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Genetic mapping of the wheat leaf rust resistance gene Lr19 and development of translocation lines to break its linkage with yellow pigment

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Abstract

The leaf rust resistance locus *Lr19*, which was transferred to wheat (*Triticum aestivum*) from its relative *Thinopyrum ponticum* in 1966, still confers broad resistance to most known races of the leaf rust pathogen *Puccinia triticina* (*Pt*) worldwide. However, this gene has not previously been fine-mapped, and its tight linkage with a gene causing yellow pigmentation has limited its application in bread wheat breeding. In this study, we genetically mapped *Lr19* using a bi-parental population from a cross of two wheat-*Th. ponticum* substitution lines, the *Lr19*-carrying line 7E1(7D) and the leaf rust-susceptible line 7E2(7D). Genetic analysis of the F_2 population and the $F_{2:3}$ families showed that *Lr19* was a single dominant gene. Genetic markers allowed the gene to be mapped within a 0.3 cM interval on the long arm of *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 the *Th. ponticum* chromosome 7E1. Two translocation lines with short *Th. ponticum* chromosome fragments carrying *Lr19* were identified using the genetic markers closely linked to *Lr19*. Both translocation lines were resistant to 16 *Pt* races collected throughout China. Importantly, the linkage between *Lr19* and yellow pigment content was broken in one of the lines. Thus, the *Lr19* linked markers and translocation lines developed in this study are valuable resources in marker-assisted selection as part of common wheat breeding programs.

Key message

The leaf rust resistance gene *Lr19*, which is present on the long arm of chromosome 7E1 in *Thinopyrum ponticum* was mapped within a 0.3 cM genetic interval and, translocation lines were developed to break its linkage with yellow pigmentation.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important staple crops produced for human consumption throughout the world, with an average production of ~ 3.47 tons per hectare from 2016 to 2020 (Rollar et al. 2021; FAO 2022). However, global wheat production is threatened by leaf rust, caused by *Puccinia triticina* (*Pt*), which is the most destructive and widespread rust in wheat growing regions. Infection with *Pt*, causes average yield losses more than 1% globally (Savary et al. 2019) and even up to 70% among susceptible cultivars (Aktar-Uz-Zaman et al. 2017). The most efficient and environmentlly sustainable method of controlling this devastating pathogen is the development of resistant wheat cultivars (Xu et al. 2022).

To date, 82 wheat leaf rust resistance (*Lr*) genes have been formally catalogued (Dinh et al. 2020; Kumar et al. 2021; Bariana et al. 2022; Xu et al. 2022), nearly half of which were derived from wild wheat relatives (Prasad et al. 2020). Of the 82 known *Lr* genes, only nine 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;

Kolodziej et al. 2021; Athiyannan et al. 2022; Lin et al. 2022). Furthermore, *Pt* evolution has allowed some races to overcome specific *Lr* genes (Bolton et al. 2008). Ongoing protection against leaf rust therefore requires identification of novel *Lr* genes or further application of known effective *Lr* genes (Sibikeev et al. 1996; Xu et al. 2022).

The resistance gene *Lr19*, transferred to bread wheat from *Thinopyrum ponticum* (Knott and Sharma 1966), still kept broad resistance to *Pt* races in many regions of the world (Zhang et al. 2022), even several *Pt* races have potentially 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 of *Th. ponticum* and common wheat do not pair normally, irradiation was employed to induce translocation of a *Th. ponticum* chromosome segment carrying *Lr19* from a wheat-*Th. ponticum* substitution line into wheat (Knott and Sharma 1966). The same research group produced a translocation line, referred to as Agatha (K11695), with the long arm of *T. aestivum* chromosome 7D replaced by the homoeologous chromosome of *Th. ponticum* (Knott and Sharma 1966; Knott and Dvořák 1977a; Kim et al. 1993). To reduce the alien segment, Marais (1992) generated a new translocation line with a shorter alien segment on chromosome 7B, *Lr19*-149, using the Chinese Spring *Ph1b* mutant (CS *ph1bph1b*).

