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ORIGINAL ARTICLE

Mapping and characterization of the recessive leaf rust resistance gene *Lr83* on wheat chromosome arm 1DS

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Abstract

Key message **The leaf rust resistance gene in Thatcher wheat derivative 78–1 was mapped to chromosome 1DS with SNP markers and designated as *Lr83*.**

Abstract 'Thatcher' wheat near isogenic line RL6149, a putative derivative of *Triticum dicoccoides*, was previously determined to carry leaf rust resistance gene *Lr64* on chromosome arm 6AL and a second gene temporarily named *LrX* on chromosome arm 1DS. The objective of this study was to map and characterize *LrX* in a population of recombinant inbred lines (RILs) that segregated for a single gene. Thatcher line 78–1 with *LrX* was crossed with Thatcher and individual F_2 seedlings and F_6 RILs were evaluated for leaf rust response. The 208 F_2 plants segregated for a single recessive gene and 148 F_6 lines for a single gene. The RILs and parents were characterized by genotyping by sequencing (GBS). Six GBS markers and five Kompetitive Allele-Specific PCR (KASP) markers were used to map *LrX* on the distal region of chromosome arm 1DS. *LrX* was 1 centiMorgan (cM) proximal to marker *K-IWB38437* and 0.4 cM distal to GBS marker *1D_9037138*. Line 78–1 was crossed with Thatcher wheat lines with *Lr21*, *Lr42*, and *Lr60* for allelism tests. *LrX* mapped 19.49 cM from *Lr21* and 11.93 cM from *Lr42*. In the cross of line 78–1 with the Thatcher line with *Lr60*, one recombinant in 1,003 F_2 plants was found. *LrX* and *Lr60* are at tightly linked loci on the distal region of chromosome arm 1DS. The gene in line 78–1 was designated as *Lr83*. Cytological examination of RL6149 provided no evidence of transfer of a chromosome segment of an A- or B-genome chromosome to chromosome 1D.

Introduction

Leaf rust, caused by *Puccinia triticina* Eriks., is a widespread and commonly occurring disease of wheat. On a worldwide basis, leaf rust is the leading cause of yield loss in wheat due to disease (Savary et al. 2019). The leaf rust pathogen is highly variable for biotypes or races that differ in ability to infect and cause disease on wheat lines with different leaf rust resistance genes. In the USA, 30–60 races are detected on an annual basis (Kolmer and Fajolu 2022). Currently 82 leaf rust resistance genes (Xu et al. 2022; McIntosh et al. 2022) in wheat have been mapped to chromosome locations and assigned gene designations. However, many of these genes no longer provide resistance, since the *P. triticina* population is highly dynamic as new virulent races increase rapidly in response to the release of cultivars with effective resistance genes. In the Great Plains region of the USA, cultivars with resistance genes *Lr2a*, *Lr21, Lr24, Lr26, Lr37,* and *Lr39* have all selected virulent races of *P. triticina* in the last 30 years (Kolmer 2019).

Given the dynamic nature of *P. triticina* populations, it is important to characterize germplasm for leaf rust resistance to find new effective resistance genes that can potentially be used in wheat breeding programs to improve leaf rust resistance. The leaf rust resistance gene *Lr64* derived from the Thatcher wheat near isogenic line RL6149 was mapped to chromosome 6AL (Kolmer et al. 2019). In the same set of F_7 recombinant inbred lines (RILs) that was used to map *Lr64*, a second unexpected leaf rust resistance

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gene that mapped to the distal region on chromosome arm 1DS also segregated. Kompetitive Allele-Specific PCR (KASP) markers based on 90 K Infinium iSelect single-nucleotide polymorphic (SNP) markers (Wang et al. 2014) were used to map the 1DS resistance gene. The objective of this study was to further map the 1DS leaf rust resistance gene in a set of RILs that segregated for a single gene with an increased number of markers, to characterize the resistance response with a number of *P. triticina* isolates, and to cytologically assess the putative wild emmer (*Triticum dicoccoides*) origin of the gene.

