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ORIGINAL PAPER

# Characterization and mapping of complementary lesion-mimic genes *lm1* and *lm2* in common wheat

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**Abstract** A lesion-mimic phenotype appeared in a segregating population of common wheat cross Yanzhan 1/Zaosui 30. The parents had non-lesion normal phenotypes. Shading treatment and histochemical analyses showed that the lesions were caused by light-dependent cell death and were not associated with pathogens. Studies over two cropping seasons showed that some lines with more highly expressed lesion-mimic phenotypes exhibited significantly lower grain yields than those with the normal phenotype, but there were no significant effects in the lines with weakly expressed lesion-mimic phenotypes. Among yield traits, one-thousand grain weight was the most affected by lesion-mimic phenotypes. Genetic analysis indicated that this was a novel type of lesion mimic, which was caused by interaction of recessive genes derived from each parent. The *lm1* (*lesion mimic 1*) locus from Zaosui 30 was flanked by microsatellite markers *Xwmc674* and *Xbarc133*/*Xbarc147* on chromosome 3BS, at genetic distances of 1.2 and 3.8 cM, respectively, whereas *lm2* from Yanzhan 1 was mapped between microsatellite markers *Xgwm513* and *Xksum154* on chromosome 4BL, at genetic distances of 1.5 and 3 cM, respectively. The linked microsatellite makers identified in this study might be useful for evaluating whether potential parents with normal phenotype are carriers of lesion-mimic alleles.

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## Introduction

Many plant mutants such as the *spotted leaf* series (*spl1*, *2*, *3*, *4*, *5*, *6*, *7* and *9*) in rice (Sekiguchi and Furuta 1965; Kinoshita 1995), *disease-like lesions 1* (*dll1*) in *Arabidopsis thaliana* (Pilloff et al. 2002) and a line Ning7840 in wheat (Li and Bai 2009) produce lesion phenotypes resembling the hypersensitive response (HR) associated with certain disease resistances when grown in the absence of pathogens. Such mutants are called lesion mimics. With artificial mutagenesis, large numbers of lesion mimics were obtained in rice (Li et al. 2005; Takahashi et al. 1999; Wu et al. 2008; Yin et al. 2000), barley (http://www.crpmb.org/mlo/), *Arabidopsis thaliana* (Dietrich et al. 1994; Shirano et al. 2002), maize (Walbot et al. 1983) and wheat (Kamlofski et al. 2007; Kinane and Jones 2001; Nair and Tomar 2001).

At least 12 lesion-mimic alleles, or their counterpart 'normal' alleles have been cloned, including *Spl11* and *Spl7* in rice (Yamanouchi et al. 2002; Zeng et al. 2004), *Mlo* in barley (Buschges et al. 1997), *HLM1*, *LSD1*, *SSI2*, *CPN1*, *MOD1*, *ACD11* and *ACD2* in *Arabidopsis thaliana* (Balagué et al. 2003; Brodersen et al. 2002; Dietrich et al. 1997; Jambunathan et al. 2001; Kachroo et al. 2001; Mach et al. 2001; Mou et al. 2000), *Les22* and *Lls1* in maize (Gray et al. 1997; Hu et al. 1998). Extensive studies of lesion mimics in plants provided available insights into

