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Genetic mapping of the wheat leaf rust resistance gene Lr19 and development of translocation lines to break the linkage with PSY-E1

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Abstract

The *Lr19* locus, which was transferred to wheat from the wheat relative *Thinopyrum ponticum* in 1966, still confers broad resistance to most known leaf rust isolates worldwide. However, this gene has not been fine mapped to day, and its tight linkage with the yellow pigment gene *PSY-E1* has limited the deployment of *Lr19* in bread wheat breeding. In this paper, we genetically mapped *Lr19* using a bi-parental population obtained by crossing two wheat-*Th. ponticum* substitution lines, 7E1(7D) (carrying *Lr19*) and 7E2(7D) (susceptible to leaf rust). Genetic analysis of the F_2 population and $F_{2:3}$ families showed that *Lr19* is a single dominant gene, and it was successfully mapped within a 0.3 cM interval on the long arm of the *Th. ponticum* chromosome 7E1, flanked by markers *XsdauK3734* and *XsdauK2839*. To reduce the size of the *Th. ponticum* chromosome segment carrying *Lr19*, the Chinese Spring *Ph1b* mutant was employed to promote recombination between the homoeologous chromosomes of the wheat chromosome 7D and 7E1. Two translocation lines with short *Th. ponticum* chromosome fragments carrying *Lr19* were identified using markers closely linked to *Lr19*, and in one of the lines the linkage between *Lr19* and *PSY-E1* was broken. Both translocation lines were resistant to 16 *Pt* isolates collected from China. Thus, the *Lr19* linkage markers and translocation lines developed in this study can be used for marker-assisted selection in common wheat breeding programs.

Key Message

The leaf rust resistance gene *Lr19* on the long arm of chromosome 7E1 was mapped within a 0.3 cM genetic interval, and translocation line were developed to break the linkage with *PSY-E1*.

Introduction

Wheat (*Triticum aestivum L.*) is one of the most important crops for human nutrition, with an average production of ~ 3.47 tons per hectare from 2016 to 2020 (Rollar et al. 2021; FAO 2022). However, wheat production worldwide is threatened by leaf rust caused by *Puccinia triticina* (*Pt*), which is the most destructive and widespread rust in wheat growing regions, causing yield losses higher than 1% globally (Savary et al. 2019) and even up to 70% for susceptible cultivars (Aktar-Uz-Zaman et al. 2017). The most efficient and eco-friendly method to reduce the threat of wheat leaf rust is developing resistant cultivars (Xu et al. 2022).

To date, 108 genes conferring resistance to wheat leaf rust have been formally catalogued (Dinh et al. 2020; Rothwell et al. 2020; Kumar et al. 2021; Xu et al. 2022). Almost half of these leaf rust resistance (*Lr*) genes are derived from wild relatives (Prasad et al. 2020). Among the 108 known *Lr* genes, only 11 have been cloned (Feuillet et al. 2003; Huang et al. 2003; Cloutier et al. 2007; Krattinger et al. 2009; Moore et al. 2015; Thind et al. 2017; Dracatos et al. 2019; Wang et al. 2019; Dinh et al. 2022). In addition, along with the evolution of *Pt*, some *Lr* genes have lost the ability to confer resistance to leaf rust (Bolton et al. 2008). Therefore, searching for new sources of *Lr* genes and combining effective *Lr* genes is necessary to protect wheat against leaf rust (Sibikeev et al. 1996; Xu et al. 2022).

One example of an effective *Lr* gene is *Lr19*, which was transferred to wheat from *Thinopyrum ponticum* in 1966 (Knott and Sharma 1966). So far, there are no *Pt* races which are virulent to the leaf rust resistance gene *Lr19* in many parts of the world (Zhang et al. 2022), although some new pathogen races have overcome the *Lr19*-mediated resistance in Mexico, Russia, Ukraine, and India (Sibikeev et al. 1996; Elyasi-Gomari and Panteleev 2006; Xiao et al. 2016). Because the chromosomes between *Th. ponticum* and common wheat do not pair normally, Knott and Sharma used irradiation to translocate a *Th. ponticum* chromosome segment carrying *Lr19* from a wheat-*Th. ponticum* substitution line to wheat and produced four translocation lines (Knott and Sharma 1966). In the fourth translocation line (T4), referred to as Agatha (K11695), the long arm of chromosome 7D was replaced with the homoeologous chromosome of *Th. ponticum* (Knott and Dvořák 1977a; Kim et al. 1993). However, T4 still has a large alien segment that does not completely pair with the wheat chromosome, and it carries a large linkage block (Marais and Marais 1990). Marais (1992) used the Chinese Spring *Ph1b* mutant (CS *ph1bph1b*) to create a new line with a shorter translocation segment designated as *Lr19*-149 (Prins et al. 1997). And Marais et al. (2001) obtained four secondary translocations using *Lr19-149*, but the size of the alien segment remains unknown.

