Research Progress on Cloning and Mechanism of Rice Lesion Mimic Genes

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Abstract  The formation of rice lesion mimic was affected by gene mutation, metabolic disorder, accumulation of active oxygen, hormone imbalance, blocked ion channels, out of control programmed cell death process, abiotic stress and so on. The process of disease was usually accompanied by the improvement of plant resistance, and the expression of defense system related genes increases. At present, the study of disease-like mutants mainly focused on the mapping, cloning and functional analysis of mutant genes. The mechanism of rice lesion mimic mutants was reviewed, especially the location, coding and function of the cloned disease like genes In this paper. The mechanism of programmed cell death in rice disease like cells was summarized, and the prospect of disease like mutants and rice breeding practice was put forward. It was of great significance to further analyze the regulatory mechanism of defense response and breeding application of rice lesion mimic mutant gene.

Keywords  Rice; Lesion mimic; Gene cloning; Mechanism

Rice (Oryza.sativa L.) is one of the main grain crops and a series of heritable changes will be found during its production, while some can be visible to the naked eye. Rice lesion mimic (RLM) refer to the necrotic spot spontaneously formed with the development of rice. It could be divided into two types, one is diffuse, which is mainly produced by the inhibition of programmed cell death (PCD). A point on its leaves is stimulated by external factors, resulting in cell necrosis and progressive spread around, and finally spread to the whole leaf or even the whole plant, that is, uncontrolled spread of disease spots. Another is initial, these mutants are mainly produced by the excitation of programmed cell death, which occurs randomly in many parts of the leaf without external excitation, thus forming spontaneously, but not spreading (Hoisington et al., 1982; Lorrain et al., 2003). Therefore, it is of great significance to study the disease resistance mechanism of rice mutant, and breeding application.

1 Source and Performance Characteristics of Rice Lesion Mimic Mutants

The lesion mimic mutant (LMM) was first found in maize (Lu et al., 2012). In subsequent decades, the first rice lesion mimic mutant -sekiguchi lesion was found, which is a recessive mutant produced by natural mutation (Imaoka et al., 2008). Rice lesion mimic mutant is mainly produced by natural and artificial mutation. Natural mutation is mainly induced by environmental factors such as temperature, humidity, germs or radiation. Most of these are meaningless mutations that tend to be eliminated, and is not easy to happen (Xiao et al., 2017). Artificial mutagenesis is formed by physical, chemical and biological methods. Physical mutagenesis mainly induces plant seeds with the help of high energy radiation such as X and γ ray, which leads to the mutation of chromosome structure of plant cells, makes the genome sequence change accordingly. Chemical mutagenesis is induced by some alkylating agents, base nucleic acid analogues and other chemical mutagens. The principle of action is similar to physical mutagenesis. Biological mutagenesis is to use genetic engineering techniques such as T-DNA insertion or transposon tag to mutagenesis, so that the plant genome can produce site-directed mutagenesis or frameshift mutagenesis. The phenotype of some mutants appears at the seedling stage, while the phenotype of some mutants does not appear on the plant until late growth or heading stage (Liu et al., 2004; Wang et al., 2004).
The production rice lesion mimic will also be affected by temperature, light and other ecological factors. Arase et al. (2000) found that the phenotypic characteristics of rice lesion mimic mutant m1009 were affected by temperature. When the temperature was lower than 20℃, the lesion mimic mutant phenotype appeared, but no obvious characteristics were found at higher than 25℃. Yamanouchi et al. (2000) found that the production of spl7 mutant is related to ultraviolet light and high temperature conditions. Wu et al. (2008) found that after the mutant spl19 appeared, the leaf curl, dry, narrow and seed setting rate decreased and other phenomena also appeared.