Several studies have been carried out to construct a map of *Lr19*. Marais (1991) selected 29 lines with gamma irradiation-induced deletions in the translocated chromosome segment containing *Lr19*. Restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) markers were then used to generate a deletion map of *Lr19* from the 29 deletion mutants (Kim et al. 1993; Prins et al. 1996; Prins and Marais 1998; Groenewald et al. 2005). However, the resolution of the deletion map was too low to provide stable, closely-linked markers for *Lr19* that could be used in high-throughput screening for *Lr19* transfer. Therefore, attempting to get shorten translocation lines, require a precise map and tightly linked markers of *Lr19*.

Fine-mapping of *Lr19* is particularly desirable because the *Th. ponticum* chromosome carrying *Lr19* also contains a gene for yellow pigment (*Y*) (Knott 1968). Yellow pigment is an undesirable trait in bread wheat and has therefore limited the use of *Lr19* in wheat breeding (Knott 1980). Attempts to separate *Y* from *Lr19* have included recombination between homoeologous chromosomes and ethyl methane sulfonate (EMS) mutant generation. EMS treatment yielded two mutants (‘Agatha-28-4’ and ‘Agatha-235-6’) that produced white flour (Knott 1980), but these lines show impaired agronomic performance (Knott 1989). Homoeologous recombination induced by CS *ph1bph1b* in *Lr19*-149 and its secondary recombinants successfully separated *Y* from *Lr19* (Marais 1992; Marais et al. 2001). However, the resulting lines have a high rate of self-elimination during male meiosis, limiting their use in common wheat breeding programs (Zhang et al. 2005). Zhang et al. (2005) created a new 7EL(7DL) interstitial recombinant line, 1–96, that carries *Lr19* without the meiotic self-elimination previously reported in 7EL(7BL) translocation *Lr19*-149, and it was shown without yellow pigment scored by spectrophotometer. Notably, Agatha-28-4 and Agatha-235-6 have differing levels of yellow pigment content (Knott 1980), Zhang and Dubcovsky (2008) demonstrated that this difference was due to a nonsynonymous substitution mutation in the carotenoid biosynthesis gene *PSY-E1* of Agatha-28-4, while Agatha-235-6

without any mutation within *PSY-E1* gene or upstream sequence. Experiments with gamma irradiation have also produced several leaf rust-resistant mutants with white or partially white endosperm (Marais 1991). These results suggested that there may be at least two yellow pigment loci on the translocated 7E1 chromosome possibly (Knott 1984; Marais 1991; Zhang and Dubcovsky 2008). Completely breaking the linkage between *Lr19* and yellow pigment loci will allow broader application of *Lr19* in common wheat breeding.

The objectives of this study were as follows: (1) to map the *Lr* gene *Lr19* and develop markers closely linked to *Lr19*; (2) to break the linkage between *Lr19* and yellow pigment and create wheat-*Th. ponticum* translocation lines with short alien fragments using marker assisted selection. The genetic markers and wheat lines generated in this study are expected to increase the use of naturally pathogen-resistant cultivars in wheat breeding, promoting sustainable food security in China and throughout the world.

Materials and methods

Plant materials

The F_1 hybrids (n = 15), F_2 segregating populations (n = 1273), and $F_{2:3}$ families were developed by crossing the wheat-*Th. ponticum* substitution lines 7E1(7D) (resistant) and 7E2(7D) (susceptible) (Knott et al. 1977b), 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*, K11695 (Kim et al. 1993), was crossed with CS *ph1bph1b* to induce homoeologous recombination. Wild emmer wheat (*T. dicoccodes*) accession AS15 (AABB), *Aegilops tauschii* accession 2147 (DD), and the common wheat cultivar Jimai22 were used to evaluate 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 wheat lines carrying Lr19 **with shorter alien chromosome segments**

The original Robertsonian translocation line K11695 was crossed to CS *ph1bph1b*. The F_1 plants were self-pollinated and the resulting F_2 population was genotyped with *Ph1* molecular markers and *Lr19* linked markers to select individuals that were homozygous for *ph1b* but heterozygous for *Lr19*. Such plants were self-crossed and *Lr19* linked marker analysis was performed on the progenies to identify plants with potentially shortened *Th. ponticum* chromosome segments. These plants were tested for leaf rust resistance as described below (**Leaf rust resistance evaluation**) and analyzed using GISH (**Cytogenetic analysis**) to determine the physical size of the shortened alien chromosome segments.