Since the chromosomes from *Th. ponticum* and wheat do not pair normally (Knott and Sharma 1966), a genetic map of the *Lr19* locus on chromosome 7E1 has not been well established (Zhang et al. 2011). A series of deletion maps of *Lr19* have been developed using 29 mutants with deletions in the translocated chromosome segment containing *Lr19* (Prins et al. 1996; Prins and Marais 1998). Although the deletion map contains molecular markers surrounding *Lr19*, the order of these markers is still unknown (Groenewald et al. 2005). In addition, the *Th. ponticum* chromosome carrying *Lr19* also contains many other genes, such as *PSY-E1* and the wheat stem rust resistance gene *Sr25* (Knott 1984). The *PSY-E1* gene causes a distinctly yellow flour, which is undesirable in bread wheat breeding and limits the use of *Lr19* (Knott 1980). To remove the yellow pigment, Knott (1980) produced two mutants with low pigment levels using ethyl methane sulfonate (EMS). Although the yellow pigment level was reduced, the alien chromosome segments is still large and confers many detrimental agronomic traits in these mutants. Identification of recombinants in the *Lr19* locus is necessary to identify the causal gene and to separate it from loci responsible for undesirable traits. This is particularly important given the potential of *Lr19* to not only confer resistance against *Pt* but also improve yield (Rosewarne et al. 2015) and enhance quality traits (Rai et al. 2019).

The objectives of this study were to (1) map the *Lr* gene *Lr19* and develop markers closely linked to *Lr19*, (2) break the linkage between *Lr19* and *PSY-E1* and create wheat-*Th. ponticum* translocation lines with short alien fragments using marker assisted selection.

Materials And Methods

Plant materials

The F_1 (n = 15) hybrids, F_2 (n = 1273) segregating populations, and $F_{2:3}$ family were developed by crossing the wheat-*Th. ponticum* substitution line 7E1(7D) carrying *Lr19* to another wheat-*Th. ponticum* substitution line 7E2(7D) (Knott et al. 1977b), which is susceptible to leaf rust, for genetic analysis and mapping of the leaf rust resistance gene *Lr19*. The population constructed from 7E1(7D) and 7E2(7D) was previously used to clone the fusarium head blight resistant gene *Fhb7* located on 7E2 (Wang et al. 2020).

A wheat-*Th. ponticum* Robertsonian translocation line carrying *Lr19*, in which the long arm of chromosome 7E1 replaced the long arm of chromosome 7D, designated as K11695 (Kim et al. 1993), was crossed with CS *ph1bph1b* to induce homoeologous recombination. Wild emmer AS15 (AABB), *Aegilops tauschii* 2147 (DD), and the common wheat cultivar Jimai22 were used to determine the specificity of the designed markers. *Th. ponticum* was used as the source of DNA probe for fluorescent genomic in situ hybridization (GISH), while wheat cultivar Chinese Spring (CS) was used as block DNA.

Development of Lr19**-carrying lines with shorter alien chromosome segments**

The original Robertsonian translocation line K11695 was crossed to CS *ph1bph1b*. The F_1 plants were self-pollinated. The F_2 population was genotyped with *Ph1* molecular markers and *Lr19* linkage markers to select individuals that were homozygous for *ph1b* while heterozygous for *Lr19*. The selected F_2 plants were self-crossed. By performing *Lr19* linkage marker analysis, the progenies derived from the heterozygous plants with potentially shortened *Th. ponticum* chromosome segments were tested for leaf rust resistance (as described in “Leaf rust resistance evaluation”) and analyzed using genomic in situ hybridization (GISH) (as described in “Cytogenetic analysis”) to determine the physical size of the shortened alien chromosome segment.