2 Mutation Mechanism of RLM

2.1 Genic mutation
The mutation or abnormal expression of disease resistance and stress resistance related genes in rice may lead to the deviation of signal transmission pathway, which may lead to the passive occurrence or elimination of stress response, resulting in programmed cell death, and then produce rice lesion mimic mutant. Yamanouchi et al. (2002) found that the Spl7 gene was the first lesion mimic mutant gene found in rice, which was obtained by \( \gamma \) radiation mutagenesis of japonica rice varieties. Its traits were controlled by a single invisible gene and located on chromosome 5. At low temperature in the field, the mutant spl7 appeared from tillering stage to maturity stage, but under sunlight condition, no mutant appeared at high or low temperature. This indicates that the mutant phenotype is affected by temperature and light. Spl7 gene coding, a transcription factor regulated by light and temperature, changes the structure of the protein so that it cannot be expressed normally, and then loses its function, which eventually leads to the formation of lesion mimic.

2.2 Metabolic disturbance
The process of plant growth and metabolism is regulated by a variety of enzymes and proteins. Decreased activity or loss of function of these enzymes or proteins will cause metabolic disorders in plants, leading to lesion mimic mutant. At the same time, in the process of chlorophyll synthesis, the expression of pigmentogen deaminase and other genes related to the synthesis pathway was inhibited, which also caused the lesion mimic mutant. Sun et al. (2011) found that the mutation of chlorophyll synthesis related genes in rice resulted in the decrease of fecal porphyrinogen III oxidase activity, accumulation of fecal porphyrinogen III in rice plants, and disturbance of the metabolism of chlorophyll and heme. Sakuraba et al. (2013) showed that the rice lesion mimic mutant fgl because of the frameshift mutation in the second exon of the gene associated with the original chlorate redox enzyme coding, the synthesis of the original chlorophyll ester redox enzyme B was terminated in advance, which in turn led to the disorder of chlorophyll metabolism, and the rice plant leaves appeared lesion mimic.

2.3 Reactive oxygen species accumulation
The production of many lesion mimic mutants is closely related to the accumulation of reactive oxygen species in plants. Reactive oxygen species (ROS) is a kind of metabolites with high oxidation activity and certain toxicity. Reactive oxygen species usually maintain a low level of dynamic balance in plants. Once the accumulation of reactive oxygen species (ROS) in plants is large, it will cause damage and accelerate the process of plant senescence. Lin et al. (2012) found that the main reason for the noel to produce lesion mimic phenotype was the significant increase in hydrogen peroxide content in plants, which in turn activated nitrate reductase, resulting in a large accumulation of nitric oxide in rice.

2.4 Hormonal imbalance
As hormone signaling molecules, ethylene, salicylic acid and jasmonic acid play an important role in the signaling process in plants. And metabolic disorders of these hormones may lead to the production of lesion mimic. Jiang et al. (2009) found that the increase of endogenous salicylic acid level of ssil2 induced a significant increase in the expression of key factors regulating rice resistance WRKY45 resulting in lesion mimic mutant.
2.5 Lonic channel blocked
In plant cells, cytoplasmic ions play an important role in the defense system in plants. Ion channels associated with abnormal gene expression, which may cause metabolic disorders and then cause lesion. Balague et al. (2003) found that HLM1 genes play an important role in cyclic nucleotide-gated channels, which is activated by cGMP and cAMP to allow K⁺ and Na⁺ to pass through, thus hlm1 mutants to produce lesion mimic when they exhibit abnormal disease-resistant allergic reactions. Ca²⁺ signaling also plays a very important role in the immune regulation in plants.

2.6 Rice PCD process lost control
PCD is a very critical process of rice lesion mimic. Zeng et al. (2004) found that mutant spl11 is able to encode U-Box/ARM proteins with E3 ubiquitin ligase functions. U-Box/ARM proteins negatively regulate the defense response and cell death process in rice. Spl11 interact with SPIN1 and RBS1 proteins, leading to early termination of translation of spl11 proteins, suggesting that spl11 proteins may negatively regulate plant PCD and defense responses through the ubiquitin pathway. Liu et al. (2017) found that rice lesion mimic mutant lm-ZH phenotypic characteristics are caused by OsCUL3a dysfunction. OsCUL3a is able to interact with OsNPR1 in vivo and mediate its 26S proteasome degradation process, which can negatively regulate rice PCD process by targeting degradation.