Materials and methods

Phenotyping and inheritance

The F_6 line 78–1 from a cross of Thatcher and RL6149 that lacked *Lr64,* but had a leaf rust resistance gene temporarily designated *LrX* on chromosome arm 1DS based on seedling infection type and flanking KASP markers (Kolmer et al. 2019), was crossed with Thatcher and F_2 seedlings were tested for leaf rust response with *P. triticina* race isolate BBBDB 1–1, which is avirulent to all of the leaf rust resistance genes in the standard US differential set (Kolmer and Fajolu 2022), except for virulence to the Thatcher wheat line with *Lr14a*. The F_2 seedlings, 78–1, and Thatcher were grown in segmented trays with 48 seedlings per tray, in a greenhouse at 20–23 °C. The seedlings were inoculated when the primary leaves were fully expanded at 7 d with a suspension of urediniospores in Soltrol 170 light mineral oil (Phillips Petroleum, Bartlesville, OK). After inoculation, the plants were dried for 1 h and placed in a dew chamber for 16 h at 18 °C. The seedlings were returned to a greenhouse bench at 20–23 °C with supplemental lighting for a 16 light period and fertilized with a 20–20–20 NPK solution. Infection type (IT) was scored 10 days after inoculation: plants with IT 0 (immune response); (hypersensitive fleck), 1 (small necrotic uredinia), and 2 (small size uredinia with prominent chlorosis) were considered resistant, and plants with IT 3 (moderate size uredinia lacking necrosis or chlorosis) to 4 (large uredinia) were considered susceptible. Plants that had mixed IT were described with the most common IT first. Symbols "–" and "+" denoted smaller or larger uredinia, respectively. Two hundred and sixteen F_2 plants were advanced to F_6 RILs. The F_6 RILs and parents were grown in segmented trays with 4–6 seedlings/RIL and were inoculated in two separate tests with isolate BBBDB 1–1 at 7 d when the primary leaves were fully expanded. The F_2 and F_6 RIL segregation ratios were assessed for conformity to expected Mendelian ratios by Chi-square tests (Steel and Torrie 1980).

Genotyping

The RILs that segregated for response in either test were not considered further for genotyping. The final number of RILs was also reduced to accommodate the genotyping procedure. High quality DNA from 148 RILs and parents was isolated using the method recommended for Diversity Arrays Technology (DArT) (Triticarte Pty Ltd, Canberra, Australia) (Akbari et al. 2006). DNA samples were processed at the University of Minnesota Genomics Center (Minneapolis, MN), using the genotyping-by-sequencing (GBS) (Poland et al. 2012) method. DNA was digested with *Msp*I and *Pst*I to generate 96-plex libraries and ligated with barcode adapters. The DNA libraries were sequenced on one lane of a Novaseq S1 flowcell to generate 100 base pair single end reads (Illumina, San Diego, CA). Only reads that had an average minimum quality score (Q) > 30 were retained. Depth for each RIL exceeded 5 M reads on average.

The filtered GBS reads were aligned with the reference genome Chinese Spring IWGSC RefSeq v2.1 (Zhu et al. 2021) assembly using default parameters in the Burrows-Wheeler Aligner 0.7.5a (Li and Durbin 2009), which was also used to index the reference genome. Variant calling was done using SAMtools 1.6 and BCFtools 1.6 (Li et al. 2009). Alleles were filtered for a minimum read depth of 10, minimum minor allele frequency of 3%, and maximum missing information of 20%. All heterozygous SNPs were converted to 'H' and missing alleles to 'N.' The SNPs that were H or N for either parent were removed, as were monomorphic SNPs. The SNPs were filtered to obtain sets of markers at 10 or 20% of missing data, respectively.

