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Lesion mimic associates with adult plant resistance to leaf rust infection in wheat

Tao Li · Guihua Bai

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Abstract Lesion mimics (LM) that resemble plant disease symptoms in the absence of plant pathogens may confer enhanced plant disease resistance to a wide range of pathogens. Wheat line Ning7840 has adult plant resistance (APR) to leaf rust (*Puccinia triticina*) and shows LM symptoms at heading. A recessive gene (*lm*) was found to be responsible for LM in Ning7840 and located near the proximal region of chromosome 1BL using a population of 179 recombinant inbred lines (RIL) derived from the cross Ning7840/Chokwang. Genomic in situ hybridization showed that Ning7840 carries the short arm of 1R chromosome from rye (*Secale cereale* L.), on which the race-specific gene *Lr26* resides. The RILs were infected with the isolate PRTUS 55, an isolate virulent to *Lr26*, at anthesis in two greenhouse experiments. The result showed that the lines with LM phenotype had a significantly higher rust resistance than the non-LM lines. Composite interval mapping consistently detected a QTL, *Qlr.pser.1BL*, for APR on chromosome 1BL. *Qlr.pser.1BL* peaked at *lm* and explained up to 60.8% of phenotypic variation for leaf rust resistance in two greenhouse experiments, therefore, *lm* from Ning7840 may have pleiotropic effects on APR to leaf rust.

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T. Li
Department of Plant Pathology,
Kansas State University, Manhattan, KS 66506, USA

G. Bai (✉)
USDA-ARS Plant Science and Entomology Research Unit,
Manhattan, KS 66506, USA
e-mail: guihua.bai@ars.usda.gov

Introduction

Lesion mimics (LM) resemble leaf disease symptoms but occur spontaneously without attack by any pathogen. Both dominant and recessive LM mutants have been reported in several plant species such as *Arabidopsis* (Pilloff et al. 2002; Ishikawa et al. 2003; Ishikawa 2005), barley (Rostoks et al. 2003), rice (Kang et al. 2007; Mori et al. 2007), and maize (Hu et al. 1998; Penning et al. 2004). To date, at least 12 genes have been cloned in different plant species associated with LM (Boch et al. 1998; Greenberg et al. 2000; Mach et al. 2001; Brodersen 2002; Shirano et al. 2002; Yamanouchi et al. 2002; Lorrain et al. 2004; Wang et al. 2005; Rostoks et al. 2006; Lee and McNellis 2009). In maize, several genetic loci may contribute to LM phenotypes because a high frequency of non-allelic LM mutations was observed (Walbot et al. 1983). Phenotypes of LM mutants can range from a hypersensitive-reaction (HR)-like fleck to large chlorotic or necrotic lesions.

Genes that regulate expression of LM symptoms in plants may play an important role in signal pathways of plant defense (Lorrain et al. 2003; Campbell and Ronald 2005; Johal et al. 2005). Some mutants with LM phenotype confer broad-spectrum resistance to diverse pathogens. In *Arabidopsis*, two LM mutants, the *lsd1* (lesion simulating disease) and *acd* (accelerated cell death), showed resistance to the bacterial pathogen *Pseudomonas syringae* and oomycete *Peronospora parasitica* (Greenberg and Ausubel 1993; Dietrich et al. 1994; Rate et al. 1999). In barley, the *mlo* (a powdery mildew resistance locus o) mutant that expressed LM conferred race-nonspecific resistance to all known races of the powdery mildew pathogen (*Blumeria graminis* f.sp. *hordei*) (Jarosch et al. 1999). In rice, LM mutants *cdr1* (cell death and resistance), *cdr2*, *cdr3*, and *ebr3* (enhanced blast resistance) showed increased resistance

to the rice blast fungus (*Magnaporthe grisea*), and *ebr3* also showed enhanced resistance to the bacterial pathogen *Xanthomonas oryzae* pv. *oryzae* (Takahashi et al. 1999; Campbell and Ronald 2005). Some *spl* (spotted leaf) mutants conferred race-nonspecific resistance to rice blast and bacterial blight (Yin et al. 2000). For example, *Spl18* mutant showed enhanced resistance to blast disease through accumulation of phytoalexins and up-regulation of pathogenesis-related (PR) proteins (Mori et al. 2007). Rice mutant *blm* (blast lesion mimic) spontaneously formed necrotic lesions and provided a high level of resistance against all known races of the rice blast fungus (Jung et al. 2005). LM phenotypes were also observed when known disease resistance-related genes such as genes for beta-glucanase and *NPR1* (non-expressor of PR1) homolog were overexpressed in rice (Nishizawa et al. 2003; Chern et al. 2005). These observations suggest that LM may spontaneously activate expression of defense genes. Some LM mutants have been used as models for deciphering cell death signal pathways because they spontaneously display programmed cell death and constitutive defense responses (Lorrain et al. 2003).