2.7 Abiotic stress
When rice is stressed by abiotic factors such as light and temperature, the plant will make a series of immune responses in order to adapt to the external environment. In this process, PCD, ROS and immune defense system will be turned on, which will lead to the occurrence of lesion mimic. The mutant lm-psl was affected by light. Under the condition of lighting, the spots of the mutant were not appeared, but after the restoration of light, the lesion mimic phenotype appeared again, and its photosynthetic pigment content was significantly reduced (Xia et al., 2019). Wang et al. (2004) found that high temperature would lead to a decrease in the number of lesion mimic mutants. However, at the later stage of rice growth, the inhibitory effect of temperature on the phenotype of disease-like spot was not obvious.

3 Cloning of Rice Lesion Mimic Genes
Through long periods of natural selection, rice has evolved a sophisticated immune defense system against pathogens from the outside world. Among the lesion mimic genes that have been mapped and cloned, many genes show resistance to rice diseases, and this resistance is usually associated with disease-like phenotypes. By analyzing the function of 20 lesion mimic mutation genes, it was found that they almost participated in different signal pathways and defense pathways (Table 1). The cloning and function of different types of lesion mimic mutations were summarized.

\( \text{spl11} \) mutant was obtained from indica rice varieties induced by EMS. It was controlled by a single invisible gene and located on chromosome 12 of rice. The leaves of \( \text{spl11} \) mutant showed iron rust spots from late tillering stage to mature stage, and it was found that the resistance of \( \text{spl11} \) mutants to rice blast and bacterial blight was higher than that of wild type (Zeng et al., 2004). \( \text{SPL11} \) gene encodes an E3 ubiquitin ligase. Gene sequence analysis showed that the mutation site resulted in the production of a stop codon, which could not form a complete transcription product, and eventually led to the formation of leaf lesions.