programed cell death and disease resistance pathways. *Spl11*, which encodes a U-box/armadillo repeat protein with E3 ubiquitin ligase activity, may negatively regulate cell death (Zeng et al. 2004). The recessive mutation *spl11* confers a broad-spectrum resistance to rice blast and bacterial blight (Yin et al. 2000). Similarly, the barley lesion-mimic gene, *mlo*, confers resistance to all isolates of *Blumeria graminis* f. sp. *hordei* (Jørgensen 1992). The dominant *Mlo* allele, predicted to contain at least six transmembrane helices, and located in the plasma membrane, probably has a dual negative control function in leaf cell death and disease resistance responses (Buschges et al. 1997). Due to the highly effective resistance, that it confers, the *mlo* allele is widely used in barley breeding as a source of powdery mildew resistance (http://www.crpmb.org/mlo/). The *HLM1* gene of *Arabidopsis* encodes a cyclic nucleotide-gated channel (CNGC4), that is permeable to both $K^+$ and $Na^+$ ions and is activated by both cGMP and cAMP. The *hlm1* allele also exhibits increased resistance to a virulent strain of *Pseudomonas syringae* pv. *tomato*. HLM1 is hypothesized to constitute a common downstream component of signaling pathways leading to HR or resistance (Balagué et al. 2003). However, the phenotypes of some lesion mimics are obviously not directly associated with defense response and may result from disruptions of cellular physiology. For example, rice *Spl7* encodes a heat stress transcription factor (HSF) associated with the accumulation of environmental stresses, and the light-dependent *Les22* gene in maize encodes an uroporphyrinogen decarboxylase, a key enzyme in the biosynthetic pathway of chlorophyll and heme in plants (Hu et al. 1998; Yamanouchi et al. 2002).

There are relatively a few studies concerning lesion mimic in wheat. Recently, Krattinger et al. (2009) cloned *Ltn1* which encodes a putative ABC transporter and confers durable partial resistance to leaf rust, stripe rust and powdery mildew. The gene causes spontaneous necrosis in the wheat leaf tips and edges, which is similar to lesion-mimic phenotype. Kamlofski et al. (2007) described a lesion-mimic HLP (hypersensitive-like phenotype) with enhanced adult resistance to leaf rust. Nair and Tomar (2001) reported a flecking mutant C591 (M8) that was a stable, developmentally programed, dominant lesion-mimic mutant under monogenic control. Symptoms started appearing only after the boot leaf stage. Yield components of these two mutants did not differ significantly from those of normal phenotypes. A mutant (M66) with severe leaf flecking showed complete and stable resistance to powdery mildew and enhanced resistance to both yellow rust and brown rust (Boyd and Minchin 2001; Boyd and Smith 2002; Kinane and Jones 2001). Recently, Li and Bai (2009) reported a spontaneous lesion-mimic phenotype from wheat line Ning7840 which may have pleiotropic effects on adult plant response to leaf rust. A recessive gene was mapped on the long arm of chromosome 1B.

We recently observed segregation of a lesion-mimic (LM) phenotype in a wheat breeding population. In this study, we genetically characterized a light-dependent LM phenotype, identified and mapped the underlying genes, and investigated the influence of the phenotype on agronomic traits in the field.

## Materials and methods

### Plant materials

One hundred ninety-eight single seed descent derived $F_8$, $F_9$, $F_{10}$ recombinant inbred lines (RILs) of 'Yanzhan 1' × 'Zaosui 30' were evaluated for the lesion-mimic phenotype. Yanzhan 1 is a commercial wheat variety released in 2000 in Henan, China, and Zaosui 30 is an early maturing line bred in the 1980s at the Chinese Academy of Agricultural Sciences, Beijing.

### Investigations of the lesion-mimic phenotype and agronomic traits

Under field conditions, $F_8$ and $F_9$ plants were grown in Beijing (39.54°N) in 2005 and 2006 and $F_{10}$ plants were planted in Yuanmou (25.44°N), Yunnan province, in 2008. The 198 RILs tested for LM phenotype were arbitrarily divided into 6 scores based on flag leaf symptoms as follows: viz. 1, no visible specks, the parental phenotype; 2, chlorotic specks; 3, brown lesions, low severity (≤25%); 4, brown lesions, medium severity (25–50%); 5, brown lesions, high severity (50–75%); 6, brown lesions, very high severity (≥75%). The score of each RIL was taken shortly before a majority of plants had undergone senescence. Plants with score 1 were considered normal and those with scores of 2 or higher were classified as having lesion-mimic phenotypes.

