Fine mapping of a recessive leaf rust resistance locus on chromosome 2BS in wheat accession CH1539

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Abstract Leaf rust (LR), caused by *Puccinia triticina* (*Pt*), is one of the most important fungal diseases of wheat worldwide. The wheat accession CH1539 showed a high level of resistance to leaf rust. A mapping population of 184 recombinant inbred lines (RILs) was developed from a cross between the resistant accession CH1539 and the susceptible cultivar SY95-71. The RILs showed segregating infection responses to *Puccinia triticina* Eriks. (*Pt*) race THK at the seedling stage. Genetic analysis showed that leaf rust resistance was controlled by a monogenic gene, and the potential locus was temporarily named *LrCH1539*. Bulked segregant analysis (BSA) using a 35 K DArTseq array located *LrCH1539* on the short arm of chromosome 2B. Subsequently, a genetic linkage map of *LrCH1539* was constructed using the developed 2BS chromosome-specific markers, and its flanking markers were *sxau-2BS136* and *sxau-2BS81*. An F₂ subpopulation with 3619 lines was constructed by crossing the resistant and susceptible lines selected from the RIL population. The inoculation identification results showed that *LrCH1539* was recessively inherited and was fine-mapped to a 779.4-kb region between markers *sxau-2BS47* and *sxau-2BS255* at the end of 2BS. The linkage marker analysis showed that the positions of *LrCH1539* and *Lr16* were the same, but the identification results of the resistance spectrum indicated that the causal genes of the two might be different. The resistant materials reported in this study and the cosegregation marker can be used for marker-assisted selection breeding of leaf rust-resistant wheat cultivars.

Keywords Wheat · Leaf rust · Seedling resistance · Fine-mapping

Introduction

Leaf rust, caused by *Puccinia triticina* Eriks. (*Pt*), is a worldwide disease of wheat (Huerta-Espino et al. 2011; Kolmer 2005). It occurs more frequently than other rusts and is more common worldwide (Ellis et al. 2014). In recent years, leaf rust has become an increasingly significant disease in the major wheat
production regions of China (Li et al. 2014; Zhang et al. 2020), and more than 15 million hectares of wheat are affected by leaf rust annually (Gao et al. 2019). The use of disease-resistant cultivars is the most efficient and environmentally friendly way to prevent yield losses from this disease worldwide (Pink 2002).

To date, 80 permanently named and numerous temporarily designated leaf rust resistance genes (Lr genes) and quantitative trait loci (QTLs) have been reported in wheat (Kumar et al. 2021). Although the number of designated Lr genes is increasing annually, new LR races with new virulence(s) that can overcome some of these Lr genes will likely occur (Ren et al. 2015). Lr genes such as Lr1, Lr3, Lr3bg, Lr10, Lr11, Lr14a, Lr16, and Lr26, which are common in Chinese wheat cultivars, have been nearly ineffective when applied alone (Gao et al. 2019; Li et al. 2010; Zhao et al. 2013). Therefore, to ensure the genetic resistance of wheat, it is essential to identify new or effective resistance genes in different germplasms of wheat varieties or related species worldwide.

Meanwhile, fully use the discovered resistance resources and improve the efficiency of breeding selection, it is necessary to develop more effective selection markers for resistance genes, which will also lay the foundation for cloning genes and studying their resistance mechanisms. Currently, only few race-specific seedling resistance genes, including Lr1 (Cloutier et al. 2007), Lr10 (Feuillet et al. 2003), Lr14a (Kolodziej et al. 2021), and Lr21 (Huang et al. 2003), and few adult plant resistance genes, including Lr13 (Hewitt et al. 2021; Yan et al. 2021), Lr22a (Thind et al. 2017), Lr34 (Krattinger et al. 2009), and Lr67 (Moore et al. 2015), have been cloned. The remaining large number of leaf rust resistance genes/QTLs have not been finely mapped and cannot be efficiently used in marker-assisted selection (MAS).

The wheat accession CH1539 developed by our laboratory has shown a high level of resistance to leaf rust in the field environment for many years. In this study, genetic analysis was performed on the resistance of the RIL constructed by CH1539 and a susceptible parent using the race THK. BSA combined with a wheat DArTseq array was used to determine the gene locus of resistance to leaf rust; a subpopulation was constructed to fine map this gene, and a cosegregation marker was developed to better use the germplasm.