DNA of the RILs and parents was also genotyped with KASP markers *K-IWB38437, K-IWB577, K-IWA713,* and *K-IWB14612* (Table 1) that were used to map the 1DS leaf rust resistance gene in Kolmer et al. (2019). KASP genotyping followed the standard thermocycler protocol with reagent mixtures of 5 µL described in the LGC Genomics KASP product manual (https://biosearch-cdn.azureedge.net/assetsv6/KASP-genotyping-chemistry-User-guide.pdf). PACE Genotyping Master Mix (3CR Biosciences) was used. Fluorescence was read using a StepOnePlus Real-Time PCR System, and genotypes were determined using the StepOne v2.3 software (Applied Biosystems, Waltham, MA). KASP markers were also developed for the GBS-derived markers *1D_9037138* and *1D_10592628* (Table 1).

Mapping

Selected GBS markers on chromosome arm 1DS and the 1DS KASP markers were combined with the reactions of

Table 1 Sequences of KASP markers for the leaf rust resistance gene *LrX* on chromosome arm 1DS

KASP	Thatcher allele	78–1 allele	Allele-1-forward primer*	Allele-2-forward primer*	Common reverse primer
K-1D_9037138	G	A	GAAGGTGACCAAGTTCATGCTG GTTATTGTCACTCAGTCCAGCG	GAAGGTCGGAGTCAACGGATTA GGTTATTGTCACTCAGTCCAGCA	TAGCTTCCCGGCAGACACGGTT
K-1D_10592628	G	A	GAAGGTGACCAAGTTCATGCTA TCTTCACTGCATCAGCTTTGG	GAAGGTCGGAGTCAACGGATTCC TATCTTCACTGCATCAGCTTTGA	GAGATCAACCTGCCACCTGTGCA
K-IWB38437	T	C	GAAGGTGACCAAGTTCATGCTGCTACAATGGCTAGTGTGATCT	GAAGGTCGGAGTCAACGGATTGCTACAATGGCTAGTGTGATCC	ACAAATAGGGCATGGTACCTTT
K-IWB577	T	C	GAAGGTGACCAAGTTCATGCTCAAACTGAACGAGCAGGTGAT	GAAGGTCGGAGTCAACGGATTCAAACTGAACGAGCAGGTGAC	TCTCAATCCTAAGCATCCGGTTTA
K-IWA713	G	A	GAAGGTGACCAAGTTCATGCT CAACAGGAACTCGGGAGGG	GAAGGTCGGAGTCAACGGATTCAACAGGAACTCGGGAGGA	TGCTGTGAGATAACCGCCA
K-IWB14612	T	C	GAAGGTGACCAAGTTCATGCTCCACAGTCCACACAAAGCATAT	GAAGGTCGGAGTCAACGGATTCCACAGTCCACACAAAGCATAC	TGAGGTCTCGTGTTAATAACTGC

*Each allele-specific forward primer was tailed with a FAM (GAAGGTGACCAAGTTCATGCT) or HEX (GAAGGTCGGAGTCAACGGATT) oligo sequence

the F_6 RILs to map the segregating leaf rust resistance gene with MapMaker v2.0 for MacIntosh (Lander et al. 1987) using the Kosambi map function with logarithm of odds (LOD) of 10 and r of 0.3. Map order was confirmed with R/qtl (Broman et al. 2003).

Allelism tests

Line 78–1 was crossed with the Thatcher near isogenic lines with *Lr21* (RL6043) (McIntosh et al. 1995), *Lr60* (RL6172) (Hiebert et al. 2008), and *Lr42* (Thatcher*4/WGRC11) (Cox et al. 1994; J. A. Kolmer, unpublished data), and F_2 seedlings were inoculated with isolate BBBDB 1–1. Infection types were evaluated 10 d after inoculation. Seedlings that had higher IT than either parent were selected and grown out to F_3 lines. Twenty-five seedlings of each F_3 line were inoculated with isolate BBBDB 1–1 at 7 d after planting and evaluated for IT at 10 d post-inoculation. Lines that segregated for plants that had low and high IT were considered as being derived from F_2 plants heterozygous for leaf rust resistance gene(s), and lines that had plants that were all either resistant or susceptible were considered as being derived from F_2 plants that were either homozygous for a resistance gene, segregating for both genes in repulsion, or lacked both resistance gene(s).