Only a few examples of LM have been reported in wheat. In these cases, LM mutants were induced by either mutagen treatment or a transgenic approach (Boyd and Minchin 2001; Boyd et al. 2002; Anand et al. 2003; Kamlofski et al. 2007). Some of these mutants expressed HR-like phenotypes in a pathogen-free environment, and exhibited enhanced resistance to powdery mildew and rusts (Boyd and Minchin 2001; Boyd et al. 2002; Anand et al. 2003; Smith et al. 2004; Kamlofski et al. 2007). However, our knowledge of LM effect on wheat disease resistance is limited and the chromosome locations of the genes underlying the LM trait have not been determined.

Ning7840 is a Chinese wheat line that shows LM symptoms on the leaf blade around heading and resistance to leaf rust at both seedling and adult plant stages. The LM symptom in Ning7840 resembles typical initial response of wheat to leaf rust infection. However, whether LM has a pleiotropic effect on the expression of leaf rust resistance in Ning7840 remains unknown. The objectives of this study were to (1) characterize genes for LM trait in Ning7840 and locate it to a specific chromosome arm and (2) elucidate the genetic relationship between LM and APR to leaf rust resistance.

Materials and methods

Plant materials and LM evaluation

A population of 179 F_8 recombinant inbred lines (RIL) was developed from the cross of Ning7840/Chokwang by single seed descent. Ning7840 is a Chinese wheat line that has both leaf rust resistance and LM; Korean wheat cultivar Chokwang is susceptible to leaf rust and does not have LM. Two parents and their RILs were vernalized at 4°C in a growth chamber for 7 weeks and transplanted into 4″ × 4″ plastic pots on a greenhouse bench at 17 ± 2°C (night) and 22 ± 5°C (day) with supplemental light for 12 h. Each experiment was arranged in a randomized-complete-block design with two replicates (pots) and five plants per replicate. The experiment was repeated twice in the greenhouses at Kansas State University, Manhattan, KS, in fall 2007 and spring 2008. LM symptoms were scored at anthesis and recorded as presence (1) or absence (0) of LM symptoms on the flag leaf. A consensus LM rating for each RIL was derived based on consistent ratings from the two experiments and used for mapping.

Leaf rust evaluation

To identify an appropriate isolate for rust evaluation at adult plant stages, 15 isolates of *Puccinia triticina* were used to inoculate the two parents at the seedling stage. Inoculum was prepared by making a spore suspension in solitron and sprayed uniformly onto the leaves. Inoculated plants were incubated in a moist chamber at 15°C with 100% relative humidity overnight and then moved to a greenhouse bench at 17 ± 2°C (night) and 22 ± 5°C (day) with supplemental light for 12 h. Rust symptoms were scored 14 days after inoculation on a scale ranging from 1 (resistant) to 4 (susceptible) (McIntosh et al. 1998) where 1 = pustules with a few or no spores surrounded by necrotic tissue, 2 = small spore-producing pustules surrounded by necrotic tissue, 3 = spore-producing pustules surrounded by chlorotic tissue, and 4 = large spore-producing pustules surrounded by green tissue. To determine adult plant leaf rust resistance, the rust culture virulent to both parents at seedling stage was chosen to inoculate both parents and their RILs at anthesis in fall 2007 and spring 2008 in a greenhouse at Kansas State University, using the same method as described for seedling inoculation. Rust inoculation was performed immediately after LM evaluation in the same experiments. Adult plant resistance (APR) was scored 14 days after inoculation as described for the seedling test (McIntosh et al. 1998). In spring of 2007, both parents and RILs were also evaluated for rust resistance under natural infection conditions at the Rocky Ford Research Farm of Kansas State University, Manhattan, KS, and rust was scored at anthesis as described for the greenhouse experiments.