Leaf rust resistance evaluation

A *Pt* isolate, 12-15-32 (provided by Prof. Wenxiang Yang at Hebei Agricultural University), was used to test wheat resistance in the parental lines 7E1(7D), 7E2(7D), K11695, and CS *ph1bph1b*, in the progenies of the mapping populations, and in 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 inoculated, then incubated at 21℃ with 100% relative humidity (RH) for 12 h in the dark. The seedings were then transferred to a greenhouse set at 22 ± 2℃ with 70% RH under a 12/12 h light/dark cycle. Susceptibility was score at 14 d post-inoculation (dpi) using modified Stakman scale described by Dakouri et al. (2013) as follows: 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* races (17-5-473-202, 05-8-62- -201, 17-5-473-203, 2-25-544-1-182-203, 17-9-509-202, 17-9-569-201, 45–201, 09-7-964-1, 09-5-1215-1-1, 12-7-236-1, 12-7-200, 13-5-9-1, 12-7-201-1, 13-5-28, 12-206-1, 12-7-186) collected from various locations in China were used to test the resistance spectra of *Lr19*.

Cytogenetic analysis

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

DNA extraction, molecular marker analysis, and statistical analysis

All gDNA extractions were performed with a standard CTAB method as described by Huang et al. (2000). DNA concentration and quality were assessed using a NanoDrop 2000 spectrophotometer and 1.0% agarose gels electrophoresis.

Molecular marker analysis was performed to construct a genetic map of *Lr19*, identify homozygous *ph1b* F_2 plants, and select homoeologous recombinants induced by the *Ph1b* mutation. The parental lines 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), respectively. The markers *AWJL3*, *PSR128*, and *PSR574* were used to detect the *ph1b* allele in F_2 plants using multiplex touchdown PCR (Roberts et al. 1999). PCR was conducted on a Bio-Rad T100™ Thermal Cycle instrument in a 10 μl reaction volume containing 100 ng gDNA, 10 μM each of the forward and reverse primers, and 5 μl of 2×Taq Mix (Vazyme, P111-01), and ddH_2O to 10 μl. The PCR program was as follows: one cycle of 5 min at 95℃, 35 cycles of 30 s at 94℃, 30 s at 55–60℃

(depending on the specific primer pair), and 1 min at 72℃, 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.

To investigate the mode of *Lr19* inheritance, 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 (Kosambi 1943) and visualized with MapDraw V2.1 (Liu et al. 2003). A LOD score of 3.0 was used as the threshold for linkage.

Yellow pigment content analysis

Carotenoids concentrations in flour samples by mixing 2–3 g of each sample with 15 ml of extraction solution (2:1:1 N-hexane : acetone : ethanol with 0.01% butylated hydroxytoluene) in a 50 ml centrifuge tube with ultrasonication for 30 min. The mixed samples were centrifuged at 4000 × g for 10 min, then the supernatant was transferred to a new 50 ml centrifuge tube. Each pellet was re-extracted following the same steps until the resulting supernatant appeared colorless. Supernatant samples were evaporated under nitrogen gas, and reconstituted in a mixed solution of methanol : methyl tert-butyl ether. The resulting solution was passed through a filter with a 0.22 μm filter for further LC-MS analysis. Carotenoid contents were detected by MetWare (http://www.metware.cn/) based on the AB Sciex QTRAP 6500 LC-MS/MS platform.

Results

Genetic analysis of resistance to leaf rust

The parental line 7E1(7D), which carries *Lr19*, was determined to be fully resistant to *Pt* 12-15-32, whereas 7E2(7D), which does not contain *Lr19*, was highly susceptible (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. Thus, the results indicated that *Lr19* was a single dominant gene for resistance to leaf rust on chromosome 7E1 (Table 1).

Table 1
Genetic analysis of resistance to *Puccinia triticina* 12-15-32 among selected parental wheat lines and progenies at the seedling stage.

Parents	Generation	Phenotype and number of tested plants			Chi-squared
		All resistant	Segregating	All susceptible	$\chi^2_{(3:1 \text{ or } 1:2:1)}$
7E1(7D)	Resistant parent	16		0	
7E2(7D)	Susceptible parent	0		16	
	F_1	16		0	
	F_2	202		67	$\chi^2_{3:1} = 0.001$
	$F_{2:3}$	63	139	67	$\chi^2_{1:2:1} = 0.42$
$\chi^2_{3:1} = 0.001$. Not statistically significant at $p = 0.05$ (1 degree of freedom).					
$\chi^2_{1:2:1} = 0.42$. Not statistically significant at $p = 0.05$ (2 degrees of freedom).					