Recombination frequency (RF) between linked leaf rust resistance genes was calculated based on 2 times the square root of the frequency of susceptible F_2 plants (*f*s), ($RF = 2\sqrt{fs}$) (Kuspira and Bhambhani 1984). Genotypes of F_2 plants were confirmed in F_3 progeny tests. The recombination frequency was converted to map distance using the Kosambi mapping function (Kosambi 1943). Thatcher, line 78–1, and the Thatcher lines with *Lr21, Lr42*, and *Lr60* were inoculated with nine *P. triticina* isolates that represented different races. All race designations were based on the five letter code used to describe virulence phenotypes in US populations of *P. triticina* (Kolmer and Hughes 2017). Thatcher lines with *Lr21, Lr42,* and *Lr60* were genotyped with KASP markers developed from GBS markers *1D_9037138* and *1D_10592628*, and KASP markers *K-IWB38437, K-IWB577*, *K-IWA713*, and *K-IWB1461*.

Cytology

Mitotic metaphase chromosome preparations from root tips of RL6149 (PBI accession C96.15) and Thatcher followed Lang et al. (2018). Fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH) procedures were performed as described in Li et al. (2020). For FISH, oligo probes Oligo-pSc119.2 and Oligo-pTa535 were 5′-end labeled with 6-carboxyfluorescein (6-FAM) and 6-carboxytetramethylrhodamine (Tamra) (Merck, Bayswater, Australia) producing green and red signals, respectively. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen Life Science, Carlsbad, CA, USA) and pseudo-colored blue.

FISH enabled identification of individual chromosomes. For GISH, total genomic DNA from *T. urartu* (genome AA) and *Aegilops tauschii* (genome DD) was labeled with fluorescein-12-dUTP and tetramethyl-rhodamine-5-dUTP (Roche, Basel, Switzerland), which fluoresced green and red, respectively. Total genomic DNA of *Ae. speltoides* was used as a blocker at a probe-to-blocker ratio of 1:10. GISH enabled distinction of the A-, B-, and D-genome chromosomes and identification of detectable intergenomic translocations if present.

Results

Inheritance and mapping

Individual F_2 seedlings of Thatcher × line 78–1 segregated 57 resistant with IT ;22^- to 2^+, and 151 susceptible with IT 3^+ to 4 when tested with isolate BBBDB 1–1, fitting a 1:3 ratio ($\chi^2 = 0.64$, $p = 0.42$) and indicating that 78–1 carries a recessive resistance gene. Over two tests, the F_6 RILs segregated 67 resistant and 81 susceptible, fitting an expected segregation of 1:1 ($\chi^2 = 1.31$, $p = 0.25$) and confirming a single gene in 78–1. After alignment of the GBS reads with the reference genome and filtering based on < 20% missingness and minimum read depth of 10, 11 SNPs on chromosome 1D were segregating in the RILs. Six markers that were completely linked with other markers were removed, and a preliminary map based on *LrX* and the GBS-derived markers *1D_9037138, 1D_10592628, 1D_12018690, 1D_15705818*, and *1D_20457400* were constructed. A final linkage map that combined the GBS markers and KASP markers for *IWB38437*, *IWB577*, *IWA713*, and *IWB14612* on chromosome arm 1DS (Table 1) and the resistance locus was constructed. *LrX* mapped to the distal region of chromosome arm 1DS, 1 cM proximal to *K-IWB38437* and 0.4 cM distal to *1D_9037138* (Fig. 1). The entire linkage map covered 48.1 cM.