Leaf rust resistance evaluation

A *Pt* isolate, 12-15-32 (provided by Prof. Wenxiang Yang at Hebei Agricultural University), was used to test the resistance of the parents (7E1(7D), 7E2(7D), K11695 and CS *ph1bph1b*), the progenies of the mapping populations, and the wheat lines with shortened alien chromosome segments. At least 16 plants from each material were tested to confirm the phenotypes. Two-week-old seedlings were used to test the resistance to leaf rust. The inoculated seedlings were placed at 21℃ and 100% relative humidity for 12 h in darkness and then transferred to a greenhouse set at 22 ± 2℃ with a humidity of 70% and illumination for 12h. The infection types were recorded using the 0 to 4 Stakman scale modified by Dakouri et al. (2013) at 14 days post inoculation. According to the 0 to 4 Stakman scale: 0 = no flecks or uredinia, ; = hypersensitive flecks, 1 = small uredinia with necrosis, 2 = small uredinia with cholorsis, 3 = moderate size uredinia, 4 = large uredinia.

Sixteen *Pt* isolates collected from China were used to test the resistance spectrum of *Lr19*. This work was conducted in Wenxiang Yang’s lab at Hebei Agricultural University.

Cytogenetic analysis

The chromosomes were prepared for GISH and fluorescence in situ hybridization (FISH) following the method described by Kato et al. (2004). The total genomic DNA of *Th. ponticum* was used as a probe, while the genomic DNA from CS was used as block DNA. GISH analysis was performed as described by Li et al. (2021). FISH analysis was performed as described by Huang et al. (2018) using TAMRA (6-carboxytetramethylrhodamine)-labeled oligonucleotides pAs1-1, pAs1-3, pAs1-4, pAs1-6, AFA-3, and AFA-4 and FAM (6-carboxyfluorescein)-labeled oligonucleotides pSc119.2-1 and $(GAA)_{10}$ as probes. The chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI), and images were captured with a fluorescence microscope (Olympus BX60, Tokyo, Japan) equipped with a charge-coupled device camera.

DNA extraction, molecular marker analysis, and statistical analysis

Genomic DNA extraction from the parents and progeny from the aforementioned crosses (described in “Plant materials”) was performed using a standard CTAB DNA extraction method as described by Huang et al. (2000). The DNA concentration and quality were assessed using a NanoDrop 2000 spectrophotometer and electrophoresis on 1.0% agarose gels.

Molecular marker analysis was performed to construct a genetic map of *Lr19*, identify homozygous *ph1b* F_2 plants, and select the homoeologous recombinants induced by *ph1b*. The parents 7E1(7D), 7E2(7D), K11695, and CS *ph1bph1b* were analyzed for polymorphisms using the simple sequence repeat (SSR) markers and expressed sequence tag (EST) markers described in Zhang et al. (2011) and Guo et al. (2015). Three markers, *AWJL3*, *PSR128*, and *PSR574*, used to detect the *ph1b* allele in the F_2 plants were amplified using multiplex touchdown PCR (Roberts et al. 1999). PCR amplification was carried out using a Bio-Rad T100™ Thermal Cycle in a 10μl reaction volume containing 100 ng genomic DNA, 10μM forward primer, 10μM reverse primer, and 5μl 2 ×Taq Mix (Vazyme, P111-01), with the remaining volume consisting of ddH_2O. The PCR program was as follows: one cycle for 5 min at 95℃, 35 cycles for 30 s at 94℃, 55–60℃ (depending on the Tm) for 30 s, 72℃ for 1 min, and a final extension at 72℃ for 10 min. The PCR products were separated on an 8% non-denaturing polyacrylamide gel or 1% agarose gel.

For investigating the mode of inheritance of *Lr19*, Chi-square (χ^2) tests were used to determine if the observed data deviated from the expected segregation ratio. A genetic linkage map was constructed with JoinMap 4.0 using the Kosambi function to calculate map distance and a LOD score of 3.0 as the threshold for linkage. The linkage map was drawn using MapDraw V2.1.

Pigment content analysis

To determinate the lutein concentration in flour, a flour sample (2–3 g) was mixed with 15 ml of extracting solution (N-hexane : acetone : ethanol [2:1:1] with 0.01% butylated hydroxytoluene) in a 50 ml centrifuge tube using ultrasonic concussion for 30 min, and then centrifuged at 4000g for 10 min. The supernatant was transferred to a new 50 ml centrifuge tube and the residue was re-extracted repeating the steps above till the supernatant was colorless. The supernatant was collected and dried by N_2, and

the dried precipitate was used to determine the concentration of lutein by liquid chromatography. The content of lutein calculated as followed: Content (μg/g) = Concentration/(Weight*0.8). This work was conducted in collaboration with Dr. Xuebin Zhang at Henan University.