Genotyping and cytological methods

Two-week-old wheat leaves of each RIL were dried in a freeze dryer (ThermoSavant, Holbrook, NY, USA) for

48 h and ground to fine powder in a Mixer Mill (MM 300, Rotsch, Germany) for DNA isolation using the CTAB method (Saghai-Maroof et al. 1984). Two DNA bulks were constructed separately from ten LM and ten non-LM RILs for marker screening. A total of 610 SSR primers, including BARC (Song et al. 2005), GWM (Röder et al. 1998), WMC (Somers et al. 2004), GDM (Pestsova et al. 2000), CFA, CFD (Guyomarc'h et al. 2002, Sourdille et al. 2003) and covering all 21 wheat chromosomes (at least 8 from each short arm and 18 from each long arm), were screened between the parents. Polymorphic primers between the parents were used to screen the bulks, and the polymorphic primers between the bulks were then used to screen all RILs. All SSR markers available on 1BL were screened after markers on 1BL were found to be associated with LM. A 10-μL PCR mixture used for SSR analysis contained 50 ng templates DNA; 1 mM each of reverse, M13-tailed forward primers, and a M13 primer labeled with fluorescence-dye of FAM, VIC, PET or NED; 0.2 mM each of dNTP; 1× PCR buffer; 2.5 mM $MgCl_2$; and 0.6 U of *Taq* Polymerase. A touch-town PCR program was used for PCR amplification following Yu et al. (2006). Amplified PCR fragments were separated in an ABI 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). All marker data were scored using GeneMarker 1.6 (Softgenetics LLC, State College, PA, USA) and visually inspected twice to remove ambiguous data.

Rye genomic DNA was used as probe, and wheat DNAs from Ning7840, Aurora and Chokwang were used as templates. Genomic probe labeling, hybridization, and washing followed Langdona et al. (2000). Fluorescent images were captured by a cooled CCD camera and assigned false color.

Data analysis

A genetic linkage map was constructed with the markers using JoinMap version 3.0 (Plant Research International, Wageningen, The Netherlands). Genetic distance between markers was measured using centiMorgans (cM) based on the Kosambi function (Kosambi 1944). Threshold of logarithm of odds value was set at 4.0 to claim linkage between markers with a maximum recombination fraction at 0.4. Analysis of variance (ANOVA), correlation, and *t*-tests were carried out by using SAS software (SAS Institute Inc., Cary, NC, USA).

Single marker analysis and composite interval mapping were performed using WinQTL Cartographer version 2.5 (North Carolina State University, Raleigh, NC, USA). Mean rust score for each RIL collected from each individual experiment (field and greenhouse) and from combined two greenhouse experiments were used for QTL analysis. Threshold for declaring a significant QTL was determined by 1,000-permutation tests.

Results

LM in parents and RIL

In the absence of any visible pathogen, small yellowish spots appeared randomly on both sides of green leaves of Ning7840 around the heading stage. The spots were not observed in Chokwang under the same conditions. The RILs segregated as LM and non-LM types. About half of the RILs produced LM spots of varied sizes and densities. Some LM RILs had more but smaller spots than those in Ning7840, while others had fewer but larger spots. As plants grew, a small, brownish dot appeared in the center of yellowish spots in some RILs (Supplemental Fig. 1). The consensus LM ratings over three experiments showed 1:1 segregation ($P = 0.0856$), suggesting that a single Mendelian factor mainly controls LM in the population. Variation in the size and density of spots among RILs suggested that some modifying genes and/or environmental factors might also affect LM expression in the population. The F_1 plants from the cross of Ning7840/Chokwang showed non-LM phenotypes, suggesting that the gene underlying LM is recessive, therefore designated as *lm*. Pedigree analysis indicated that none of the parents of Ning7840 (Aurora/Anhui 11//Sumai 3) has LM, suggesting that the LM in Ning7840 is most likely from a recent natural mutation during breeding process.

Chromosome location of *lm*

A total of 610 SSR primers were screened between two parents, 216 primers displayed polymorphism between parents, but only markers from 1B, including *Xgwm264.1*, *Xwmc85.1*, *Xgwm131.2*, *Xbarc181*, and *Xbarc61*, showed polymorphism between LM and non-LM bulks, suggesting that the *lm* is on chromosome 1B.