Genetic mapping of the Lr19 locus

Based on previously constructed genetic maps (Zhang et al. 2011; Guo et al. 2015), we used four SSR markers (*XBe605194*, *XBg262436*, *XBarc76*, *XBe445653*), two EST markers (*Xcfa2240*, *XsdauK66*) and one *PSY-E1*-specific marker (*PSY-E1*) located on the long arm of chromosome 7E1, all of which were expected to be polymorphic between the parental lines, 7E1(7D) and 7E2(7D) (Table S1, Fig. S2). We first confirmed the linkage of these markers to *Lr19* by testing them in 269 plants from the F_2 population. Characterization of leaf rust resistance phenotypes and the genotypes at these markers allowed us to construct a primary genetic linkage map for *Lr19*. The map showed that *Lr19* was flanked by the SSR markers *XBg262436* and *XBarc76*, which were 2.4 cM and 1.7 cM away from *Lr19*, respectively (Fig. 1, Table S2).

To further fine-map *Lr19*, a set of dominant or co-dominant sequence-tagged site (STS) markers were developed for 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). The specificity of each newly developed STS marker for 7E1(7D) or 7E2(7D) was confirmed (Fig. S2). Using 269 F_2 plants from the 7E1(7D)/7E2(7D) cross as describe above, a total of five newly designed STS markers were verified to be linked to *Lr19*. Subsequent screening of the remaining 1004 F_2 plants revealed 93 recombinants between *XBg262436* and *XBarc76*. The 93 recombinants and their $F_{2:3}$ families were then phenotyped for resistance to the *Pt* isolate 12-15-32 (Table S3). Using the genotype and phenotype data, *Lr19* was mapped to the region between the

markers *XsdauK3734* and *XsdauK2839*, with genetic distances of 0.2 cM and 0.1 Cm, respectively (Fig. 1).

Creation of translocation lines with shorter Lr19 -containing alien segments

To develop *ph1b*-induced homoeologous recombinants between *Th. ponticum* chromosome 7E1 and wheat chromosome 7D, the CS *ph1bph1b*/K11695 F_2 population was constructed and screened using molecular markers for *ph1b* (*PSR128*, *SR574*, and *AWJL3*) and *Lr19* (*XBe605194*, *XBg262436*, *XBarc76*, *XBe445653*, and *XsdauK66*). Of the 538 individuals in the F_2 population, 126 were homozygous for *ph1b*, and a total of 61 plants homozygous for *ph1b* and heterozygous for *Lr19* were obtained (Table S4). We then screen the resulting F_3 families (n = 3819) with *Lr19*-linked markers (Table S5). This analysis revealed 111 7E1/7D recombinants between the markers *XBe605194* and *XsdauK66* (Table S6). Of these 111 individuals, there were 50 recombinants between *XBE605194* and *XBG262436* (recombinant types 1 to 5), 43 recombinants between *XBg262436* and *XBarc76* (recombinant types 6 to 9), three between *XBarc76* and *XBe445653* (recombinant types 10 and 11), and 15 between *XBe445653* and *XsdauK66* (recombinant types 12 to 15). Recombinant types 2, 4, 6, 7, 8, 9, 10, 13, 14, and 15 retained relatively large proximal segments of the *Th. ponticum* chromosome, whereas some recombinant types (namely 1, 5, 11, and 12) had lost the *Lr19* locus. This left only type 3 recombinants as candidates.

To identify lines carrying *Lr19* and shorter alien chromosome segments, 1436 plants derived from type 3 recombinant individuals were genotyped with the five co-dominant markers. Ultimately, only two individuals were found with recombination between the markers *XBarc76* and *XBe445653*, which were designated 9–80 and 1–40 (Table S7).

Translocation lines genotyping and phenotyping

To confirm the presence of the *Th. ponticum* alien segment carrying *Lr19* in the two selected translocation lines (9–80 and 1–40), the lines were genotyped with linked markers. The distal flanking markers *XBe605194* and *XBe445653* were negative, but all the intervening linked markers were positive. This finding supported the hypothesis that the proximal breakpoint occurred between markers *XBe605194* and *XBg262436*, and that the distal breakpoint occurred between *XBarc76* and *XBe445653*. There was a genetic distance of ~ 20 cM between the two breakpoints (Fig. 2A, Fig. S2).