Four yield traits, including spikelet numbers (SPI), numbers of sterile spikelets per spike (SSNS), grain numbers per spike (GNS) and one-thousand grain weight (TGW) were investigated in the Beijing cropping seasons. Five plants were measured per line and their means were used for the analysis. Attempts to isolate and identify pathogens from the disease-like lesions were performed in collaboration with the Quarantine Department of CAAS.

### Histochemistry

Leaf samples were taken from lesion-mimic plants, and stained for dead cells using trypan blue staining as described by Yin et al. (2000). The leaves were

submerged in a 70°C lactic acid–phenol–trypan blue solution [2.5 mg/ml trypan blue, 25% (w/v) lactic acid, 23% water-saturated phenol, 25% glycerol, and $H_2O$] for 10 min, and then heated over boiling water for 2 min and left to stain overnight. After staining, a chloral hydrate solution (25 g in 10 ml of $H_2O$) was used for destaining for 3 days. Leaves were finally equilibrated with 70% glycerol and mounted on slides for assessment with a light microscope.

### Microsatellite analysis

One hundred twenty-four $F_8$, $F_9$ and $F_{10}$ RILs that showed the most consistent phenotypes over the three generations, including 78 normal and 46 lesion-mimic lines, were chosen for microsatellite analysis. Total DNA was extracted from leaves following the method described by Devos et al. (1992) with minor modifications. Based on published maps (Somers et al. 2004; Yu et al. 2004), 698 wheat microsatellite markers listed in GrainGenes 2.0 (http://www.wheat.pw.usda.gov/) were screened for polymorphisms between the two parents. A lesion-mimic bulk consisting of equal amounts of DNA from 15 RILs with score 6 was constructed for screening SSR markers associated with the LM phenotype following the strategy of bulked segregant analysis (Michelmore et al. 1991), with the two parents as controls. Polymorphic markers were used to analyze the susceptible bulk. Markers detecting bands of only one parent in the mimic bulk were considered to be possibly linked to a target gene(s), and therefore were used to genotype all the selected RILs.

Each PCR reaction was conducted in a total volume of 20 µl containing 2 µl template DNA (40–60 ng), 0.16 µl Tag DNA polymerase (5 U/µl, Fermentas), 2 µl 10 × PCR buffer [200 mM $(NH_4)_2SO_4$, 750 mM Tris–HCl (pH 8.8 at 25°C), 1% Tween 20], 1.44 µl 25 mM $MgCl_2$, 0.16 µl dNTP (25 mM), 1.6 µl primers (2 µM). Amplifications were performed for 35 cycles at 94°C for 45 s, 50–60°C (depending on microsatellite primers) for 45 s and 72°C for 45 s with a final extension at 72°C for 10 min.

The samples of 3.5 µl PCR products were mixed with 2 µl loading (98% formamide, 0.3% of each bromophenol blue and xylene cyanol, and 10 mM of EDTA), denatured at 95°C for 5 min and chilled on ice. Electrophoresis was carried out in 5% denatured polyacrylamide gel with 1 × TBE (90 mM Tris-borate, 2 mM EDTA) at 50 W for 90 min. Gels were then silver stained using the method of Tixier et al. (1997).

### Statistical analysis

One-way ANOVA of RIL scores during each of three seasons and a student's $t$ test comparing the normal phenotype (score 1) and various LM phenotypes (scores 2, 3, 4, 5 and 6) on yield traits were performed with SPSS software version 15 (SPSS Inc, Chicago, IL, USA). The genetic segregation ratio of normal and LM phenotypes was tested for goodness of fit to the expected ratio by chi-square test. A linkage map of SSR markers was constructed using MAPMAKER/EXP ver. 3.0 program (Lander et al. 1987). As both the genetic segregation analysis and the bulked segregant analysis indicated that the LM phenotype was controlled by complementary genes, the genetic distance between a target gene and a marker was estimated by solving the following equation derived by the maximum-likelihood method:

$$\frac{n_1}{2-R} - \frac{n_2}{1+R} - \frac{n_3}{R} + \frac{n_4}{1-R} = 0 \quad (1)$$

where $R$ is the recombination fraction between the target gene and the linked marker; and $n_1$, $n_2$, $n_3$ and $n_4$ are observed numbers of RILs showing the normal phenotype and parent 1 (or parent 2) marker type, normal phenotype and parent 2 (or parent 1) marker type, LM phenotype and parent 1 (or parent 2) marker type, and LM phenotype and parent 2 (or parent 1) marker type, respectively. Since the population was a RIL population, the recombinant fraction was adjusted as $r = R/[2(1 - R)]$ and then converted to genetic distance using the Kosambi mapping function (Kosambi 1944).