Genomic in situ hybridization (GISH) using rye genomic DNA as probe showed that the short arm of 1B in Ning7840 was completely replaced by the entire short arm of 1R chromosome from rye (Supplemental Fig. 2). This 1BL.1RS translocation was inherited from Aurora, one of its parents. To determine the arm location of *lm*, four rye-specific markers (*Xscm9*, *Xryenor*, *Xib267* and *Xrems1303*) from 1RS were analyzed in the RIL population. These four dominant markers were only present in Ning7840 and Aurora, and absent in Sumai3 and Anhui11, two other parents of Ning7840. That the markers from 1RS co-segregated with each other in the RIL population confirmed the result from GISH that Ning7840 carried the

Table 1 Coefficient of determination (R^2) of markers on 1BL.1RS for mean lesion mimics (LM) from spring and fall 2007 and spring 2008 greenhouse experiments at Kansas State University and leaf rust infection type (IT) evaluated in fall 2007 (IT07) and spring 2008 (IT08) greenhouse experiments at Kansas State University, and spring 2007 field experiment (ITn) at Manhattan, KS

Marker[a]	LM[b]	ITn	IT07	IT08	ITm[c]
Xscm9	0.246	0.082	0.107	0.177	0.183
lm		0.354	0.446	0.503	0.608
Xwmc85.1	0.270	0.108	0.128	0.186	0.203
Xgwm264.1	0.213	0.093	0.096	0.178	0.178
Xbarc181	0.174	0.073	0.046	0.110	0.098
Xgwm131.2	0.170	0.079	0.042	0.095	0.087
Xbarc61	0.122	0.097	0.056	0.056	0.074

[a] *Xscm9* is from 1RS of rye and co-segregated with *Xib267*, *Xrems1303* and *Xryenor* in the Ning7840/Chokwang population. The other five markers are SSR from wheat chromosome 1BL

[b] LM: Consistent lesion mimic ratings over spring and fall 2007, and spring 2008 experiments in a greenhouse at Kansas State University, Manhattan, KS

[c] ITm: mean infection type over IT07 and IT08 greenhouse experiments

Table 2 Test of independence between lesion mimics (LM) and 1RS diagnostic marker (*Xscm9*) and closest marker to *lm* on 1BL chromosome

Marker[a]	LM+[b]	LM−	Total	X^2	*P* value
Xwmc85.1N	50	28	78	23.69	1.13E-06
Xwmc85.1C	28	73	101		
Total	78	101	179		
Xscm9+	51	17	69	44.04	3.21E-11
Xscm9-	27	84	110		
Total	78	101	179		

[a] *Xwmc85.1N* represents the allele from Ning7840 and *Xwmc85.1C* represents the allele from Chokwang; *Xscm9+* represents presence of the target band in the RIL and *Xscm9−* represents absence of the target band in the RIL

[b] LM+ refers to number of the RILs that showed LM in all three experiments; LM− refers to number of the RILs that did not show LM in three experiments

1BL.1RS translocation and recombination between 1RS and 1BS did not occur in the population. Single marker analysis indicated that LM was significantly correlated with most of the tested markers on 1BL.1RS (Table 1), but it did not co-segregate with 1RS (Table 2) because some RILs that carried 1RS did not show LM. The results suggested that *lm* was linked to 1RS, but unlikely on 1RS chromosome. Marker *Xgwm264.1* was previously mapped to 1BL chromosome proximal to the centromere, therefore, *lm* was more likely on the long arm of 1B chromosome within the deletion bin C1BL6-0.32 between marker *Xwmc85.1* or *Xgwm264.1* and the centromere (Supplemental Fig. 3).