GISH and FISH were also performed to determine the chromosomal composition of the two *Lr19*-carrying lines. The positive control, the Robertsonian translocation line K11695, showed signals corresponding to the *Th. ponticum* probe across nearly the entire long-arm derived from chromosome 7E1. However, the translocation lines that were presumed to carry shorter alien chromosome fragments, 1–40 and 9–80, showed *Th. ponticum* signals only on the terminal end of the long arm. After removing the GISH signals, the same slides were analyzed with FISH. The results indicated that the translocations occurred on chromosome 7D (Fig. 2B). These cytological results suggested that 1–40 and 9–80 carried only small alien chromosome segments from the terminus of chromosome 7E1, consistent with the results of the genetic marker analysis (Fig. 2A).

To determine whether *Lr19* remains a useful breeding target for wheat grown in China, we evaluated the resistance performance of the two translocation lines to 16 *Pt* races collected throughout the country. As expected, the negative control CS *ph1bph1b* was susceptible to all 16 races, whereas the donor 7E1(7D) was resistant. Importantly, both the translocation lines carrying *Lr19* were resistant to all 16 *Pt* races (Fig. 3A), confirming the validity of *Lr19* as a breeding target.

Assessment of the linkage between Lr19 and yellow pigment in the translocation lines

To establish whether the translocation lines carried the yellow pigment gene, flour color was compared between CS *ph1bph1b* (the white flour-producing negative control) and several lines carrying *Lr19*. Translocation line 1–40 and CS *ph1bph1b* produced white flour, whereas 9–80 and 7E1(7D) produced yellow flour (Fig. 3B). To quantify these results and identify the responsible locus, we measured levels of carotenoid components in each of the lines. Lutein the most abundant carotenoid associated with the *Lr19*-linked carotenoid biosynthesis gene *PSY-E1*, was significantly reduced in the translocation line 1–40 compared to both line 9–80 and 7E1(7D) (Fig. 3B, Fig. S3). Using a *PSY-E1*-specific marker, we examined the presence of *PSY-E1* in the lines carrying *Lr19*. *PSY-E1* was detected in 7E1(7D), K11695, and 9–80 but not in 1–40 (Fig. S2). These results demonstrated that the linkage between *Lr19* and yellow pigment was broken in translocation line 1–40, which lacked the lutein gene *PSY-E1*. This may have been due to the smaller alien chromosome segment present in 1–40 compared to 9–80 (Fig. 2A).

Discussion

Leaf rust is a persistent threat to wheat production worldwide, necessitating identification of novel effective resistance genes. Although 82 genes conferring leaf rust resistance have been identified in wheat (Dinh et al. 2020; Rothwell et al. 2020; Kumar et al. 2021; Xu et al. 2022), most have lost their effectiveness due to pathogen evolutions (Rani et al. 2020). New resistance genes are often sourced from wild species that are closely related to wheat (Dinh et al. 2020). For example, a total of four *Lr* genes have been transferred from *Thinopyrum* species into wheat. These *Lr* genes (*Lr19*, *Lr24*, *Lr29*, and *Lr38*) are known to have originated from the *Thinopyrum* chromosome arms 7EL, 3EL, 7ES, and 6EL, 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 rates between alien and wheat chromosomes have prevented the construction of high-resolution genetic maps and the development of markers closely linked to these *Lr* genes, limiting their use in wheat breeding. To address this issue, we here sought to fine-map a key *Lr* gene, *Lr19*.

The first instance of a fine-mapped alien gene in wheat was the *Fusarium* head blight resistance gene *Fhb7*, which was mapped and cloned using two wheat-*Th. ponticum* substitution lines, 7E1(7D) and 7E2(7D). This was possible because the alien chromosomes 7E1 and 7E2 could pair relatively freely during meiosis (Wang et al. 2020). A previous study indicated that *Lr19* was also located on the long arm of the chromosome 7E1 (Zhang et al. 2011). Thus, to fine-map *Lr19*, we first constructed an F_2 population using the same wheat-*Th. ponticum* substitution lines and five linked markers that we designed based on

sequencing data from *Thinopyrum elongatum*, 7E1(7D), and 7E2(7D) (Wang et al. 2020). Genetic analysis revealed that *Lr19* was located on the chromosome 7E1 within a 0.3 cM interval between the markers *XsdauK3734* and *XsdauK2839*, and further analysis localized the gene still more precisely in this region (Fig. 1).