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**Materials and methods**

**Plant materials and Pt races**

An RIL mapping population containing 184 F$_{2:10}$ lines was developed from the cross between the resistant accession CH1539 and the susceptible cultivar SY95-71. The accession CH1539 developed by the College of Agriculture, Shanxi Agricultural University (Shanxi Key Laboratory of Crop Genetics and Molecular Improvement) has a high level of resistance to leaf rust, and the wheat cultivar SY95-71 developed in the 1990s by the Wheat Research Institute of Sichuan Agricultural University is susceptible to prevalent Pt races in China. The susceptible control is Mingxian 169. Additionally, a high-resolution mapping population comprising 3619 F$_2$ plants was constructed by crossing resistant line #36 and susceptible line #4 of the RIL population. In order to determine whether LrCH1539 was dominant or recessive, 925 of 3619 F$_2$ plants were divided into three groups (F$_2$-1, F$_2$-2, F$_2$-3) and used to test the infection type in response to Pt race THK.

The wheat cultivar Selkirk was donated by Wentai Zhang, Gansu Academy of Agricultural Sciences, and RL6005 was provided by Dr. Minjie Liu, College of Plant Protection, Shanxi Agricultural University, China. A set of core germplasms containing 262 wheat varieties that are widely used in Chinese breeding programs (Chen et al. 2019) was used to detect the frequency of the LrCH1539 allele.

The pathotype of P. triticina was collected from the wheat-growing region in northern China through single spore separation and pure culturing. The Pt race was designated according to the system of Long and Kolmer (1989) and provided by Dr. Minjie Liu. A total of 31 races were used in the experiment (Table S1). The avir/vir formulas for THK were as follows: Lr3ka, Lr9, Lr13, Lr14b, Lr18, Lr21, Lr24, Lr25, Lr28, Lr29, Lr38/Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr3bg, Lr10, Lr11, Lr12, Lr14a, Lr15, Lr16, Lr17, Lr20, Lr22a, Lr22b, Lr23, Lr26, Lr30, Lr32, Lr33, Lr36, and Lr39.

**Testing for seedling reactions**

In the greenhouse, resistance identification of the tested material and the susceptible control Mingxian 169 was carried out using Pt races at the seedling
stage. Seeds were planted in a plastic growth chamber with five plants for each line and repeated twice. When the first leaves were fully expanded, inoculations were performed by dusting with urediniospores. Inoculated seedlings were subsequently incubated in the dark at 18 °C and 100% relative humidity (RH) for 24 h. The seedlings were then placed in a growth chamber at 16–21 °C and 70% RH. The infection types (ITs) were scored approximately 14 days later based on the 0–4 Stakman Scale modified by Roelfs et al. (1992). ITs: 0 = no visible symptoms; = necrotic or chlorotic flecks without any uredinia; 1 = small uredinia surrounded by necrosis; 2 = small to medium uredinia surrounded by chlorotic or necrosis; 3 = medium-sized uredinia without chlorosis or necrosis; 4 = large-sized uredinia without chlorosis or necrosis. “+” and “-” were used when uredinia were somewhat larger or smaller than normal for the ITs. ITs of 0–2 and 3–4 were considered resistant and susceptible, respectively (Long and Kolmer 1989).

A chi-square ($\chi^2$) test was used to determine whether the observed segregation ratio of the phenotypic data fits the expected genetic ratios. The $\chi^2$ analysis was performed in Microsoft Excel (version 2010) using the “chitest” function to calculate the $\chi^2$ and p-value.

DNA extraction and bulk segregant analysis (BSA)

The genomic DNA of all tested wheat lines was extracted using a modified CTAB method (Hill-Ambroz et al. 2002) and detected by 1% agarose electrophoresis. The concentration was measured, and the DNA samples were diluted to a final concentration of 50 ng/μL and stored at −20 °C for later use.

BSA was performed to determine the chromosomal location of leaf rust resistance in CH1539. Equal amounts of DNA from 21 homozygous resistant RILs (HR, ITs: 0–1) and 21 homozygous susceptible RILs (HS, ITs: 3–4) were pooled to constitute the respective resistant and susceptible bulks. The two parents and bulks were genotyped with the wheat 35 K DArT-seq array (Diversity Arrays Technology Pty Ltd). Polymorphic SNPs between parents were considered to be associated with $LrCH1539$ when the score values of resistant bulk and CH1539, susceptible bulk and SY95-71 were consistent. The sequences of DArTseq markers linked to resistance were blasted against the genome assembly of T. aestivum cv. Chinese Spring (CS) (International Wheat Genome Sequencing Consortium (IWGSC) RefSeq v1.0, https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/) (IWGSC 2018) to obtain their physical positions.