## Results

### Lesion-mimic phenotype and its effects on yield traits

The lesion-mimic phenotype became most obvious at the booting stage. The necrotic lesions developed progressively from lower leaves to the flag leaf (Fig. 1a). At the early stages, lesions were discrete, small (<5 mm) and light green. They did not develop further in score 2 plants, but gradually turned brown with surrounding tissues becoming dry in the lesion mimics (scores 3–6) (Fig. 1b). In severely affected plants, lesions were scattered over leaf blades and leaf sheathes and even over glumes and awns (Fig. 1c, d). Finally, the leaves became narrow and rolled and the entire plants dried up and died earlier than the normal plants. Interestingly, the lesions remained clearly visible on leaf blades, leaf sheathes and flower tissue of senescent plants (Fig. 1e). Possible pathogens, triggering the LM phenotype, could not be isolated at various developmental stages of lesions, suggesting that the phenotype was not due to infection by pathogens. Presumably, physiological disturbance of some key metabolic or synthetic pathway or death-controlling genes were involved.

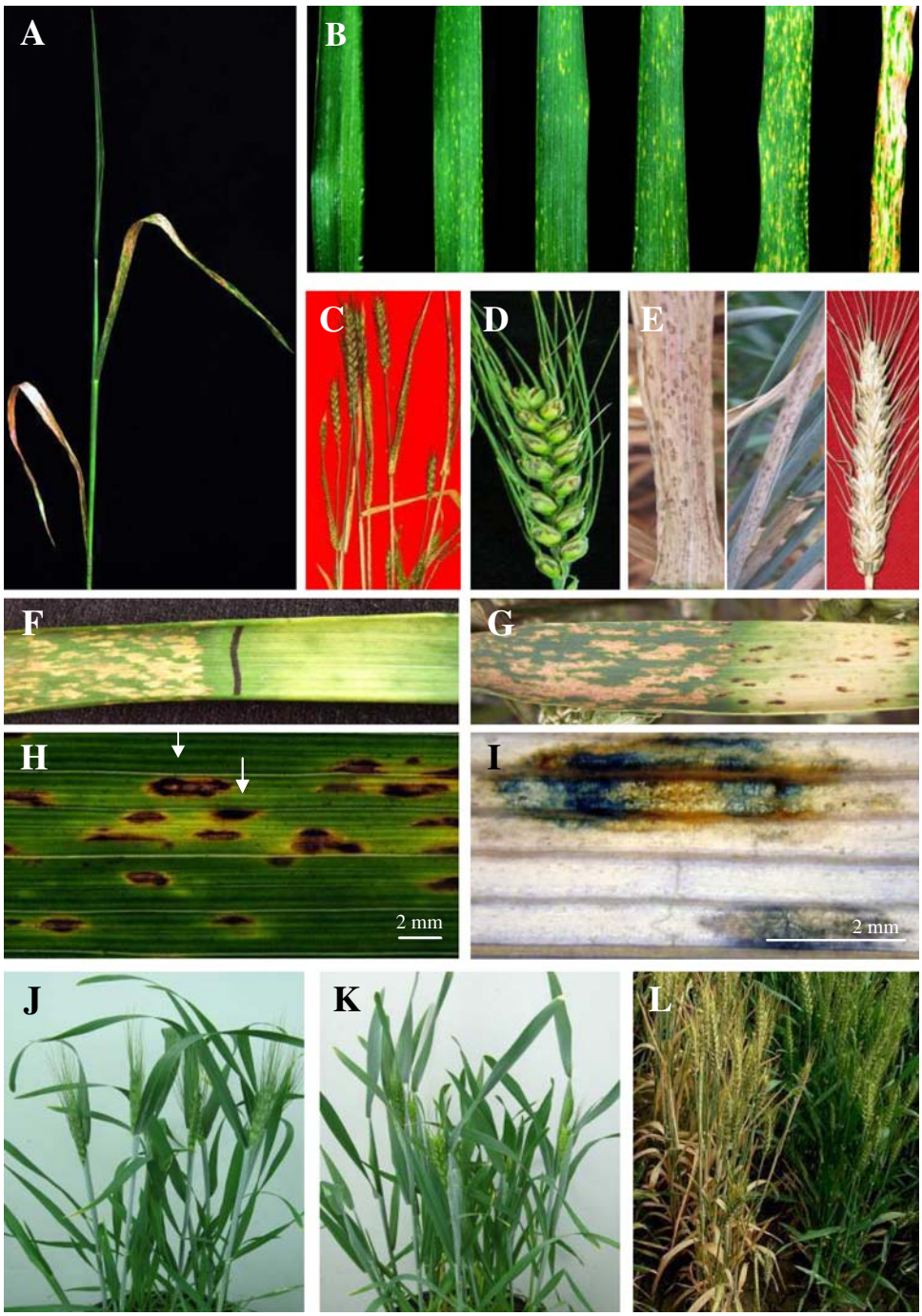


**Fig. 1** Phenotypes and histology of mimic lesions. **a** Phenotype of one plant at flag leaf emergence; **b** range of phenotypes (and scores) on flag leaves of different RILs; **c**, **d** lesion-mimic phenotypes on leaf blades, leaf sheathes and glumes; **e** the LM phenotype on