Results

Genetic analysis of resistance to leaf rust

The parental line 7E1(7D) carrying *Lr19* was resistant to *Pt* 12-15-32 with an infection type of 0 according to the Stakman scale, while 7E2(7D) was highly susceptible with an infection type of 4 (Fig. S1). Genetic analysis was carried out to investigate the inheritance mode of leaf rust resistance in the parents (7E1(7D) and 7E2(7D)) and in the F_1 hybrids and F_2 and $F_{2:3}$ populations derived from a 7E1(7D)/7E2(7D) cross challenged with the isolate *Pt* 12-15-32. Like the resistant parent 7E1(7D), the F_1 plants were resistant. Next, we tested 269 F_2 plants; 202 were resistant to isolate *Pt* 12-15-32 while 67 were susceptible. A χ^2 test indicated that leaf rust resistance segregated as a single dominant allele in the F_2 population ($\chi^2_{3:1}$ = 0.001) (Table 1). Thus, we concluded that resistance to *Pt* 12-15-32 conferred by 7E1(7D) was provided by a single dominant gene, which was already designated as the *Lr19* locus. To confirm this result, 269 $F_{2:3}$ progenies from selfing of the F_2 individuals were evaluated in assays with isolate *Pt* 12-15-32. Genetic analysis revealed that the $F_{2:3}$ progenies exhibited a segregation ratio of 1(resistant):2 (segregating):1 (susceptible) ($\chi^2_{1:2:1}$ = 0.42) (Table 1); therefore, this $F_{2:3}$ segregation ratio corroborates the above conclusion that *Lr19* is a single dominant locus conferring resistance to leaf rust.

Genetic mapping of the Lr19 locus

According to the genetic maps constructed previously, four SSR markers, two EST markers and one *PSY-E1*-specific marker located on the long arm of chromosome 7E1 (*XBe605194*, *XBg262436*, *XBarc76*, *Xcfa2240*, *XBe445653*, *XsdauK66*, *PSY-E1*) were polymorphic between the parental lines, 7E1(7D) and 7E2(7D) (Zhang et al. 2011; Guo et al. 2015) (Table S1, Fig. S2). Firstly, the linkage of these makers to *Lr19* was successfully confirmed by testing the 269 F_2 plants. Combined with the leaf rust resistance phenotypes, a primary genetic linkage map for *Lr19* was constructed, showing that *Lr19* was flanked by SSR markers *XBg262436* and *XBarc76*, with respective genetic distances of 2.4 cM and 1.7 cM (Fig. 1, Table S2).

To fine map *Lr19*, a set of dominant or co-dominant sequence-tagged site (STS) markers were developed within the interval between *XBg262436* and *XBarc76* using the CS reference genome (URGI, IWGSC RefSeq v1.0) and *Thinopyrum elongatum* genome and the re-sequencing data of 7E1(7D) and 7E1(7D) (Wang et al. 2020) (Table S1). All the newly developed STS markers were confirmed for specificity to 7E1(7D) or 7E2(7D) (Fig. S2). Using the 269 F_2 plants, a total of five newly designed STS markers were verified to be linked to *Lr19*.

Next, we screened the remaining F_2 population with 1004 plants, and 93 recombinants were obtained between *XBg262436* and *XBarc76*. The 93 recombinants and their $F_{2:3}$ families were phenotyped for resistance to the *Pt* isolate 12-15-32 (Table S3). Using the genotype and phenotype data, *Lr19* was mapped between the markers *XsdauK3734* and *XsdauK2839*, with respective genetic distances of 0.2 cM and 0.1 cM (Fig. 1).