SSR marker assays and genotyping

Based on the results of polymorphic SNPs in the array, the specific-chromosome SSR primers were developed and named with the prefix “sxau” (Shanxi Agricultural University) followed by a consecutive number. A total of 157 designed markers and twelve markers linked with known $Lr$ genes were used to screen the parents and bulks to confirm their polymorphism before genotyping the entire RIL population, and the screened markers were then used to construct a linkage map. Another 120 specific chromosome markers were also designed for fine mapping $LrCH1539$. Information of the markers located on the linkage map in this paper is listed in Table S2.

PCR amplification was performed on a C1000 Touch thermal cycler. The PCR amplification reaction mixture volume was 10 μL: 5.0 μL of 2×Taq PCR Master Mix (Tiangen Biochemical Incorporation, Beijing), 2.0 μL of ddH2O, 1.0 μL (2 mmol/μL) of each primer, and 1.0 μL (50 ng/μL) of DNA template. PCR amplification program: pre-denaturation at 94 °C for 5 min; denaturation at 94 °C for 30 s, renaturation at 55–68 °C (determined by the annealing temperature of each primer) for 30 s, extension at 72 °C for 60 s, a total of 35 cycles; final extension at 72 °C for 10 min and preservation at 12 °C. The PCR amplification products were detected by electrophoresis through 8% nondenaturing polyacrylamide gels (the mass ratio of Acr to Bis was 29:1) for 50–70 min. After silver nitrate staining and formaldehyde solution dyeing, the results were photographically observed.

Genetic linkage map and gene annotation

The linkage map for the CH1539/SY95-71 RIL population was constructed using the JoinMap v4.0 software (www.kyazma.nl), and the Kosambi map function was used to convert recombination rates to centimorgans (Kosambi 1943). A logarithm of odds (LOD) of 3.0 was set to declare genetic linkages.
MapDraw V2.1 was used to draw the linkage map (Liu and Meng 2003).

The flanking markers were subjected to BLAST against IWGSC RefSeq v1.0 to obtain the physical location of the target gene. Then, gene annotation of the refined LrCH1539 interval was retrieved from the above mentioned IWGSC RefSeq v1.0. Expression data for the genes in leaf tissues and under pathogen treatment were obtained using the Wheat Expression Browser database (http://www.wheat-expression.com, Ramírez-González et al. 2018).

Result

The reaction of parents and RILs to Pt races

The parents CH1539 and SY95-71 and the RIL population were evaluated for their reactions to the Pt race THK at the seedling stage. CH1539 was highly resistant to THK with IT of ;–2 (Fig. 1); however, SY95-71 was highly susceptible to THK with IT of 4 (Fig. 1). The susceptible Mingxian 169 developed large-sized uredinia without chlorosis (Fig. 1). Meanwhile, the RILs were segregated for their reaction to THK, and the reactions ranged from highly resistant (IT =1) to highly susceptible (IT =4). Of the 184 RILs evaluated, 86 (two missing data) and 91 lines were resistant, and 96 and 93 lines were susceptible in the two duplications, respectively. The segregation of resistant and susceptible RILs fit a single gene segregation ratio of 1:1 (Table 1). Through the phenotypic analysis of the three groups of F2 subpopulations, the separation of resistant lines and susceptible lines conformed to a separation ratio of 1:3 (Table 1), indicating that the resistance of CH1539 to Pt race THK is controlled by a single recessive gene, tentatively named LrCH1539.

Molecular mapping of LrCH1539

A total of 106 SNPs showed polymorphisms between the DNA bulks after genotyping by the 35 K DArT-seq array. According to the physical location of the polymorphic SNPs, 60 (56.6%) of these SNPs were located on chromosome 2B, and no more than 11 (10.4%) were located on other single chromosomes (Fig. 2A). These results indicated that SNPs in 2B were extremely likely to be associated with the resistance locus.