Leaf rust resistance

Fifteen different leaf rust cultures including one avirulent culture, PRTUS BBB, as control, were used to inoculate seedlings of Ning7840 and Chokwang. Ning7840 showed a high level of resistance to ten cultures and susceptibility to five others. The resistance response of Ning7840 to seedling rust infection resembled that of leaf rust resistance gene *Lr26* derived from 1RS, suggesting that the seedling resistance of Ning7840 to leaf rust was likely mediated by the race-specific resistance gene *Lr26*. Thirteen isolates could clearly differentiate rust resistance between Ning7840 and Chokwang except for PRTUS 55 and PRTUS 49 at the seedling stage. The isolate PRTUS 55 was chosen to evaluate adult plant resistance to leaf rust because it was virulent to both parents in the seedline test. When inoculated with PRTUS 55 after heading, Ning7840 consistently showed moderate resistance with infection type (IT) of 22+ in the two greenhouse experiments, and Chokwang showed IT of 4 in fall 2007 and 33+ in spring 2008 (Fig. 1). The RIL population segregated for resistance to PRTUS 55, ranged from moderately resistant to highly susceptible with most RILs being susceptible (Fig. 1). Although the average IT of the RIL population in the 2007 experiment was slightly higher than that of the 2008 experiment, a significant correlation was observed between the ITs of RILs from the two greenhouse experiments ($P < 0.0001$) (Table 3). Transgressive resistant RILs were not observed in either greenhouse experiment. In the field experiment, rust severities of Ning7840 and the RILs were slightly lower than those in the greenhouse experiments, and Ning7840 was highly resistant (IT = 1), and Chokwang was moderately susceptible (IT = 3). About 13% of RILs showed resistance as high as Ning7840. Significant positive correlations of IT among RILs were observed between field and greenhouse data ($P < 0.0001$) (Table 3).

Relationship between LM and rust resistance

Significant negative correlation were observed between LM ratings and leaf rust IT from the two greenhouse experiments ($r = -0.71$, $P < 0.0001$) and one field experiment ($r = -0.51$, $P < 0.0001$) (Table 3). In general, RILs with LM showed a significantly lower IT than non-LM RILs in all three experiments (Fig. 2), suggesting that the expression of LM likely played an important role in enhancing leaf rust resistance in adult plants. It is more likely that *lm*, not *Lr26*, mediated APR in Ning7840. Interaction was not found between *lm* and *Lr26* (Table 4).

Single marker analysis showed that all markers on 1BL chromosome were significantly associated with adult plant

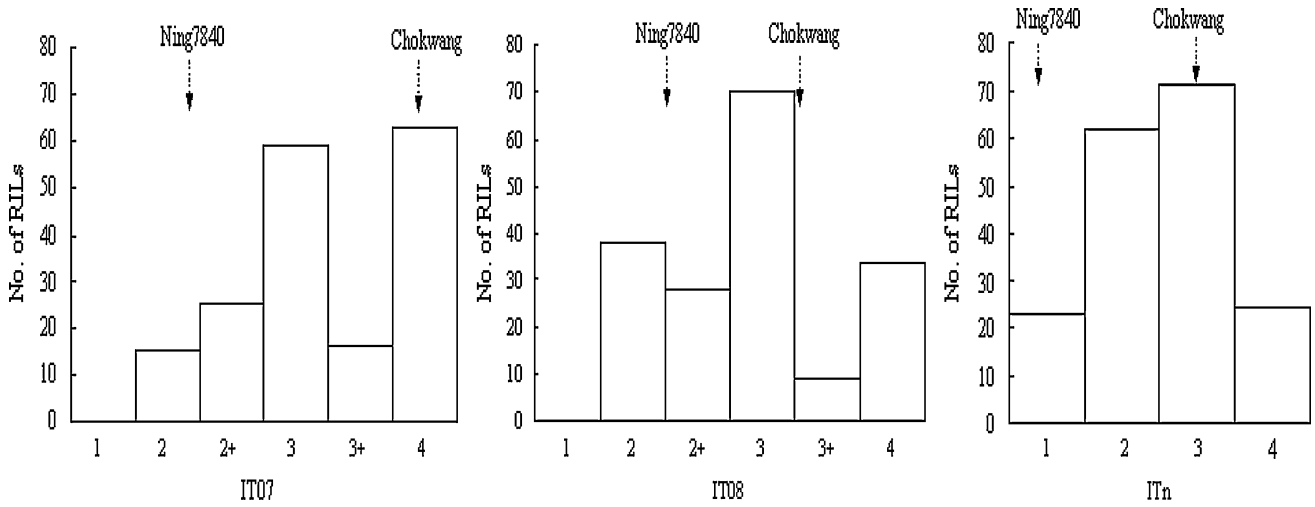

Fig. 1 Frequency distributions of leaf rust infection types (IT) for parents and recombinant inbred lines (RIL) from both greenhouse and field experiments. IT07 and IT08 refer to infection type (IT) from fall 2007 and spring 2008 greenhouse experiments respectively, and ITn refers to IT from spring 2007 field experiment at Manhattan, KS. IT scale: 1(R) = pustules with few or no spores surrounded by necrotic tissue, 2(MR) = spore-producing pustules surrounded by necrotic tissue, 3(MS) = spore-producing pustules surrounded by chlorotic tissue, and 4(S) = spore-producing pustules surrounded by green tissue; + = uredinia somewhat larger than normal for the IT