A precise genetic map plays an important role in breeding efforts. Tightly-linked flanking markers can be used for marker assistant selection (MAS) of important traits and to clarify the relative positions of target genes and nearby undesirable genes. For example, although *Lr19* confers broad resistance to most leaf rust races worldwide (Zhang et al. 2022), its linkage with one or more yellow pigment genes has limited its use in bread wheat breeding (Knott 1980). Multiple studies have endeavored to separate the yellow pigment gene(s) from *Lr19*. Here, after fine-mapping *Lr19* and developing closely-linked genetic markers, we constructed a secondary segregating population, which was screened to determine recombination distances with respect to *Lr19*. We ultimately identified two translocation lines with relatively small regions of alien chromatin, maximizing the chances that they would contain *Lr19* but not the yellow pigment locus or loci. Line 9–80 (~ 62 Mb of alien chromosome content) carried the carotenoid biosynthesis gene *PSY-E1* and showed significant but incomplete reduction of lutein contents, implying the existence of other loci on 7E1L that regulate yellow pigmentation (Fig. S3). *PSY-E1* was deleted in line 1–40 (~ 40 Mb of alien chromosome content), which showed similar lutein levels as the control line CS *ph1bph1b* and produced white flour (Fig. 2A, Fig. 3B, Fig. S3). These results demonstrated the powerful use of a MAS-based strategy to allow precise identification of desired translocation lines, enabling much more rapid attainment of desired traits compared to traditional phenotype-based screening (Marais 1992; Niu et al. 2014); using this method, we identified a line (1–40) that can be used directly in wheat breeding.

Although *Lr19* has been reported to confer broad resistance to *Pt* races in many regions of the world (Zhang et al. 2022), several *Pt* races may have overcome *Lr19*-mediated resistance in areas such as Mexico, Russia, Ukraine, and India (Sibikeev et al. 1996; Elyasi-Gomari and Panteleev 2006; Xiao et al. 2016). We therefore tested the resistance of the two selected translocation lines (9–80 and 1–40) to 16 virulent leaf rust races collected from China, and found that both lines were resistant to all 16 races. These results confirmed that introducing *Lr19* to common wheat remains a valuable approach in Chinese breeding programs aiming to provide leaf rust resistance.

Conclusion

Leaf rust is a globally distributed and economically devastating disease affecting cereal crops. The causal agent rapidly develops resistance to host genetic resistance, although the resistance gene *Lr19* has remained effective. We here fine-mapped *Lr19* to a 0.3 cM region on the long arm of chromosome 7E1 and developed genetic markers to identify genotypes carrying *Lr19*. We used these markers to select two translocation lines, 1–40 and 9–80, with relatively short *Th. ponticum* fragments to minimize the chances of transferring the *Lr19*-linked yellow pigment gene. Both translocation lines conferred resistance to 16 *Pt* races collected from China; most importantly, genotyping and phenotyping verified that the *Lr19* and yellow pigmentation was broken in 1–40. These newly-developed genetic markers are

suitable for future use in MAS for *Lr19* in wheat breeding, and the translocation line is a valuable new resource for direct germplasm improvement.

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 revised the manuscript. All authors have read and approved the final version of the manuscript.

Data Availability The datasets generated or analyzed for this study are available from the corresponding author upon 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 is not under consideration for publication elsewhere. The experiments in this study comply with the current laws in China.