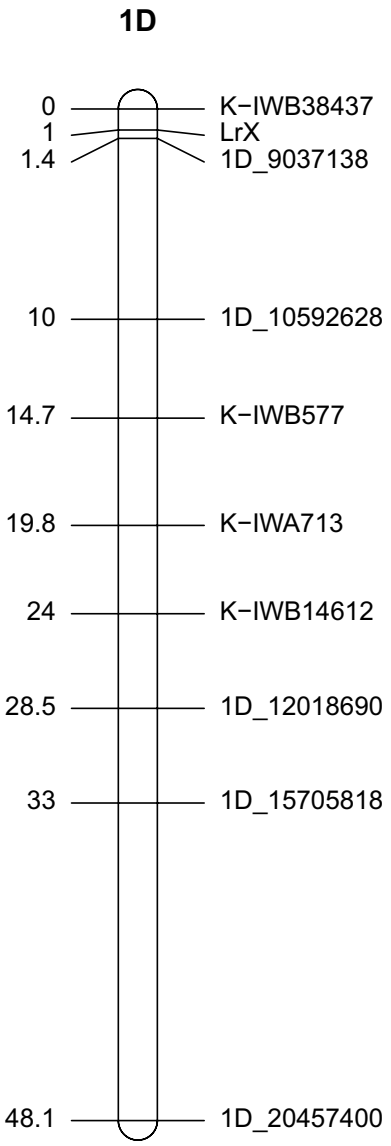

Fig. 1 Genetic map of chromosome arm 1DS derived from F_6 recombinant inbred lines of Thatcher line 78–1 with LrX, crossed with Thatcher. All distances are in centiMorgans

Allelism tests

Leaf rust resistance genes *Lr21, Lr42,* and *Lr60* are also located on chromosome arm 1DS. Tests of allelism were conducted to determine the relationship of *LrX* with each of these genes. To isolate BBBDB 1–1, line 78–1 had IT ;22^- and the Thatcher line with *Lr21* (Tc*Lr21*) had IT ;22^+. From two F_2 families of 78–1 × *TcLr21*, 654 F_2 plants had equal or lower IT than the parents, and 43 had IT higher than either parent. When progeny tested, six of the latter plants were classified homozygous susceptible with IT 3^+4, a recombination rate of 0.186 and map distance of 19.49 cM between *LrX* and *Lr21*. Two of the F_3 lines had plants that were all resistant (IT ;1–2^-), and 35 lines had plants that segregated for IT ;1–3^+.

The Thatcher line with *Lr42* had IT ;12^- to isolate BBBDB 1–1. From one F_2 family of 78–1 × Tc*Lr42*, there were 253 plants with equal or lower IT than the parents and 39 with IT higher than either parent. With isolate BBBDB 1–1, progeny tests of the latter 39 plants identified one homozygous susceptible (IT 3^+) F_3 family, indicating a recombination rate of 0.117 and map distance of 11.93 cM. Sixteen of the F_3 lines segregated for plants with IT ranging from ;1 to 22^+ and 22 lines had plants with IT ranging from ;1 to 3^+.

The Thatcher line with *Lr60* had IT ;12^+ to isolate BBBDB 1–1. Four F_2 families of 78–1 × *TcLr60* included 1,003 plants with IT equal to, or lower than, the parents, and 13 had IT higher than either parent to isolate BBBDB 1–1. Twelve of the F_3 lines derived from the latter group segregated for IT ;1^- to 22^+ and one segregated for IT ;22^+ and IT 3^+. The remaining seeds of this line were planted and tested with isolate BBBDB 1–1. From this line a total of 13 plants had IT ;22^+ and 70 plants had IT 3^+, approaching a good fit to a 1:3 ratio ($\chi^2 = 3.86$, $p = 0.049$) for segregation of a single recessive gene. Thirty F_2 plants with resistant IT were progeny tested with isolate BBBDB 1–1; two lines segregated for plants with IT ;1 and 3^+, and the other 28 lines segregated for plants with IT ;1^- to ;22^+. Although the progeny tests indicated that the genes are not allelic, the genetic distance between them could not be calculated because no homozygous susceptible F_2 plants were identified.

KASP haplotypes and resistance phenotyping

The six KASP markers that mapped closest to *LrX* were used to genotype Tc*Lr21,* Tc*Lr42*, and Tc*Lr60* (Table 2). Thatcher, Tc*Lr42,* and *TcLr21* had identical alleles for all six KASP markers; line 78–1 and Tc*Lr60* had the same alleles for all six KASP markers and differed from those for Thatcher. Three resistant and nine susceptible plants from the segregating F_3 family from cross 78–1 × Tc*Lr60* were genotyped for the six KASP markers; all 12 plants had the same KASP genotypes as the parents.