Table 3 Correlation among lesion mimics (LM) and leaf rust infection types (IT) from both greenhouse and field experiments

	IT07	IT08	ITm	ITn
LM	0.64****	0.69****	0.75****	0.59****
IT07		0.55****	0.88****	0.67****
IT08			0.89****	0.45****
ITm				0.64****

**** Significant at $P < 0.0001$

LM: Consistent ratings over spring and fall 2007, and spring 2008 experiments in a greenhouse at Kansas State University, Manhattan, KS; ITm: mean infection type (IT) over fall 2007 (IT07) and spring 2008 (IT08) greenhouse experiments at Kansas State University; ITn: IT from spring 2007 field experiment at Manhattan, KS

resistance in three experiments, but *lm* showed the strongest association with leaf rust resistance in both greenhouse ($R^2 = 0.608$) and field ($R^2 = 0.354$) experiments. Composite interval mapping consistently detected a QTL (*Qlr.pser.1BL*) for leaf rust resistance in the same 1BL chromosome region from all three experiments. *Qlr.pser.1BL* peaked at *lm* and explained 60.8 and 35.4% of phenotypic variation for average leaf rust IT from the greenhouse and field experiments, respectively (Fig. 3; Table 1).

Discussion

A new type of lesion mimic in wheat

Lesion mimic on Ning7840 likely originated from natural mutation and is different from those reported previously. LM in Ning7840 expressed as small, discrete, yellowish spots exclusively on leaf blades after heading; whereas the previously reported ones were from EMS-induced mutants, started with white lesions on wheat leaves at fifth leave stage, and then spread to leaf sheaths and later to spike tissues (Kamlofski et al. 2007). Thus, the LM symptoms on Ning7840 were different from these on EMS-induced wheat mutants but resembled to those of *spl6* (spotted leaf) mutant of rice (Kang et al. 2007).

Chromosomal location of *lm*

High correlations between LM and all markers on 1RS and the two markers on 1BL (Table 1) suggests that *lm* may be either on 1RS or 1BL close to centromere. However, frequent recombinations (25%) between the 1RS diagnostic marker *Xscm9* (Saal and Wricke 1999) and *lm* (Table 2) excludes the possibility of *lm* on the 1RS chromosome, because spontaneous crossover between 1RS and 1BS should not occur at presence of the *Ph1* gene in wheat genome (Rogowsky et al. 1993). GISH result provided further evidence to support that recombination did not occur between 1BS of wheat and 1RS of rye (*Secale cereale* L.) in Ning7840 (Supplemental Fig. 2). This conclusion was also supported by co-segregation of *Xscm9* with other rye SSR markers across 1RS (Koebner 1995; Lapitan et al. 2007). Linkage mapping located *lm* at 15 cM away from the closest markers *Xwmc85.1* and *Xwmc264.1* (Supplemental Fig. 3) in the deletion bin C-1BL6-0.32 on 1BL proximal to the centromere (Sourdille et al. 2004). More recombinants between *lm* and *Xwmc85.1* than between *lm* and *Xscm9* suggested that *lm* appeared to be closer to the

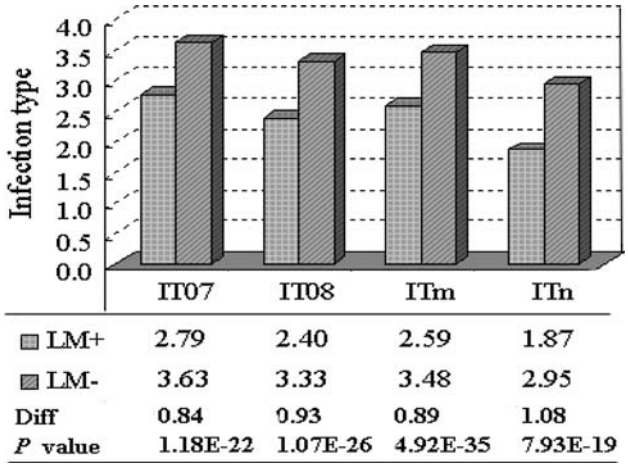

Fig. 2 A histogram to show the differences in the infection types between lesion mimic and non-lesion mimic RILs