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Figures

Figure 1

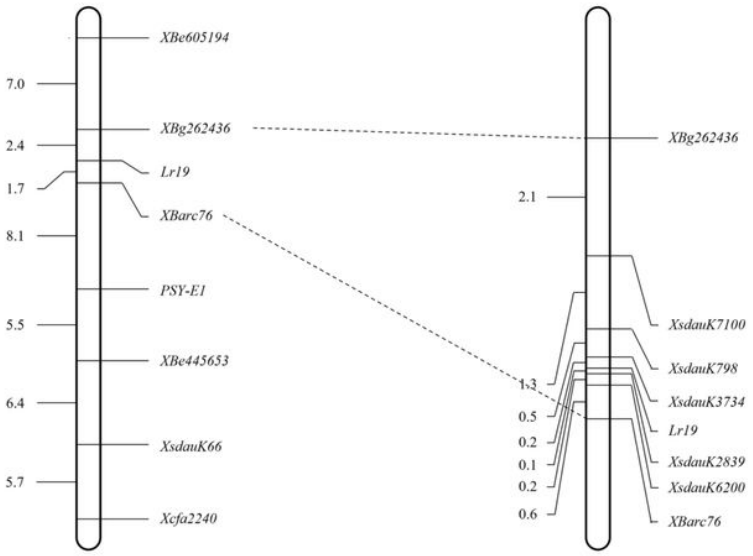


Figure 1

***Lr19* linkage maps.** Left, linkage map of chromosome 7E1, which carries *Lr19.* Right, enlarged view of the region containing *Lr19*. Markers are shown to the right of each map, and genetic distances (in cM) are shown to the left.