the leaf blade, leaf sheath and glumes after the plant die; **f** the right half of the leaf, which was hidden from light before lesions showed, failed to form lesions; **g** the right half of the leaf, which was hidden from light after lesions showed, fail to develop further; **h** leaves showing brown lesions after booting; **i** trypan blue staining at and around the sites of two lesions similar those indicated by the *arrows* in **h**; **j**–**l** Yanzhan 1 (**j**), Zaosui 30 (**k**) and RIL progenies with lesion mimic (*left*) and normal phenotypes (*right*)

## Light required for the lesion mimic

Some lesion mimics were reported to be sensitive to environmental factors such as light (Arase et al. 2000a, 2001; Gray et al. 2002; Hu et al. 1998; Mach et al. 2001). To investigate whether light influenced LM phenotype in our study, leaves were covered with aluminum foil. Leaves covered from light before lesion expression, and 2 weeks later, developed no lesions on the covered parts (Fig. 1f). When leaves were covered for 2 weeks after lesion initiation, lesions and surrounding tissues failed to develop further symptoms (Fig. 1g). These results suggested that light plays an important role in lesion expression. This is the first report in wheat that the lesion mimics are affected by light.

## The effects of LM phenotype on yield traits

The effects of LM phenotype on yield traits were observed in Beijing over two cropping seasons (Table 1). No significant