Table 4 Variance analysis of leaf rust resistance between *lm* and *Lr26*

Source	DF	SS	MS	*F*	*P*
lm	1	83.05	83.05	243.99	<.0001
Lr26[a]	1	0.12	0.12	0.37	0.5451
*lm*Lr26*	1	0.14	0.14	0.4	0.5272
Error	530	180.41	0.34		
Total	535	354.42			

[a] *Lr26* was represented by *Xscm9*

centromere than to *Xwmc85.1* (Table 2). To date the chromosome locations of the genes underlying LM has not been reported in wheat and we provide the first report on localization of an *lm* gene on the chromosome 1BL.

A new major QTL for leaf rust resistance in Ning7840

A seedling leaf rust resistance gene *Lr26* was reported on chromosome 1RS (McIntosh et al. 1998; Kosman et al. 2004). In this study, the response pattern of Ning7840 to 15 leaf rust isolates was similar to that of *Lr26* (data not shown), indicating that *Lr26* mainly mediates the seedling resistance to leaf rust in Ning7840. To determine whether the gene for APR in Ning7840 differed from *Lr26*, the isolate PRTUS 55 was selected for evaluation of APR resistance in the mapping population because it can defeat not only *Lr26* in Ning7840 but also the resistance of Chokwang. In the two greenhouse experiments, Ning7840 was moderately resistant, and Chokwang was highly susceptible to leaf rust at adult plant stage. Therefore, PRTUS 55 is an ideal isolate to differentiate APR between Ning7840 and Chokwang without interference of *Lr26*.

Composite interval mapping using leaf rust data from three experiments identified a consistently significant QTL (*Qlr.pser.1BL*) on 1BL. This QTL explained major proportion of phenotypic variation for IT (Fig. 3) The QTL is different from *Lr46* because it is mapped close to centromere, not the distal end of 1BL where *Lr46* was mapped (Mateos-Hernandez et al. 2006). High positive correlations of the IT among RILs between three experiments indicated the QTL was highly heritable and resistant to a wide spectrum of pathogens (Table 3) because a mixture of several isolates might infect plants in field conditions. This variation in the frequency distributions among experiments was likely due to environmental conditions for disease development, especially temperature after initial infection. In the greenhouse, inoculation in December (2007) produced heavier rust IT than those inoculated in early June (2008) because greenhouse temperatures during December (19–24°C) were more favorable for rust development than that in early June (up to 32°C in some days). The high greenhouse temperature in June might reduce the virulence of the pathogen isolate and slow down disease development. This phenomenon was also observed for *Lr23*. Many US leaf rust isolates produced a very low IT in Thatcher line harboring *Lr23* under high temperatures (Kolmer et al. 2007). Under the field conditions, Ning7840 showed higher resistance than that in the greenhouse experiments. This might be due to that a mixture of several isolates infected plants and some might be avirulent to Ning7840, and also due to less favorable conditions for rust infection in the field than in the greenhouse.

Relationship between *lm* and leaf rust resistance genes

A significant correlation between LM ratings and the average IT among the RILs and *lm* showed the largest effect on APR among the tested markers on 1BL (Table 1), suggesting that *lm* likely either has a pleiotropic effect on or tightly links to the QTL for leaf rust APR in Ning7840. When APR was compared between two groups of RILs contrasting in LM, the LM group showed significantly lower leaf rust infection than the non-LM group in all experiments (Fig. 2). Correlation between *Lr26* (1RS), and APR was not significant and interaction between *lm* and *Lr26* was not detected (Table 4), confirming that *Lr26* did not contribute to APR in the current study. QTL for APR peaked at *lm* locus (Fig. 3) suggested that *lm* more likely has a pleiotropic effect on leaf rust APR in Ning7840.