Creation of translocation lines carrying shorter Lr19-containing segments

To develop *ph1b*-induced homoeologous recombinants between the *Th. ponticum* chromosome 7E1 and wheat chromosome 7D, an F_2 population (CS *ph1bph1b*/K11695) of 538 plants was constructed and screened using molecular markers for *ph1b* (*PSR128*, *SR574*, and *AWJL3*) and *Lr19* (*XBe605194*, *XBg262436*, *XBarc76*, *XBe445653*, *XsdauK66*). Among the F_2 population with 538 plants, we got 126 individuals which homozygous for *ph1b*. A total of 61 plants homozygous for *ph1b* and heterozygous for *Lr19* (Table S4) were obtained at last, and their F_3 families (total of 3819 plants) were further screened using *Lr19*-linked markers (Table S5). Finally, 111 7E1/7D recombinants between the markers *XBe605194* and *XsdauK66* were obtained (Table S6). Among these individuals, 50 were recombinants between markers *XBE605194* and *XBG262436* (recombinant types 1 to 5, Table S6), 43 were recombinants between markers *XBg262436* and *XBarc76* (recombinant types 6 to 9, Table S6), 3 were recombinants between markers *XBarc76* and *XBe445653* (recombinant types 10 and 11, Table S6), and 15 were recombinants between *XBe445653* and *XsdauK66* (recombinant types 12 to 15, Table S6). Recombinant types 2, 4, 6, 7, 8, 9, 10, 13, 14 and 15 still contain relatively large proximal segments of the *Th. ponticum* chromosome. Some types have lost the *Lr19* locus already, i.e., recombinant types 1, 5, 11, and 12.

To identify the lines carrying shorter alien chromosome segments, 1436 plants derived from recombinant type 3 were genotyped with the five co-dominant markers. Finally, two individuals with recombination between markers *XBarc76* and *XBe445653* were obtained and designated 9–80 and 1–40 (Table S7).

Genotyping of translocation lines and assessment of leaf rust resistance

To confirm the presence of the alien segment from *Th. ponticum* carrying *Lr19*, the linkage markers were used to genotype 9–80 and 1–40. The distal flanking markers *XBe605194* and *XBe445653* were negative, but all the intervening linkage markers were positive. This finding supports the notion that the proximal breakpoint occurred between markers *XBe605194* and *XBg262436*, and the distal breakpoint occurred between *XBarc76* and *XBe445653*, with a genetic distance about 20 cM between the two breakpoints (Fig. 2A, Fig. S2).

GISH and FISH were also performed to determine the chromosomal composition of the *Lr19*-carrying lines. The positive control, the Robertsonian translocation line K11695, showed signals corresponding to the *Th. ponticum* probe across almost the entire long-arm derived from the chromosome 7E1. However, the translocation lines proposed to carry shorter alien chromosome fragments, 1–40 and 9–80, only showed the *Th. ponticum* signals on the terminal end of the long arm. After removing the GISH signals,

the slide was subjected to FISH analysis, and the results indicated that the translocations occurred on the chromosome 7D (Fig. 2B). These cytological results suggest that 1–40 and 9–80 only carry small alien chromosome segments from the terminus of chromosome 7E1, which is consistent with the results from genetic marker analysis (Fig. 2A).

Lr19 still confers broad resistance to most leaf rust races worldwide (Zhang et al. 2022). To determine whether *Lr19* is still useful in China, we also evaluated the resistance performance of the translocation lines against 16 *Pt* isolates collected in China. The negative control CS *ph1bph1b* was susceptible, but the translocation lines carrying *Lr19*, like the donor 7E1(7D), were resistant to all 16 *Pt* isolates (Fig. 3).

The translocation line carrying Lr19 **without** PSY-E1

The *Th. ponticum* chromosome 7E1 also carries *PSY-E1* and *Sr25* in linkage with *Lr19* (Knott 1984). Even though *Lr19* has been known to confer very broad resistance to leaf rust for several decades, the linkage with the *PSY-E1* gene causing yellow pigmentation of flour has limited its use in hexaploid wheat breeding (Knott 1980). Here, using a *PSY-E1*-specific marker, we examined the presence of *PSY-E1* in the *Lr19*-carrying lines. The *PSY-E1* marker was detected in 7E1(7D), K11695, and 9–80 but not in 1–40 (Fig. S2). Consistent with the lack of *PSY-E1*, the flour color of 1–40 was much less yellow than that of 7E1(7D), K11695, and the translocation line 9–80 (Fig. 3). *PSY-E1* is known to be involved in carotenoid synthesis, and lutein has been observed to be the most abundant type of carotenoid (Padhy et al. 2022). The lutein content in 1–40 is indeed significantly lower than that in 7E1(7D) (Fig. S3). These results demonstrate that the alien chromosome segment in 1–40 lacks *PSY-E1* and is shorter than the segment in 9–80 (Fig. 2A).