The responses of line 78–1, Tc*Lr21*, Tc*Lr42*, and Tc*Lr60* were also compared using a panel of nine *P. triticina* races (Table 3). Line 78–1 had intermediate IT of 22^+ to all isolates tested, except for isolate MBDSD 8–1 which had a slightly lower IT ;22^+. The Thatcher lines with *Lr42* and *Lr60* had slightly lower IT than 78–1 for all isolates. Isolates TBBGS 95–1 and TNBJS 44–1 had high IT of 3^+4 to Tc*Lr21* and low IT to 78–1, Tc*Lr42* and Tc*Lr60*.

Cytology

Cytogenetic characterization of RL6149 and Thatcher failed to detect a translocation of an A- or B-genome chromosome segment to chromosome 1D (Fig. 2).

Discussion

Segregation of the F_6 RILs and mapping data with previously developed KASP markers and new GBS markers confirmed that a single leaf rust resistance gene was present on the distal region of chromosome arm 1DS. This gene conditions an intermediate IT and is recessive in expression. The map order and cM distances of the KASP markers that are in common in the new and previous (Kolmer et al. 2019) maps are very similar. In both maps, the closest KASP marker to the 1DS gene is *K-IWB38437*, with *K-IWB577* 13.7 cM proximal to *LrX* in the current map, and 11.2 cM proximal in the previous map.

Table 2 Alleles for KASP markers for line 78–1, Thatcher and Thatcher (Tc) wheat lines with leaf rust resistance genes on chromosome 1DS

Wheat line	KASP marker					
	K-IWB38437	*K-*1D_*9037138*	*K-1*D_*10592628*	*K-IWB577*	*K-IWA713*	*K-IWB14612*
78–1	C	A	A	C	A	C
Tc*Lr60*—RL6172	C	A	A	C	A	C
Tc*Lr21*—RL6043	T	G	G	T	G	T
Tc*Lr42*—Tc*4/WGRC11	T	G	G	T	G	T
Thatcher	T	G	G	T	G	T

Table 3 Infection types of line 78–1, Thatcher and Thatcher (Tc) wheat lines with leaf rust resistance genes on chromosome 1DS to nine *Puccinia triticina* isolates

Wheat line	Isolate								
	MNPSD 33–3	TFTSB 21–3	TCRKG 108–3	MBDSD 8–1	TBBGS 95–1	KFBJG 64–1	MCTNB 185–1	TNBJS 44–1	MJBJG 180–1
78–1	22^+	22^+	;2	;22^+	2^+	22^+	22^+	22^+	22^+
Tc*Lr21*- RL6043	2^+	2^+	;12^-	2^+	3^+4	22^-	2^+	3^+4	;12
Tc*Lr42*-Tc*4/WGRC11	;12^-	;12^-	22^-	;2^-	22^+	2	;12^-	2^+	22^-
Tc*Lr60* -RL6172	;2	;12^-	12	;12^+	;22^+	;22^+	22^+;	;12^-	22+;
Thatcher	3^+4	3^+4	3^+4	3^+4	3^+4	3^+4	3^+4	3^+4	3^+4