The *lm*-associated resistance in Ning7840 is likely race non-specific because *lm* explained a large portion of phenotypic variation ($R^2 = 0.354$) for rust resistance in the field natural infection conditions where several isolates might be involved in the infection. LM showing resistance to multiple pathogens has been reported in several plant species (Greenberg and Ausubel 1993; Dietrich et al. 1994; Rate et al. 1999). In wheat, EMS-induced LM mutants exhibited enhanced resistance to powdery mildew, and stripe and leaf

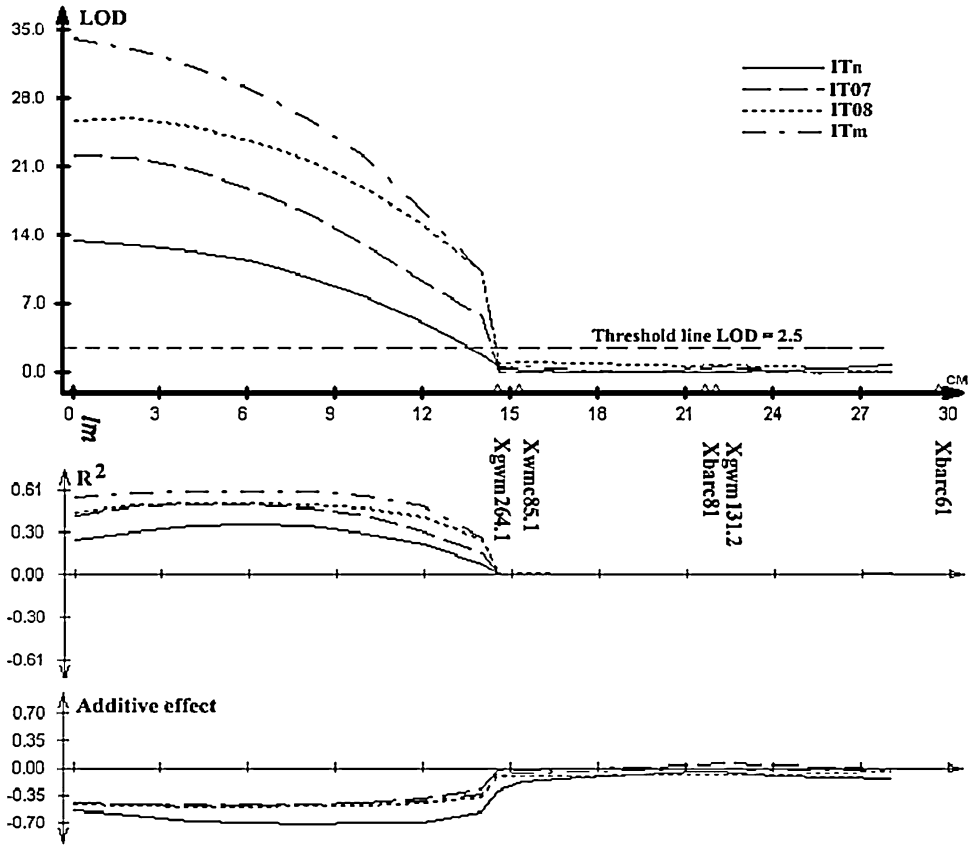

Fig. 3 The Cartographer plot of the major QTL for adult plant resistance to leaf rust on chromosome 1BL to show location of QTL peak (*top*), determination coefficients (R^2) (*middle*) and additive effects (*bottom*) The plot was derived by using composite interval mapping (CIM) of rust infection types (IT) from fall 2007 (IT07) and spring 2008 (IT08) greenhouse experiments, mean over the two greenhouse experiments (ITm), and spring 2007 field experiment (ITn) conducted at Manhattan, KS

rusts (Boyd and Minchin 2001; Boyd et al. 2002; Anand et al. 2003; Smith et al. 2004; Kamlofski et al. 2007). These findings indicate that LM can enhance plant resistance in various plant species to a wide spectrum of pathogens in a race non-specific or species non-specific manner.

Breeding crops for resistance to multiple races or pathogens is a desirable approach to improve crop productivity. In this study, we provide the first report on co-localization of the *lm* and *Qlr.pser.1BL* for APR to leaf rust on the chromosome 1BL (Fig. 3) and demonstrate that *Qlr.pser.1BL* is a new rust resistance QTL with a major effect on APR to leaf rust. The *lm* identified in Ning7840 in this study may provide a new source of race non-specific leaf rust resistance for breeding application and lay a solid foundation for further study of resistance mechanisms of *lm* in wheat.

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