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Research article

Characterization and fine mapping of a light-dependent *leaf lesion mimic mutant 1* in rice

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ABSTRACT

Plants that spontaneously produce lesion mimics or spots, without any signs of obvious adversity, such as pesticide and mechanical damage, or pathogen infection, are so-called lesion mimic mutants (*lmms*). In rice, many *lmms* exhibit enhanced resistance to pathogens, which provides a unique opportunity to uncover the molecular mechanism underlying *lmms*. We isolated a rice light-dependent *leaf lesion mimic mutant 1* (*llm1*). Lesion spots appeared in the leaves of the *llm1* mutant at the tillering stage. Furthermore, the mutant *llm1* had similar agronomic traits to wild type rice. Trypan blue and diamiobenzidine staining analyses revealed that the lesion spot formation on the *llm1* mutant was due to programmed cell death and reactive oxygen species. The chloroplasts were severely damaged in the *llm1* mutant, suggesting that chloroplast damage was associated with the formation of lesion spots in *llm1*. More importantly, *llm1* exhibited enhanced resistance to bacterial blight pathogens within increased expression of pathogenesis related genes (PRs). Using a map-based cloning approach, we delimited the *LLM1* locus to a 121-kb interval between two simple sequence repeat markers, RM17470 and RM17473, on chromosome 4. We sequenced the candidate genes on the interval and found that a base mutation had substituted adenine phosphate for thymine in the last exon of *LOC_Os04g52130*, which led to an amino acid change (Asp^{388} to Val) in the *llm1* mutant. Our investigation showed that the putative coproporphyrinogen III oxidase (CPOX) encoded by *LOC_Os04g52130* was produced by *LLM1* and that amino acid Asp^{388} was essential for CPOX function. Our study provides the basis for further investigations into the mechanism underlying lesion mimic initiation associated with *LLM1*.

Introduction

Plants have developed a battery of molecular defense mechanisms to protect themselves from pathogen attacks, such as the hypersensitive response (HR), which induces programmed cell death (PCD), and the formation of lesion mimics, which inhibit pathogen spread in plant host tissues (Negishi et al., 1997; Lam et al., 2001). Some mutants that have developed spontaneous necrotic lesions in the absence of pathogen infection, abiotic stress and mechanical damage, are called lesion mimic mutants (LMM) (Xu et al. 2014). Localized cell death in LMM resembles that caused by HR, and most of these mutants display increased expression of pathogenesis-related genes, which results in enhanced resistance to many different pathogens (Lorrain et al. 2003).

A number of LMM mutants have been isolated from a variety of plant species, including barley, maize, *Arabidopsis* and rice (Lorrain et al. 2003). In rice, more than 50 LMMs have been identified, which encode proteins that belong to various functional categories (Feng et al. 2013). For example, *spotted leaf 11* (*SPL11*) encodes a U-box/armadillo repeat protein endowed with E3 ubiquitin ligase activity (Zeng et al. 2004; Vega-Sanchez et al. 2008); *SPL5*/*OsSL5* encodes a putative splicing factor 3b subunit 3 that regulates gene expression

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(Chen et al. 2012; Ge, et al. 2014) and *RLIN1* encodes a putative coproporphyrinogen III oxidase (CPOX) in the tetrapyrrole biosynthesis pathway (Sun et al. 2011). In general, the molecular mechanism underlying lesion mimic formation in plants is complicated. More importantly, several rice LMM exhibit enhanced disease resistance and thus studies of these LMM would provide insight into the mechanisms underlying HR, PCD and immunity (Takahashi et al. 1999; Wu et al. 2008).

Previous studies have demonstrated that chloroplast defects in plants can result in light-dependent lesion mimic phenotypes. When a plant fails to dissipate excess excitation energy (EEE), numerous reactive oxygen species (ROS) are generated in the chloroplasts, which triggers a HR and initiates the appearance of a light-dependent lesion mimic (Asada, 1999; Karpinski et al. 1999; Niyogi, 2000; Mullineaux and Karpinski, 2002; Fryer et al. 2003). However, chlorophyll degradation is also accompanied by HR and PCD (Mach et al. 2001). This is highlighted by the light-dependent lesion mimic mutants: *lsd1*, *acd1* and *acd2*, which show defects in EEE dissipation or in chlorophyll catabolism caused by photo-oxidative damage and the formation of ROS (Mach et al. 2001; Pruzinska et al. 2003; Mateo et al. 2004; Wituszynska et al. 2015).

In this study, we characterized a light-dependent leaf lesion mimic mutant 1 (*llm1*). This mutant started to display lesion spots around leaves at the tillering stage without any obvious changes to plant architecture. Trypan blue and diamiobenzidine (DAB) staining analyses revealed that PCD and ROS were accompanied by the formation of lesion spots in *llm1*. Transmission electron microscopy (TEM) assay showed that the degradation of chloroplasts might be the first step in the formation of lesion spots by *llm1*. Meanwhile, we also found that the *llm1* plants display enhanced resistance to several blight bacterial pathogens which might be attributed to enriched transcripts level of PRs. Genetic analysis of *llm1* indicated that the lesion mimic phenotype was controlled by a single recessive nuclear gene, *LLM1*, located on chromosome 4 between simple sequence repeat (SSR) markers RM17470 and RM17473 in a 121-kb region containing 17 putative open reading frames (ORFs). Sequencing of the ORFs revealed that a substitution mutation from adenine phosphate (A) to thymine (T) in the last exon of *LOC_Os04g52130*, which encoded a putative CPOX. This led to a missense mutation from Asp^{388} to Val in the amino acid sequence. These results suggest that *LOC_Os04g52130* is *LLM1* and that Asp^{388} is probably essential for *LLM1* function.