**Table 1** Relationship between LM score and four yield traits during two cropping seasons in Beijing

| Trait | LM score | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| SPI | | | | | | |
| 2005 | 20.0 ± 2.2[a] | 20.4 ± 1.6 | 20.5 ± 2.7 | 20.0 ± 2.3 | 18.7 ± 2.0 | 19.8 ± 1.8 |
| 2006 | 20.0 ± 1.8 | 20.1 ± 2.0 | 19.3 ± 1.6 | 19.1 ± 2.9 | 18.8 ± 2.4 | 20.0 ± 1.7 |
| SSNS | | | | | | |
| 2005 | 2.2 ± 1.1 | 2.4 ± 0.5 | 2.7 ± 0.2 | 2.8 ± 0.8 | 2.9 ± 0.8 | 3.1 ± 0.8** |
| 2006 | 1.7 ± 1.0 | 1.8 ± 1.2 | 1.9 ± 0.8 | 2.0 ± 1.1 | 2.1 ± 0.7 | 2.4 ± 1.0** |
| GNS | | | | | | |
| 2005 | 40.8 ± 5.9 | 40.6 ± 5.8 | 39.7 ± 4.7 | 38.8 ± 7.5 | 37.8 ± 5.7 | 37.7 ± 6.6* |
| 2006 | 42.3 ± 6.6 | 41.8 ± 6.2 | 36.6 ± 4.9 | 36.4 ± 7.1* | 36.2 ± 3.1** | 35.5 ± 7.5** |
| TGW | | | | | | |
| 2005 | 42.2 ± 6.6 | 41.1 ± 5.3 | 40.3 ± 7.8 | 36.5 ± 5.2* | 35.7 ± 2.7** | 35.3 ± 7.4** |
| 2006 | 35.5 ± 6.5 | 35.4 ± 7.3 | 34.8 ± 5.5 | 28.4 ± 3.7** | 26.6 ± 4.0** | 25.10 ± 5.7** |

*SPI* spikelet numbers; *SSNS* numbers of sterile spikelets per spike; *GNS* grain number per spike; *TGW* one-thousand grain weight

*,** Values differ significantly from those with score 1 at $P < 0.05$ and $P < 0.01$, respectively, using Student's $t$ test

[a] Data represent averages and standard deviations of the lines with the same LM score

effects of the LM phenotype on all four yield traits were found in lines with scores 2 and 3. However, the SSNS of the lines with score 6 was significantly increased compared with the lines with score 1 in the every cropping season. The GNS and TGW of the lines with scores 4, 5 and 6 were less than those of lines with score 1. Comparing the effects of LM phenotype on four different yield traits, the severities of effects were in the order of SPI-SSNS-GNS-TGW, no effect on the SPI and the most effect on the TGW. This was consistent with the order of development of the traits. Clearly, the negative effects of LM phenotype were greatest in lines with more serious lesions and on the later developing traits.

## Histochemical studies of the lesion-mimic phenotype

Trypan blue staining was used as a histochemical indicator of cell death on leaves expressing the lesion-mimic plant phenotype (Fig. 1h). Dead cells stained deep blue at the necrotic sites, whereas surrounding normal cells remained unstained, confirming that lesions were composed of dead cells (Fig. 1i).

## Genetic basis of the lesion-mimic phenotype

Parents (Fig. 1j, k) and $F_1$ plants had neither specks nor lesions. However, the RILs showed obvious variation for the lesion-mimic trait (Fig. 1l). The RILs were clearly classified into two groups according to symptom scores. All lines exhibited consistently normal or lesion-mimic phenotypes in all three growing seasons, except for two lines, scored LM phenotype in 2005, but normal in 2006 and 2008. These two exceptions were attributed to residual segregation, or to misclassification in 2005. Excluding the two lines, the final segregation ratio in the three crop seasons was 150 normal:46 mimic (Table 2), or very close to $3:1 (\chi^2_{(3:1)} = 0.17 < \chi^2_{0.05} = 3.84, P > 0.50)$, and the relative scores of individual lines during each season were consistent ($F = 0.08$, $P > 0.05$). These data indicated that the LM phenotype was likely controlled by two recessive genes, one coming from each parent. The genes were designated *lm1* (*lesion mimic 1*) in Zaosui 30 and *lm2* in Yanzhan 1.