Discussion

Leaf rust poses a persistent threat to wheat production worldwide, and novel effective leaf rust resistance genes are urgently needed. Up to now, 108 genes conferring resistance to wheat leaf rust have been identified (Dinh et al. 2020; Rothwell et al. 2020; Kumar et al. 2021; Xu et al. 2022). However, most of these genes have lost their effectiveness due to the evolution of virulent pathotypes (Rani et al. 2020). One approach to overcoming resistance is to source resistance genes from related wild species (Dinh et al. 2020). Four *Lr* genes have been transferred from *Thinopyrum* species into wheat; these *Lr* genes, which are located on 7EL, 3EL, 7ES, and 6EL, are known as *Lr19*, *Lr24*, *Lr29*, and *Lr38*, respectively (Knott and Sharma 1966; Schachermayr et al. 1995; Friebe et al. 1996; Mebrate et al. 2007; Prasad et al. 2020). However, the low recombination rate between the alien and wheat chromosomes has prevented the construction of genetic maps and development of markers closely linked to these *Lr* genes, which has limited the use of *Lr* genes in wheat breeding. Here, to overcome this limitation, we focused on fine mapping *Lr19*.

Since the chromosomes between wheat and wild relatives could not pair normally, it is difficult to fine mapping a gene from alien species for a long time. Till the first alien gene, Fusarium head blight resistance gene *Fhb7* was mapped and cloned which located on the terminal of the chromosome 7E2, by

using two wheat-*Th. ponticum* substitution lines as parents, 7E1(7D) and 7E2(7D), in which the alien chromosomes 7E1 and 7E2 could relatively freely pair during meiosis (Wang et al. 2020). For fine mapping *Lr19*, we constructed an F_2 population using the wheat-*Th. ponticum* substitution lines, 7E1(7D) and 7E2(7D). Using the *Thinopyrum elongatum* genome and the re-sequencing data of 7E1(7D) and 7E2(7D) (Wang et al. 2020), we designed 5 linkage markers, and find that *Lr19* was located between the markers *XsdauK3734* and *XsdauK2839* on the chromosome 7E1, with respective genetic distances of 0.2 cM and 0.1 cM. The previously study indicated *Lr19* located on the long arm of the chromosome 7E1 (Zhang et al. 2011). In this study, we drew a precise map of *Lr19* and located *Lr19* in an interval with 0.3 cM. The linkage markers can be used to select lines carrying *Lr19*, and we obtained two translocation lines with *Lr19* using these markers.

PSY-E1 is known to be involved in carotenoid synthesis causing yellow flour which is undesirable in bread wheat, and simultaneously, *PSY-E1* is located on the long arm of the chromosome 7E1 and linked to the leaf rust resistance gene *Lr19*, which has limited the use of *Lr19* in bread wheat breeding (Knott 1980). The commonly used method of transferring important disease resistance genes from distantly related species into common wheat has been through the induction of homoeologous recombination between an alien chromosome and a wheat chromosome in a *ph1bph1b* background. Here, we constructed a bi-parental population by crossing CS *ph1bph1b* and K11695. We used the genetic map and *Lr19* linkage markers to identify *ph1b*-induced recombination between the chromosomes 7E1 and 7D, and eventually two translocation lines with short alien fragments carrying *Lr19* (1–40 and 9–80) were identified. In addition, the translocation line 1–40 only harbors a small alien fragment containing *Lr19* but not *PSY-E1* which was verified by both genotyping and phenotyping. And the translocation line 1–40 can be used in bread wheat breeding program directly.

To understanding the composition of the leaf resistance genes in Chinese wheat cultivars, Wu et al. (2020) identified six *Lr* genes (*Lr1*, *Lr26*, *Lr33*, *Lr34*, *Lr45*, and *Lr46*) in 100 wheat cultivars from China and 18 improved germplasms from global landrace donors, and Zhang et al. (2021) detected four known *Lr* genes (*Lr1*, *Lr26*, *Lr3ka*, *LrZH22*) and 18 potentially loci in 207 accessions from different regions in China using 90K SNP array. However, the leaf rust resistance gene *Lr19* was not detected in their studies. Besides that, *Lr19* confers broad resistance to leaf rust pathogens worldwide, although new pathogen races have been reported to have overcome the *Lr19*-mediated resistance to *Lr19* in several areas (Sibikeev et al. 1996; Elyasi-Gomari and Panteleev 2006; Xiao et al. 2016). Here, we tested the resistance of the translocation lines with shorter *Th. ponticum* translocation segments to 16 virulent leaf rust isolates in China and found that the translocation lines confer resistance to all the isolates. All this indicates that *Lr19* is not widely used in China and that transferring *Lr19* to common wheat is still a promising approach for introducing leaf rust resistance in Chinese wheat breeding programs. And our lab is currently using the translocation lines to transfer the *Lr19* gene to wheat cultivars in China.