For the polymorphic SNPs distributed on chromosome 2B, the average number of polymorphic SNPs contained in each 10 Mb was calculated using 10 Mb as a sliding window. The obtained results showed that the most polymorphic SNPs in the physical range of 0–20 Mb accounted for more than 78.3% (47) of polymorphic SNPs on 2B (Fig. 2B).

| Group            | Resistance reaction | Theoretical ratio (R:S) | \( \chi^2 \) | P value |
|------------------|---------------------|-------------------------|--------------|---------|
| RILs-rep1        | 86                  | 96                      | 1:1          | 0.71    | 0.60   |
| RILs-rep2        | 91                  | 93                      | 1:1          | 3.00    | 0.91   |
| F2-1             | 38                  | 128                     | 1:3          | 0.88    | 0.65   |
| F2-2             | 126                 | 370                     | 1:3          | 2.47    | 0.88   |
| F2-3             | 64                  | 199                     | 1:3          | 2.17    | 0.86   |

The infection type (IT) was scored in a 0–4 scale (Roelfs et al. 1992). IT 0–2, resistant; IT 3–4, susceptible. \( \chi^2 \) (0.05, 1) = 3.841
Therefore, it is speculated that there is a leaf rust resistance site at the end of the short arm of chromosome 2B.

Chromosome-specific SSR markers in the region were developed and then screened on the parents and bulks to confirm polymorphisms before being genotyped on the entire population; 35 polymorphic markers were successful in distinguishing the contrasting parents and bulks. Among the 12 markers linked to known Lr genes, one KASP and two SSR markers linked to Lr16 were also polymorphic between parents and bulks. A genetic map was constructed using one KASP and 16 SSR markers genotyped on the 184 F2:10 individuals, resulting in a linkage group spanning 16.0 cM. LrCH1539 was preliminarily located between the SSR markers sxau-2BS81/Xwmc764 and sxau-2BS136 in an interval of 1.1 cM and coseparated with sxau-2BS47 and 2BS-5175914_kwm849 (Fig. 3). According to the physical position of the markers sxau-2BS81 and sxau-2BS136 in the CS1.0 reference genome, LrCH1539 was located in the 2.4 Mb region between 5.7 and 8.1 Mb (Fig. 3).

Fine mapping of LrCH1539

To narrow down the region containing LrCH1539, we generated 3619 F2 individuals to screen for new crossovers between sxau-2BS81 and sxau-2BS136, and 33 crossovers were identified. Among the 33 crossovers, 12 showed recombination between the marker sxau-2BS136 and LrCH1539, while 21 showed recombination between the marker sxau-2BS81 and LrCH1539. Based on the 2.4 Mb interval of CS RefSeq v1.0, more primers were designed and tested on the contrasting parents and bulks. Four markers (sxau-Q2BS3, sxau-Q2BS5, sxau-2BS210, and sxau-2BS255) were polymorphic and used with sxau-2BS47 to examine 33 crossovers. The obtained results indicated that the closest flanking markers of LrCH1539 were sxau-2BS47 (with one recombination event) and
sxau-2BS255 (with six recombination events), and the marker cosegregating with \( LrCH1539 \) was sxau-2BS210 (Fig. 4). These results suggest that the \( LrCH1539 \) locus is located in a 779.4 kb region between markers sxau-2BS47 and sxau-2BS255 (6,226,584 bp–7,005,940 bp) in CS RefSeq v1.0.

Comparison with reported \( Lr \) genes in chromosome 2B

The linked markers of the \( Lr \) gene reported on chromosome 2BS were used to detect contrasting parents and bulks (Table 2, Fig. S1). The obtained
results showed that no polymorphisms of \(Xgwm630\), \(Xbarc55\), \(Xbarc7\), \(sun471\), and \(Sr39F2/R3\) were observed between CH1539 and SY95-71, and the characteristic band of \(Sr39F2/R3\) was not amplified. \(Xwmc770\), \(Xgwm374\), \(Lr13STS1\), and \(Xgwm429b\) were polymorphic between parents, but they were not polymorphic between R-bulk and S-bulk. These results suggested that these markers were not linked to \(LrCH1539\).

\(LrCH1539\) has a genetic distance of 0.8 cM from \(Xwmc764\), cosegregated with KASP marker 2BS-5175914\_kwm849 (Fig. 3), and has a linkage relationship with CAPS markers \(kwm747\) transformed from KASP markers 2BS-5175914\_kwm849 and 2BS-5194460\_kwm747, respectively (Fig. S1).

