stage were inoculated by spraying a mixture of THTT, PHTT, and THTS pathotypes each with equal proportion of urediniospores blended with a few drops of Tween 20 (0.03%) following Zhou et al. (2014). Four weeks after inoculation, leaf rust severities were scored 2–3 times at weekly intervals using the modified Cobb scale (Peterson et al. 1948). The MDS in each environment was used for subsequent data analysis and linkage mapping.

Statistical analyses

The ANOVA and correlation coefficients were performed using the AOV function of IciMapping v4.1 (http://www.isbreeding.net/). The H_b^2 of leaf rust severities was calculated using the formula $H_b^2 = \sigma^2_g/(\sigma^2_g + \sigma^2_{ge}/e + \sigma^2_\varepsilon/re)$, where σ^2_g, σ^2_{ge} and σ^2_ε are the variances of genotypes, genotype × environment interaction (G × E) and residual errors, respectively; e and r represent the number of environments and replications, respectively (Smith et al. 1998).

Linkage mapping

The 262 RILs and parents were genotyped using the Wheat 50K SNP chip (Li et al. 2021b). A high-density genetic map was constructed with 1506 backbone markers that represent 9354 polymorphic SNPs (Liu et al. 2023). QTL Cartographer v2.5 (http://statgen.ncsu.edu/qtlcart/WQTLCart.htm) was used to identify QTL associated with leaf rust resistance based on multi-environment data and BLUE values of the genes. The log of odds (LOD) threshold of 2.5 for declaring significance

of QTL was determined based on 2000 permutations, at $P < 0.01$. QTL identified in two or more environments were considered stable.

Development of KASP markers

Primers for KASP markers were designed using the PolyMarker website (http://www.polymarker.info/). Primer premixes were prepared following Yang et al. (2020), and the PCR procedure was conducted according to Li et al. (2021a). Fluorescence was detected using a PHERAstar Plus SNP (BMG Labtech GmbH, Ortenberg, Germany) and analyzed with KlusterCaller (LGC, Hoddesdon, UK).

Candidate gene prediction

Candidate genes were annotated based on the Chinese Spring reference genome (IWGSC RefSeq v2.1). The flanking sequences of SNP markers in the confidence interval of QTL were used as probes to search for candidate genes related to disease resistance by BLASTn in NCBI (http://www.ncbi.nlm.nih.gov/). The Wheat Expression Browser (http://www.wheat-expression.com/) was used to analyze gene expression patterns and select genes that are specifically and highly expressed in leaves. The steps for candidate genes selected are as follows. First, we identified all high-confidence annotated genes within the QTL regions based on IWGSC RefSeq v2.1. Second, considering the extensive research on wheat fungal diseases such as leaf rust, stripe rust, and powdery mildew, as well as the cloning of some adult plant resistance genes that can serve as references, we further refined candidate genes based on annotation information (e.g., lectin-receptor kinase proteins, F-box/LRR proteins, plant hormones, and ABC transporter proteins). Finally, using existing public databases, we determined the expression tissues of these candidate genes, prioritizing those expressed in leaves for subsequent studies.

Abbreviations

ABC	ATP-binding cassette
ANOVA	Analysis of variance
APR	Adult-plant resistance
ASR	All-stage resistance
BLUE	Best linear unbiased estimation
H_b^2	Broad-sense heritability
KASP	Kompetitive allele-specific PCR
LOD	Log of odds
MAS	Molecular marker-assisted selection
MDR	Multiple disease resistance
MDS	Maximum disease severity
Pt	*Puccinia triticina*
PVE	Phenotypic variances explained
QTL	Quantitative trait loci
RILs	Recombinant inbred lines
SNP	Single-nucleotide polymorphism
YHRVWZ	Yellow and Huai River Valleys Wheat Zone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00345-1.

Additional file1: Figure S1. Frequency distributions for maximum disease severities (MDS) in the Zhongmai 578/ Jimai 22 RIL population in four environments and BLUE values. **Figure S2.** LOD contours obtained by composite interval mapping of QTL for leaf rust resistance in the Zhongmai 578/Jimai 22 RIL population. **Figure S3.** Expression of candidate genes in different tissues of wheat.

Additional file2: Table S1. The maximum disease severities for the Zhongmai578/Jimai22 RIL population across four environments. **Table S2.** Analysis of variance of maximum disease severities in the Zhongmai 578/Jimai 22 RIL population across four environments. **Table S3.** The genotypes and maximum disease severities of 119 wheat cultivars used for validation of KASP markers. **Table S4.** Effects of QLr.caas-2B.2 and QLr.caas-5B on maximum disease severities in the natural population. **Table S5.** Candidate genes for the loci to leaf rust resistance in the Zhongmai 578/ Jimai 22 RIL population.

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Author contributions

YZ (Yue Zhang), YZ (Yong Zhang), and JL designed the research, analyzed the phenotype data, YZ (Yue Zhang) and JL drafted the manuscript. LL, FY, ZL, YT, SC, and YN performed the experiment. XX, SC, ZH, YZ (Yan Zhang), and JL revised the manuscript. All authors have read, edited and approved the current version of the manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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