Conclusion

Leaf rust is a global disease of cereal crops, and the causal pathogen for leaf rust rapidly develops resistance to control measures such as fungicides and host genetic resistance. In the present study, *Lr19* was mapped to a 0.3 cM region on the long arm of chromosome 7E1 flanked by markers *XsdauK3734* and *XsdauK2839*. Ultimately, we used these markers to select two translocation lines with shorter alien fragments, 1–40 and 9–80, with recombination induced by crosses to the CS *ph1bph1b* mutant. Both translocation lines conferred resistance to 16 *Pt* isolates collected from China in a seedling test. Genotyping and phenotyping verified that *Lr19* is no longer linked to *PSY-E1* in the translocation line 1–40. The genetic markers *XsdauK3734* and *XsdauK2839*, and the translocation lines can be used for marker-assisted selection of *Lr19* in wheat breeding and germplasm improvement directly.

Declarations

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Author contribution statement LK and HW designed the research; SX and ZL conducted the research; XW, YG, XC, WG and XL prepared the samples; YB, ML and ZY contributed to the cytogenetic analysis; NZ tested the resistance spectrum of the *Lr19* translocation lines; SX wrote the draft; LK and HW made the revision of the manuscript. All authors read and approved the manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest in the reported research.

Ethical standards I declare on behalf of my co-authors that the work described is original, previously unpublished research, and not under consideration for publication elsewhere. The experiments in this study comply with the current laws of China.

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Table 1

Table 1 is available in the Supplementary Files section.

Figures

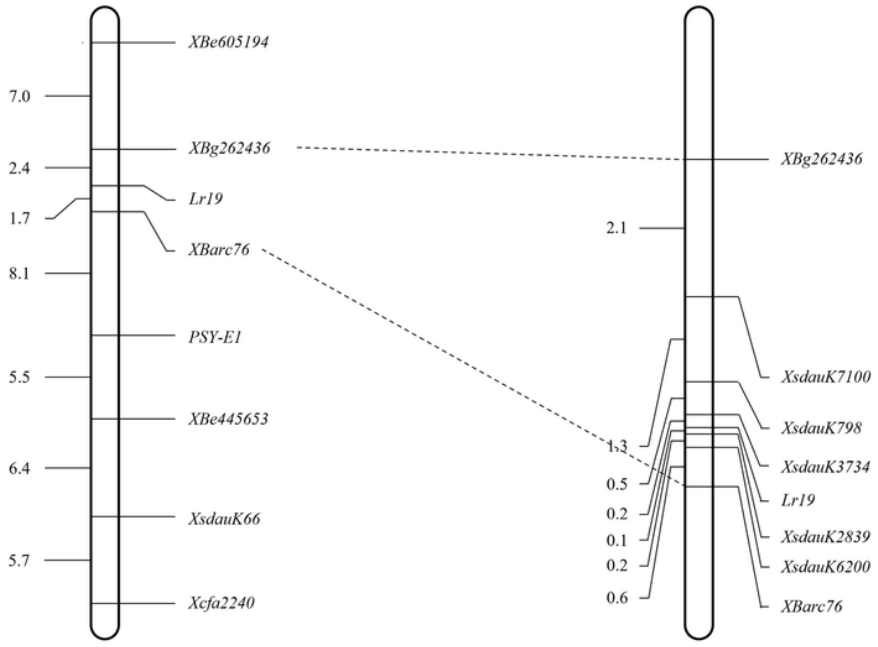


Fig.1

Figure 1

Linkage map of chromosome 7E1, which carries *Lr19*.A close-up view of the region containing *Lr19* is shown to the right. Markers are shown on the right, and genetic distances in cM are shown on the left of the chromosome.