Resistance spectrum analysis of \(LrCH1539\) and \(Lr16\)

The wheat accessions CH1539 and RL6005 showed different disease responses to 31 Chinese \(Pt\) races (Fig. 5, Table S1). Twelve of 31 \(Pt\) races, including DHK, FHK, FKT, KHJ, PGL, PHS, PKJ, PKT, PRK, PTK, THK, and TKK, were avirulent to \(LrCH1539\) but virulent to \(Lr16\) (Fig. 5). The race PBB was avirulent to \(Lr16\) but virulent to \(LrCH1539\).
Expression profiles of genes in the candidate region

*LrCH1539* was mapped to 6,226,584–7,005,940 bp on chromosome arm 2BS (Chinese Spring Ref-Seq v.1.0). There were 42 annotated genes in this region, including 14 low confidence genes and 28 high confidence genes (IWGSC 2018). To predict the *LrCH1539* gene, we analyzed the expression profiles of genes in the candidate region after being induced by pathogens using the wheat expVIP expression platform (Ramírez-González et al. 2018). Since symptoms of LR resistance begin from the early seedling stage and are maintained to the adult stage, we hypothesized that the *LrCH1539* allele should be expressed in leaves throughout the entire growth period. Nine of 42 candidate genes were expressed (above two transcripts per million) in at least ten RNA-seq samples (leaves and stress-disease, *n* = 99) at different developmental stages (Fig. 6). Eight of the nine expressed genes were high-confidence genes, which may be related to plant disease resistance.

Distribution of the *LrCH1539* allele in wheat varieties

The cosegregating marker *sxau-2BS210* of *LrCH1539* had genotyped 262 varieties which are widely used as core germplasms in Chinese breeding programs. Only 12 (4.6%) of these varieties showed the presence of the resistant CH1539 allele, and the rest showed the presence of the susceptible SY95-71 allele (Table S3). These 12 wheat varieties include two Italian varieties (Funo and St2422/464) and ten Chinese varieties (Laomai, Hongmangmai, Yangmai158, Kelao 4, Jinan 17, Xiaoyan 6, Shannong 7859, Fan 6, Zhengmai 9023, Yanzhan 1) (Fig. 7).

Discussion

The wheat accession CH1539 is a valuable source of genetic variation for biotic resistance, such as powdery mildew and stripe rust resistance (data not shown), and has a high level of resistance to leaf rust in the field. In this study, the recessive seedling resistance gene *LrCH1539* was characterized on the short arm of chromosome 2B in CH1539.

Previous studies have identified several permanently and tentatively designated *Lr* genes on chromosome 2BS, including *Lr13, Lr16, Lr23, Lr35, Lr48, Lr73, LrZH22*, and *LrA2K*. Based on the integrated genetic maps (Maccaferri et al. 2015), the markers flanked or linked with the reported genes were at least 17.6% RD (relative distance) from Xwmc764 and Xwmc661, which were flanked to *LrCH1539* (Fig. 8A). Meanwhile, there was no linkage relationship between these markers and *LrCH1539* (Table 2, Fig. S1), which indicates that the genes *Lr13, Lr23, Lr48, LrZH22*, and *LrA2K* are distinct from *LrCH1539*. *Lr35* is an adult-plant resistance gene located on a translocation chromosome fragment extracted from *Aegilops speltoides*.
(Gold et al. 1999). The selection marker Sr39F2/R3 of Lr35 could not be amplified in CH1539, and there is no Triticum speltaoides in the pedigree of CH1539. Therefore, LrCH1539 cannot be Lr35.

The dominant gene Lr73 is located between XwPt-4453 and XwPt-8760 on 2BS (Park et al. 2014), and its genetic position overlaps with LrCH1539 (Fig. 8A). The gene Lr73, sometimes referred to as the “fossil” gene, only exists in Morocco and some other Australian wheat cultivars (Park et al. 2014); thus, it is considered not to exist in the Chinese wheat background. Furthermore, unlike Lr73, LrCH1539 in this study is a recessive gene.

The molecular markers coseparated with Lr16 are also linked or coseparated with LrCH1539. Meanwhile, on the physical map, LrCH1539 is included in the region of Lr16 (Fig. 8B). CH1539, RL6005, and Selkirk were genotyped and analyzed with
markers sxau-2BS210, sxau-2BS255, sxau-2BS47, sxau-Q2BS3, sxau-Q2BS5, kwm847, and kwm747 (Table S4). According to the genotyping results of three genotypes with seven markers, CH1539, RL6005, and Selkirk had the same haplotype (combination of marker alleles). Therefore, LrCH1539 and Lr16 may be in the same chromosome interval.