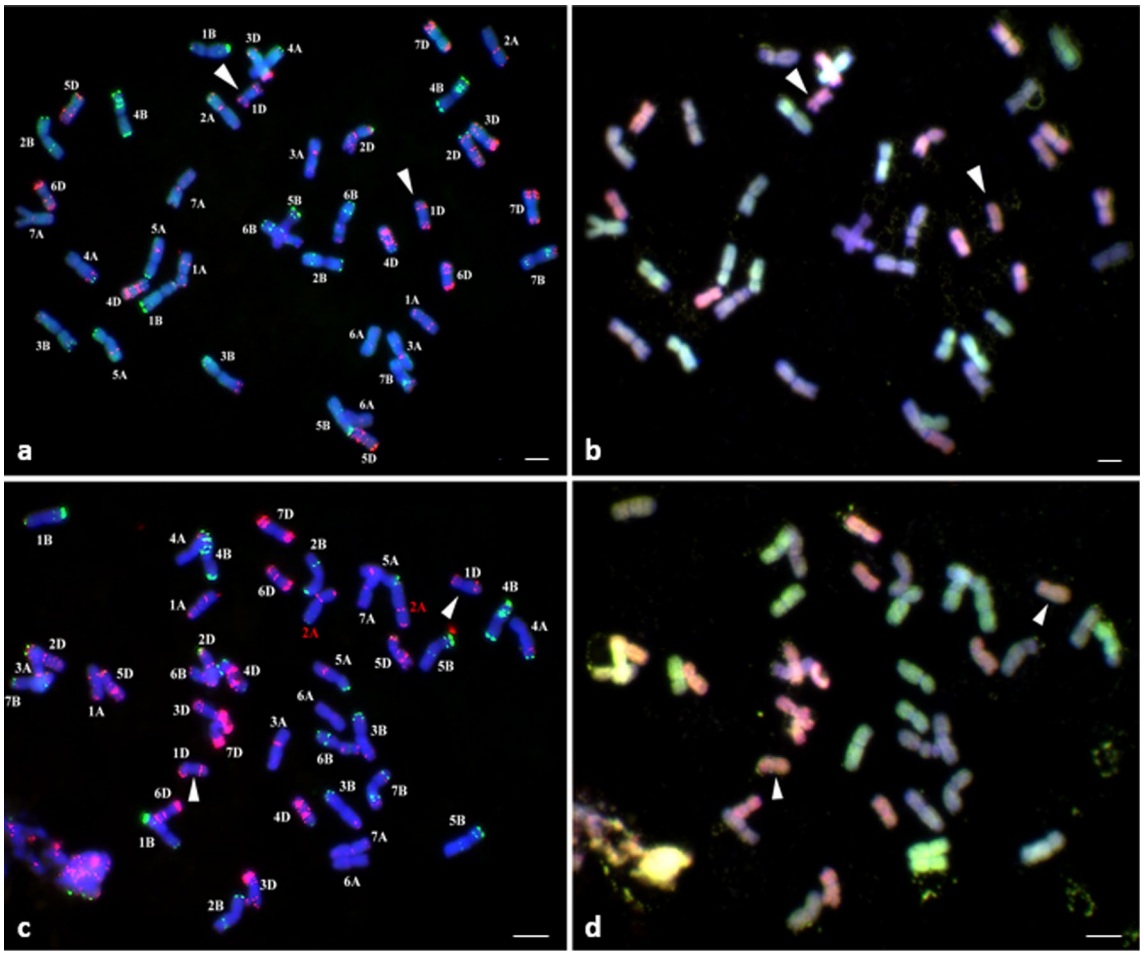

Fig. 2 Cytological analysis of chromosomes of Thatcher (**a**, **b**) and Thatcher near isogenic line RL6149 (**c**, **d**). Sequential FISH (**a**, **c**) and GISH (**b**, **d**) were performed on the same metaphase cells (**a** and **b**, **c** and **d**). Chromosomes were hybridized with probes Oligo-pSc119.2–1 labeled with 6-carboxyfluorescein (6-FAM) and Oligo-pTa535-1 labeled with 6-carboxytetramethylrhodamine (Tamra) to generate green and red signals, respectively, enabling identification of individual chromosomes. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and pseudo-colored blue. For GISH, total genomic DNA from *T. urartu* and *Aegilops tauschii* was labeled with fluorescein-12-dUTP and tetramethyl-rhodamine-5-dUTP, which fluoresced green and red, respectively. Total genomic DNA of *Ae. speltoides* was used as a blocker, and chromosomes were counterstained with DAPI and pseudo-colored blue. Arrowheads point to chromosome 1D. No A- or B-genome chromatin was detected in chromosome 1D. Bars, 10 µm