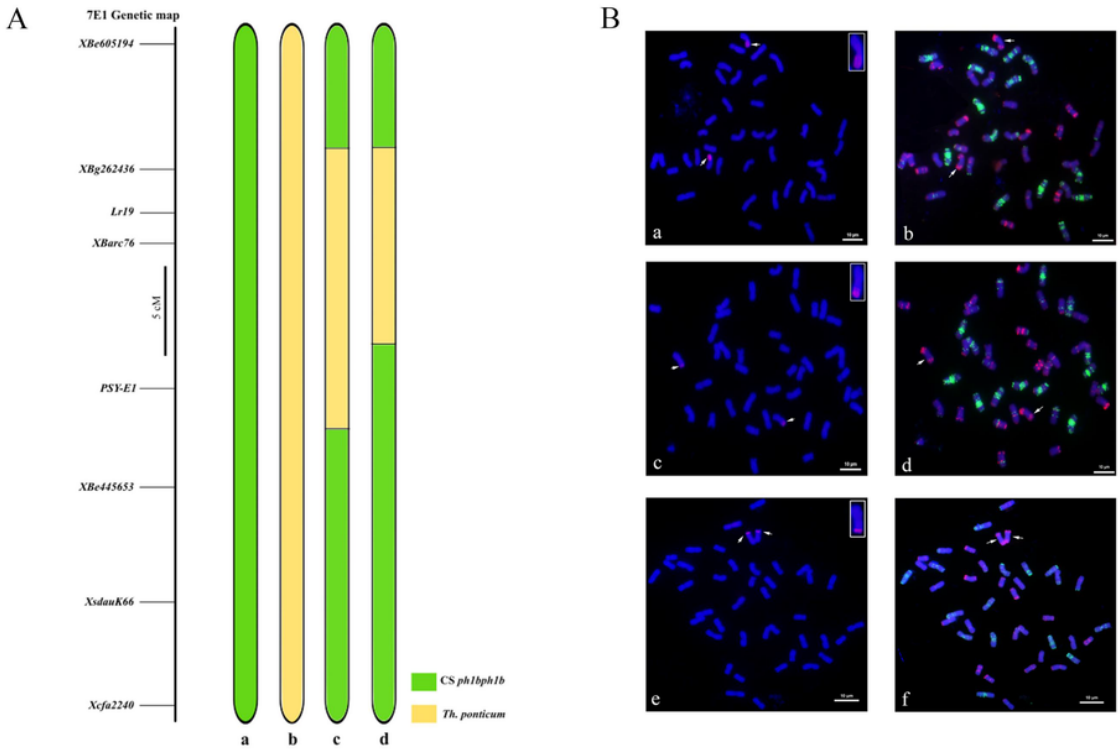


Fig.2

Figure 2

(A) The recombinant types of the wheat lines carrying *Lr19*. Yellow indicates the segments transferred from *Th. ponticum* chromosome 7E1. Green indicates the segments transferred from common wheat chromosome 7D. The genetic map and linkage markers are shown on the left. a: CS *ph1bpb1b*; b: K11695; c: 9-80; d: 1-40. (B) Cytogenetic analysis of different lines carrying *Lr19*. *Th. ponticum* chromatin detected by GISH is painted red and indicated by arrows. Common wheat chromatin detected by FISH is indicated by arrows. GISH, a: K11695, c: 9-80, e: 1-40. FISH, b: K11695, d: 9-80, f: 1-40.

Pt isolates	CS *ph1bph1b*	1-40	9-80	K11695	7E1(7D)
17-5-473-202	4	0	0	0	0
05-8-62-I-201	4	0	0	0	0
12-25-544-1-182-203	4	0	0	0	0
17-9-509-202	4	0	0	0	0
17-5-473-203	4	0	0	0	0
17-9-569-201	4	0	0	0	0
45-201	4	0	0	0	0
09-7-964-1	4	;	;	;	;
09-5-1215-1-1	4	;	;	;	;
12-7-236-1	4	;	;	;	;
12-7-200	4	0	0	0	0
13-5-9-1	4	;	;	;	;
12-7-201-1	4	;	;	;	;
13-5-28	4	0	0	0	0
12-206-1	4	0	0	0	0
12-7-186	4	0	0	0	0

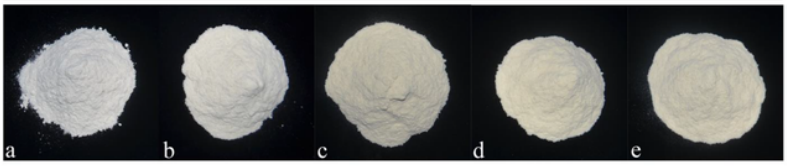


Fig.3

Figure 3

The resistance spectrum (top) and flour color (bottom) of CS *ph1bph1b* and different lines carrying *Lr19*. a: CS *ph1bph1b*; b: 1-40; c: 9-80; d: K11695; e: 7E1(7D).

Supplementary Files

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