However, the original sources of Lr16 are believed to be five wheat cultivars, Warden, Exchange, Selkirk, Etoile de Choisy, and Columbus (Harrison et al. 2015), while the pedigree of CH1539 does not correlate with Lr16. CH1539 and RL6005 produced different LR resistance responses to 13 Pt races, including THK. It is assumed that the differences in the response of CH1539 and RL6005 to leaf rust are not only due to differences in the genetic background but may also result from differences in the candidate genes for LrCH1539 and Lr16.

The wheat core germplasms were genotyped using the cosegregating marker sxau-2BS210 of LrCH1539; the CH1539 allele was detected in 12 cultivars, and the remaining 250 cultivars were the SY95-71 allele. Of the 12 cultivars, Funo and St2422/464 are Italian varieties introduced in China in the 1950s and 1970s, respectively, and have been widely used as parents in breeding programs. Five of eight Chinese wheat varieties, Yangmai 158, Xiaoyan 6, Fan 6, Zhengmai 9023, and Yanzhan 1, all have Funo and/or St2422/464 genetic backgrounds (Wu et al. 1993; Xu et al. 2009; Yuan et al. 1981; Zou 1991). The remaining two varieties, Laomai and Hongmangmai, are Chinese landraces. The former is distributed in Shaanxi Province, China, and the latter is distributed in Shandong, Hebei, and Shanxi provinces. In summary, the CH1539 allele of LrCH1539 is distributed over a wide range but at a very small frequency, representing only 4.6% of the tested germplasms. Among the 12 detected germplasms, three (25%) had a “Funo” genetic background, and four (33.3%) had a “St2422/464” genetic background. Therefore, the CH1539 allele of LrCH1539 has good application potential.

Among the nine genes in the candidate region that were significantly expressed in leaves and shoots under disease conditions, we noticed that three genes were described as related to plant disease resistance. Gene annotation of the corresponding region in Chinese Spring revealed that the gene TraesCS2B01G012400 encodes the Avr9/Cf-9 rapidly elicited protein, which is a protein produced after the plant resistance gene Cf-9 recognizes pathogens and plays a pivotal role during plant defense responses (Rowland et al. 2005; van den Burg et al. 2008). The gene TraesCS2B01G012600 encodes the StAR-related lipid transfer protein (LTP), and LTP has been classified as a member of the pathogenesis-related (PR) proteins belonging to the PR-14 group (Van Loon and Van Strien 1999). Overexpression of LTP genes enhances resistance to plant pathogens and plays an important role in plant long-distance systemic signaling in tobacco (Sarowar et al. 2009). McLaughlin et al. (2015) found that LTP can increase the glutathione content and enhance Arabidopsis resistance to a trichothecene mycotoxin; Kirubakaran et al. (2008) identified a new antifungal lipid transfer protein from wheat. Li et al. (2006) used the transient overexpression method to study the role of cloned LTP1 in wheat-powdery mildew interactions, and the obtained results indicated obvious effectiveness of LTP1 in powdery mildew resistance. The gene TraesCS2B01G012800 encodes a peptidyl-prolyl cis–trans isomerase. The activity domain of this enzyme is a common feature of immunophilins that are ubiquitous in organisms (Fisher et al. 1989). Pogorelko et al. (2014) characterized three Arabidopsis thaliana immunophilin genes involved in the plant defense response against Pseudomonas syringae, and the research showed that Arabidopsis knock-out mutations in these immunophilins result in an increased susceptibility to P. syringae, whereas overexpression of these genes alters the transcription profile of pathogen-related defense genes and led to enhanced resistance. These genes are essential in the biotic stress response of plant resistance to pathogens, and related experiments will be performed on them to screen for candidate genes of LrCH1539. Since LrCH1539 is recessive, we will focus on the loss-of-function gene which enhance resistance, like TaMlo (Wang et al. 2014), as candidate within the target region.

Conclusions

A recessive seedling stage LR resistance gene, LrCH1539, located within a 779.4 kb physical region
(6,226,584 bp–7,005,940 bp) on 2BS, was characterized in wheat accession CH1539, and the cosegregating marker sxau-2BS210 was developed. LrCH1539 and Lr16 are at the same position on the chromosome but differ in their resistance spectra.

**Author contribution** DS performed the experiments; DS and LQ analyzed the data, carried out the bioinformatics work, and wrote the manuscript; XZ and ZC conceived and supervised the experiments; XZ and XL administrated the project; LC, HG, SZ, and FC investigated the phenotype; ZC revised the manuscript. All authors have read and approved the final manuscript.

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**Declarations**

**Ethics approval** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

**Conflict of interest** The authors declare no competing interests.

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