## Mapping the lesion genes

Among 698 SSR markers tested, 231 showed polymorphisms between the two parents. Eighteen markers detected bands of only one or other parent in the bulks. Ten markers, including five (*Xbarc133*, *Xbarc147*, *Xwmc674*, *Xwmc754* and *Xgwm493*) on chromosome 3BS amplified fragments from parent Zaosui 30, and five (*Xgwm149*, *Xwmc657*, *Xksum154*, *Xgwm113* and *Xgwm513*) amplified fragments from parent Yanzhan 1 on chromosome 4BL, were used to genotype individual RILs and to construct linkage maps for each group (Fig. 2). Genetic distances of the markers from the target loci were estimated using Eq. 1. Using the linkage maps of markers and genetic distances between the target genes and the linked markers, the gene *lm1* on chromosome 3BS from Zaosui 30 was flanked by the SSR markers *Xwmc674* and *Xbarc133*/*Xbarc147*, at genetic distances of 1.2 and 3.8 cM, respectively, whereas *lm2* on chromosome 4BL from Yanzhan 1 was located between markers *Xgwm513* and *Xksum154* at genetic distances of 1.5 and 3 cM, respectively (Fig. 2).

**Table 2** Number of RILs in normal phenotype and LM phenotype during three cropping seasons

| Year | Normal phenotype | LM phenotype | | | | | |
|---|---|---|---|---|---|---|---|
| | 1[a] | 2 | 3 | 4 | 5 | 6 | Total |
| 2005 | 150 | 6 | 6 | 6 | 9 | 19 | 46 |
| 2006 | 150 | 6 | 9 | 7 | 8 | 16 | 46 |
| 2008 | 150 | 6 | 6 | 6 | 7 | 21 | 46 |

[a] Phenotype score

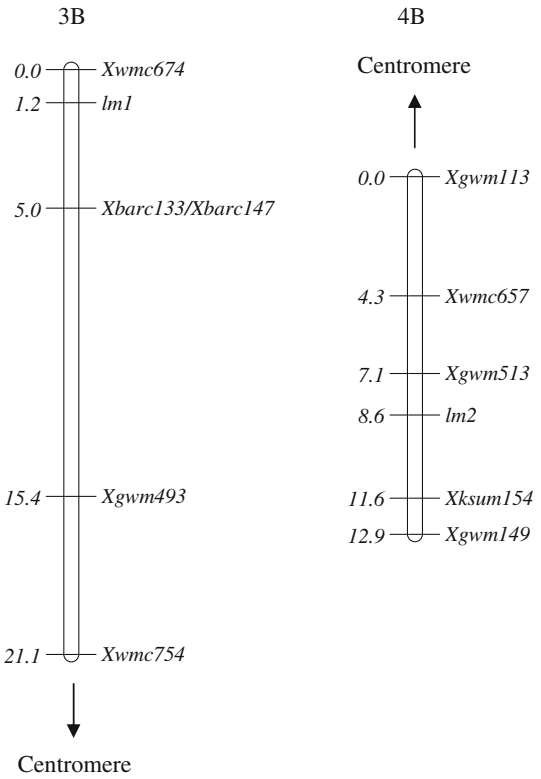


**Fig. 2** The genetic map of the regions surrounding *lm1* on chromosome 3BS and *lm2* on chromosome 4BL

## Discussion

### A novel type of lesion mimic

This study characterized a novel lesion-mimic phenotype involving discrete brown lesions on the leaf blades, leaf sheathes and flowering tissues after the booting stage in the absence of any pathogen or specific treatment and was different from other reported lesion mimics in wheat. LM in Ning7840 spontaneously showed small, discrete, yellowish spots only on the leaf blades after heading (Li and Bai 2009). In addition, the HLP mutant, induced by EMS (ethyl methane sulfonate), expressed lesions on leaf blades at the fifth or sixth leaf stage and then lesions also appeared on the leaf sheathes and flowering tissues, but were small (1–2 mm) and white in color (Kamlofski et al. 2007). The C591 (M8) mutant arising from *N*-nitroso methyl urethane treatment was controlled by a dominant gene, although flecks in this mutant were also developmentally programed and started appearing only from the boot leaf stage. Furthermore, as for the genetic basis, our LM is also a new type. In previous reports on lesion mimics, inheritance of mimic phenotypes was either partially or completely dominant, or recessive, indicative of both of gain-of-function or loss-of-function mutations. Phenotypes of *cpr22* and *ssi4* in *Arabidopsis* were partially dominant (Shirano et al. 2002; Yoshioka et al. 2001) and most of the reported lesion mimics, such as *Les1*, *Les2*, *Les3*, *Les4, Les5* in maize (Johal et al. 1995), *Spl26* and *Spl27* in rice, *dll1* in *Arabidopsis* and C591 (M8) in wheat were completely dominant (Nair and Tomar 2001; Pilloff et al. 2002; Wu et al. 2008). In contrast, some lesion mimics were controlled by recessive genes, including most of *spl* genes, all of *lm* genes, *hlm1* and *mod1* (Balagué et al. 2003; Li et al. 2005; Mou et al. 2000; Wu et al. 2008). All the above lesion mimics were caused by single genes, whereas the present LM in a polyploid species was controlled by the interaction of two recessive genes, one being present in each 'normal' parent.