2. Results

2.1. Isolating the leaf spot mutant llm1

We obtained a mutant exhibiting the leaf lesion mimic phenotype from the Xa21 EMS-induced mutation library. We then named it *leaf lesion mimic mutant 1* (*llm1*). The phenotype of *llm1* rice grown in the field was not obviously different at the seedling stage to the wild type Xa21 (WT). However, when the plants reached the tillering stage, the *llm1* plants had red brown lesions over the middle region of the leaves (Fig. 1B). We found that the yield-related agronomic traits, including the tiller number, the panicle length and seed-setting, were almost the same as for WT rice (Fig. 1A and C–F). The exception was that *llm1* plants were a little shorter (Fig. 1D).

Cell death is accompanied with leaf lesion spot appearance in *llm1*.

PCD is usually an important part of lesion spot formation. To determine whether lesion spot initiation was accompanied by PCD, we performed a trypan blue staining assay, which is a traditional method for selectively staining dead tissues or cells. The leaves of the *llm1* mutant had a large number of blue spots after staining,

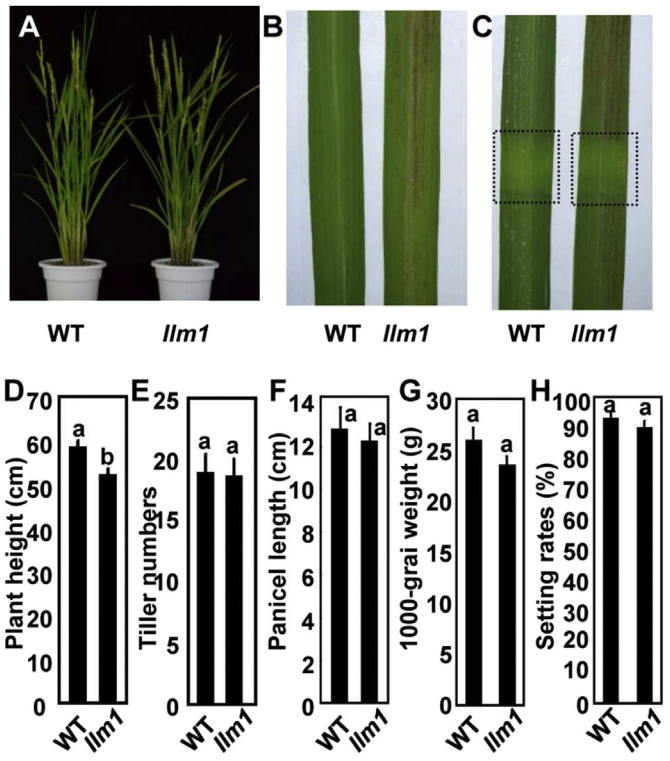

Fig. 1. Phenotype characterization of the leaf lesion mimic mutant *llm1* and wild type (WT) plants. (A) Photographs of representative plants of the *llm1* mutant (right) and WT (left) at the tillering stage. (B) Representative leaves were from the WT (left) and the *llm1* mutant (right) under natural conditions. (C) Determination of whether the *llm1* phenotype was light dependent. Leaf area photographs of the WT (left) and *llm1* (right) leaves. The areas covered with silver paper are boxed with black rectangles. (D)–(H) Statistical analysis of yield-related traits for WT and *llm1*: plant height (D), tiller numbers (E), panicle length (F), 1000-grain weight (G) and setting rate (H). The letters indicate significant differences ($P < 0.05$) between genes according to a one-way ANOVA followed by post hoc Tukey's HSD analysis.

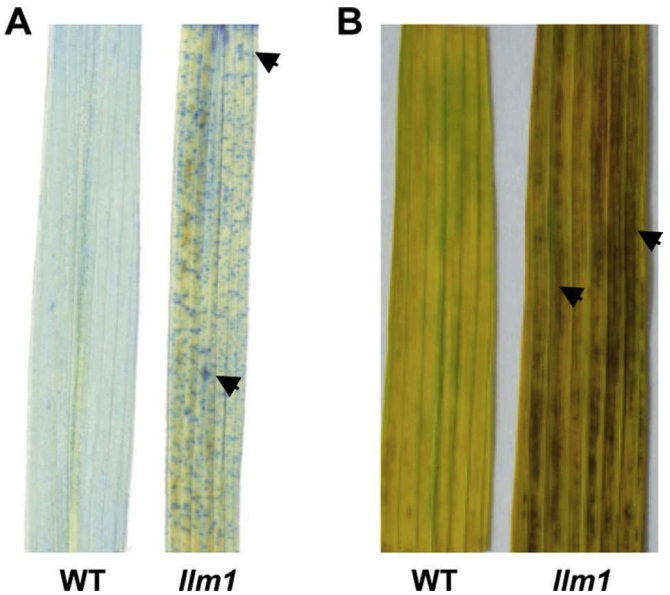

Fig. 2. Histochemical staining analysis of wild type rice (Xa21) and *llm1* mutant leaves. (A) Trypan blue staining for cell death. The black arrows indicate the dead cells in the *llm1* mutant. (B) DAB staining for H_2O_2 accumulation. The black arrows show the ROS-enriched area in the *llm1* mutant. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)