Figure 2

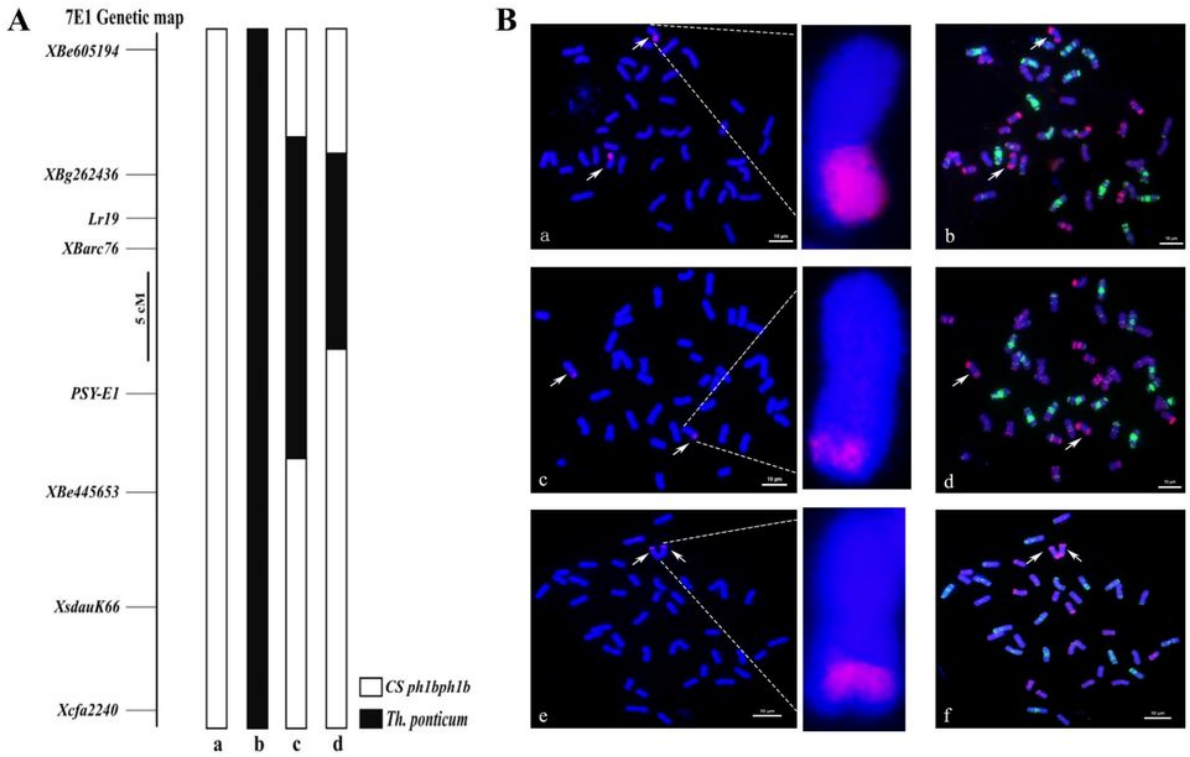


Figure 2

Translocation line analysis. (A) Recombination among selected wheat lines carrying *Lr19*. Black regions indicate segments transferred from chromosome 7E1 of *Th. ponticum*. White regions indicate segments transferred from chromosome 7D of common wheat (*Triticum aestivum*). a, CS *ph1bpb1b*; b, K11695; c, translocation line 9-80; d, translocation line 1-40. (B) Cytogenetic analysis of selected wheat lines carrying *Lr19*. (a, c, e) Genomic *in situ* hybridization (GISH) and (b, d, f) fluorescent *in situ* hybridization (FISH) were carried out in (a, b) K11695, (c, d) 9-80, and (e, f) 1-40. *Th. ponticum* chromatin was detected with GISH (shown in red and indicated with arrows). Common wheat chromatin was detected with FISH (indicated with arrows). Scale bar = 10 μm.

Figure 3

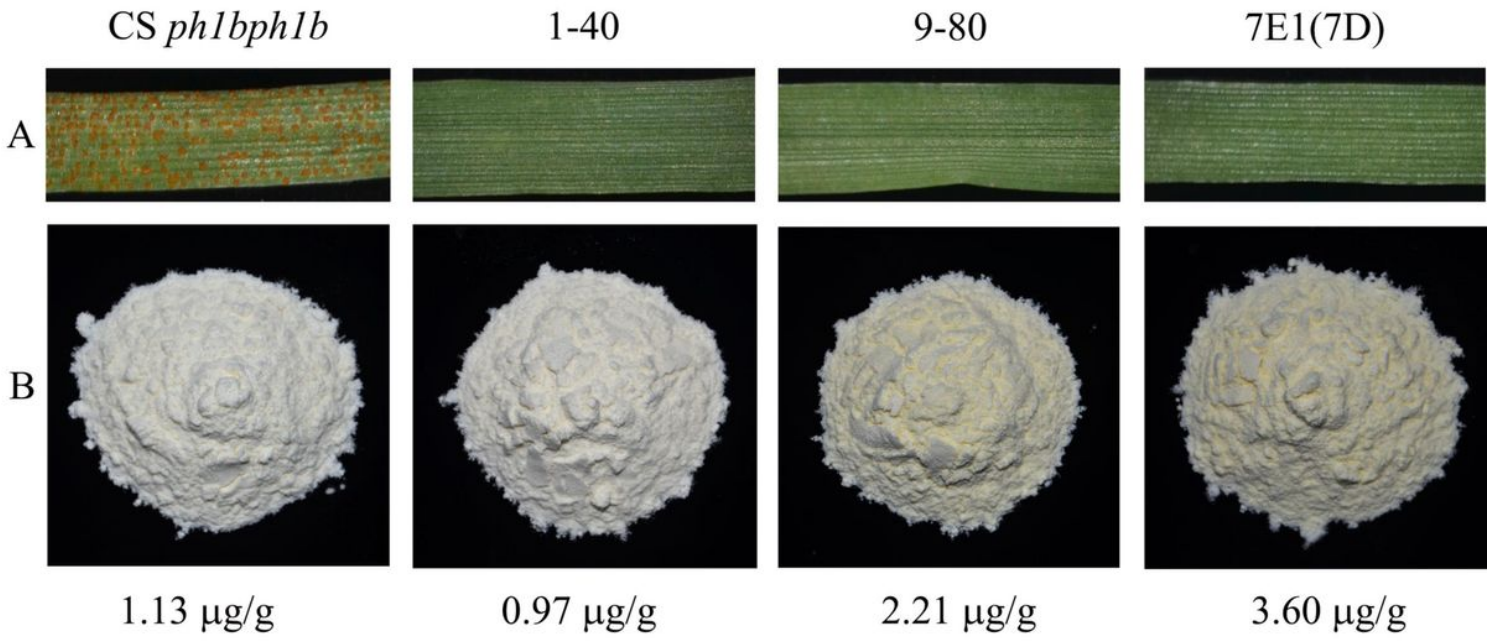


Figure 3

Leaf rust resistance and flour color among selected wheat lines. (A) Phenotypes of selected wheat lines treated with *Puccinia triticina* (*Pt*). Resistance was analyzed in a *Triticum aestivum* cv. 'Chinese Spring' *ph1b* mutant (CS *ph1bph1b*) as a negative control, the parental line 7E1(7D) as a positive control, and in translocation lines 1-40 and 9-80. (B) Flour color and lutein content of the wheat lines shown in (A).

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