Tests of allelism using F_2 plants and selected F_3 lines derived from crosses of line 78–1 with Thatcher lines possessing *Lr21* and *Lr42* indicated that *LrX* was not allelic with these genes. A considerable number of line 78–1 × Tc*Lr42* F_2 plants had higher IT than either parent, but only one F_3 line was homozygous susceptible. Since the gene in line 78–1 was recessive, most of the F_2 plants (German and Kolmer 1992) with higher IT were likely heterozygous and segregated in F_3 lines. An increased number of F_2 plants would need to be tested to calculate a more accurate map distance between the two genes. Gene *Lr42* was mapped to the distal region of 1DS in both hexaploid wheat (Gill et al. 2019) and the diploid donor *Ae. tauschii* (Lin et al. 2022). In a similar manner, a large number of F_3 lines from the line 78–1 × Tc*Lr21* cross also segregated for resistant and susceptible plants, likely caused by F_2 plants that were heterozygous for one of the two resistance genes.

Although no F_3 line from line 78–1 × Tc*Lr60* was homozygous susceptible, the presence of susceptible plants in one line derived from an F_2 plant with higher IT indicated that *Lr60* and *LrX* were not at the same locus. These genes are tightly linked in the distal region of chromosome arm 1DS. The F_2 plant from which the F_3 line was derived was heterozygous for *LrX* or *Lr60*. Since the susceptible plants from this F_3 line had KASP genotypes identical to both parents, the plants were derived from the cross and did not originate from pollen contamination from the other parents. The segregation of plants with IT lower than the two parents in F_3 lines that were homozygous resistant also indicated that line 78–1 and Tc*Lr60* had

different genes. Although over 1,000 F_2 plants from 78 to 1 × Tc*Lr60* were tested, more would be needed to increase the probability of obtaining plants that lacked either *Lr60* or *LrX*. Hiebert et al. (2008) estimated that *Lr60* was 13.5 cM to distal to *Lr21*, compared to the 19.49 cM map distance between *LrX* and *Lr21* estimated in the present work. Based on the two mapping studies, *LrX* is distal to *Lr60*.

The tetraploid *T. dicoccoides* line 8404 (Dyck 1994) was the putative source of resistance in RL6149 and therefore should be the source of *Lr64* and *LrX*. Since *LrX* mapped to chromosome 1DS, the origin of this gene, and presumably also *Lr64*, is problematic. As indicated by Kolmer (2019), the most likely possibility was that line 8404 was incorrectly identified or was a mixture of tetraploid and hexaploid genotypes, from which a hexaploid individual was the resistant source for RL6149. This is supported by the FISH and GISH analyses (Fig. 2) that provided no evidence of transfer of chromatin from an A- or B-genome chromosome to chromosome 1D, although transfer of a chromosome segment smaller than the level of detection by GISH remains a remote possibility.

LrX conditioned an intermediate IT to isolates of nine races of *P. triticina*. Wheat lines with *LrX* singly are predicted to have moderate levels of resistance in field plots based on the seedling IT. Combinations of *LrX* and other effective leaf rust resistance genes are likely to have lower infection types and improved field resistance. In the F_2 plants and F_3 lines derived from crosses of line 78–1 with Tc*Lr21*, Tc*Lr42,* and Tc*Lr60*, plants with lower IT than either parent were observed. As noted previously (German and Kolmer 1992; Samborski and Dyck 1982), combinations of effective leaf rust resistance genes, whether adult plant resistance genes only, or genes effective in both seedling and adult plants, usually exhibit lower seedling IT and lower severity levels in field plots. *LrX* is permanently designated as *Lr83*. Line 78–1 line has been accessioned in the USDA Small Grains Collection as PI 701,502, small samples of which are available upon request at the USDA-ARS Cereal Disease Laboratory or the USDA National Small Grains Collection.

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Author contribution statement JAK designed the study, developed the mapping population, phenotyped the population, mapped the population, and prepared the manuscript. PB carried out allele calling using the GBS reads, filtered the markers, and revised the manuscript. JL and PZ performed the cytological analyses and revised the manuscript. MNR designed the KASP markers, conducted the KASP assays and revised the manuscript.

Data availability Data will be made available upon reasonable request.

Declarations

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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