### Light mediates the LM phenotype

Light has been reported to promote cell death in many lesion-mimic mutants, but has not been implicated in wheat. For instance, in the *Les22* mutant, cell death is exacerbated through the production of excess porphyrin free radicals by light (Hu et al. 1998). Similarly, in plants with the *acd2* phenotype, photo-activation of the red chlorophyll catabolite triggers subsequent cell death (Mach et al. 2001). In maize *lls1* mutants, mature chloroplasts mediate light-dependent cell death (Gray et al. 2002). Furthermore, light enhances resistance to *Magnaporthe grisea* infection in the rice *sl* mutant, in which tryptamine plays an important role (Arase et al. 2000a, 2001). This is the first report in wheat that light influenced LM expression.

### Variation in LM expression

The lesion mimic studied here was controlled by the interaction of two recessive genes, which means that the *lm1lm1lm2lm2* genotype was responsible for the LM phenotype. However, as indicated by the data in Table 2 LM phenotypes scored from 2 to 6 showed considerable variations. This variable expressivity was apparently affected by developmental state, environment and genetic background. Phenotypes were consistent in relative expression over generations (years) ($F = 0.08$, $P > 0.05$) indicating

that genetic background was the most significant determinant of final phenotype.

## Influence of lesion-mimic phenotype on agronomic traits

Some lesion mimics confer resistance to pathogens. Rice lesion mimics *spl11*, *spl17* and *Spl26* showed enhanced resistance to both fungal and bacterial pathogens (Wu et al. 2008; Yin et al. 2000). In the present study, the locations of the *lm* genes in chromosome 3BS or 4BL were close to resistance genes or quantitative trait loci (QTL). For example, *Sr2*, *Lr27*, *Yrns-B1*, *stb2* and *QSng.sfr-3BS*, *QFhs.ndsu.3B* are located on the same deletion bin, 3BS8-0.78-1, as *lm1* (Paux et al. 2008). There are also resistance QTL for leaf rust, yellow rust and Fusarium head blight resistances near *lm2* in chromosome 4BL (Li et al. 2008; Lin et al. 2006; Suenaga et al. 2003; William et al. 2006). Furthermore, we found a correlation between LM and adult plant resistance to wheat powdery mildew in the field, although the value was low ($r = -0.222$, $P < 0.01$ in 2005; $r = -0.192$, $P < 0.01$ in 2006), suggesting pleiotropy or close linkage of the LM and disease resistance genes. Fine mapping and possible gene cloning are required to confirm these relationships.

Regarding the influence of the LM on crop yield, most reported lesion mimics are lower yielding than their normal counterparts, except for the HLP mutant whose yield components did not differ from those of the parent line (Arase et al. 2000b; Kamlofski et al. 2007; Kinane and Jones 2001; Kjær et al. 1990). In the present study, RILs with more highly expressed LM phenotypes exhibited significantly lower grain yields than those with normal phenotype in both cropping years, but RILs with less severe LM phenotypes were not significantly affected (Table 1). If such RILs had resistance, they could be utilized in resistance breeding. The closely linked markers identified in this study may provide a useful tool for identifying genotypic carriers of alleles determining mimic phenotypes.

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