\( \text{SPL18} \) gene is the first dominant lesion mimic gene to be discovered. It is obtained by inserting T-DNA into the mutant Nipponbare (\textit{Oryza sativa} L. ssp japonica), and it is located on chromosome 12 of rice (Mori et al., 2007). Compared with the wild type, the gene expression was mainly concentrated in the spike and leaf sheath, and the leaf expression was low, which increased the number of lesions and enhanced the resistance to rice blast. \( \text{Spl18} \) gene encodes an acyltransferase, the expression of PR1 and PBZ1 is increased, and the content of phytoalexin is also increased, which is reflected in the increase of the content of Sakura and Momilactone A.
<table>
<thead>
<tr>
<th>Gene name</th>
<th>Chromosome</th>
<th>Gene encoding</th>
<th>Gene function</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>OsNPR1</td>
<td>1</td>
<td>Transcription coactivator</td>
<td>Regulation of jasmonate and salicylic acid resistance pathway</td>
<td>Yuan et al., 2007</td>
</tr>
<tr>
<td>OsSSI2</td>
<td>1</td>
<td>Fatty acid desaturase</td>
<td>Participate in the negative regulation of rice disease resistance pathway mediated by salicylic acid</td>
<td>Jiang et al., 2009</td>
</tr>
<tr>
<td>SPL28</td>
<td>1</td>
<td>Adaptor protein complex</td>
<td>Involved in the regulation of vesicle transport in cytoplasmic matrix</td>
<td>Qiao et al., 2010</td>
</tr>
<tr>
<td>OsCUL3a</td>
<td>2</td>
<td>Cullin like ubiquitin ligase</td>
<td>Negative regulation of cell death and defense stress in Rice by targeted degradation of OsNPR1</td>
<td>Liu et al., 2017</td>
</tr>
<tr>
<td>GF14e</td>
<td>2</td>
<td>14-3-3 protein</td>
<td>Regulation of plant immune stress</td>
<td>Manosalva et al., 2011</td>
</tr>
<tr>
<td>OsHPL3</td>
<td>2</td>
<td>Lipophosphoribooxidase</td>
<td>Participate in lipid and oxygen metabolism</td>
<td>Tong et al., 2012</td>
</tr>
<tr>
<td>OsLMS</td>
<td>2</td>
<td>RNA binding protein</td>
<td>Mediated resistance response of Magnaporthe grisea</td>
<td>Undan et al., 2012</td>
</tr>
<tr>
<td>CEA62</td>
<td>2</td>
<td>Allene oxide synthase</td>
<td>Participate in mediating plant cell defense response</td>
<td>Liu et al., 2012</td>
</tr>
<tr>
<td>OsACDR1</td>
<td>3</td>
<td>RAF like mpkk kinase</td>
<td>Participate in negative regulation of rice disease resistance pathway and positive regulation of ethylene synthesis</td>
<td>Kim et al., 2009</td>
</tr>
<tr>
<td>RLIN1</td>
<td>4</td>
<td>Fecal porphyrinogen III oxidase</td>
<td>Participate in the metabolism of tetrapyrrole in Rice</td>
<td>Sun et al., 2011</td>
</tr>
<tr>
<td>SPL7</td>
<td>5</td>
<td>Heat shock transcriptional factor</td>
<td>Negative regulation of apoptosis by heat shock</td>
<td>Yamanouchi et al., 2002</td>
</tr>
<tr>
<td>OsPt1a</td>
<td>5</td>
<td>Receptor protein kinases</td>
<td>RAR1 dependent negative regulation of rice immune stress</td>
<td>Takahashi et al., 2007</td>
</tr>
<tr>
<td>SPL5</td>
<td>7</td>
<td>Sf3b3 splicing factor</td>
<td>Regulation of plant defense response and cell death by RNA splicing</td>
<td>Chen et al., 2012</td>
</tr>
<tr>
<td>OsLSD1</td>
<td>8</td>
<td>Zinc finger protein</td>
<td>Participate in the negative regulation of apoptosis</td>
<td>Wang et al., 2005</td>
</tr>
<tr>
<td>SPL29</td>
<td>8</td>
<td>UAPI gene</td>
<td>Participate in carbohydrate metabolism</td>
<td>Wang et al., 2015</td>
</tr>
<tr>
<td>FGL</td>
<td>10</td>
<td>OsPORB protein</td>
<td>Participate in chlorophyll or other cytochrome synthesis</td>
<td>Sakuraba et al., 2013</td>
</tr>
<tr>
<td>NLS1</td>
<td>11</td>
<td>CC-NB-LRR type protein</td>
<td>Participate in the signal transduction of plant immune response and defense response to pathogens</td>
<td>Tang et al., 2011</td>
</tr>
<tr>
<td>OsSL</td>
<td>12</td>
<td>Cyp71p1 protein</td>
<td>Participate in the biosynthesis of serotonin in Rice</td>
<td>Fujiwara et al., 2010</td>
</tr>
<tr>
<td>SPL11</td>
<td>12</td>
<td>U-box E3 ubiquitin ligase</td>
<td>Participate in the plant apoptosis defense</td>
<td>Zeng et al., 2004</td>
</tr>
<tr>
<td>SPL18</td>
<td>12</td>
<td>Acryltransferase</td>
<td>Positive regulation of PCD and defense response</td>
<td>Mori et al., 2007</td>
</tr>
</tbody>
</table>

Spl5 mutant was obtained from non-glutinous rice induced by γ-ray irradiation and located on chromosome 7. The mutant appeared reddish brown or tan spots 15–20 d after sowing to heading stage, and the number of spots increased with the growth of the plant (Chen et al., 2012). Spl5 gene encodes a SF3B3, which negatively regulates defense stress response and cell death by regulating RNA splicing.

OsLSD1 gene, a rice lesion mimic mutation controlled by endogenous signaling pathway, is located on chromosome 8 (Wang et al., 2005). OsLsd1 mutation will cause HR lesion. And OsLSD1 gene overexpression will accelerate plant regeneration, increase the content of chlorophyll B, improve the ability of photosynthesis, and thus promote growth, differentiation, and regeneration. OsLSD1 gene and Arabidopsis thaliana LSD gene encode proteins containing three zinc finger domains and are expressed in the nucleus.

OsPTI11A gene is a recessive gene located on chromosome 6, which is obtained by inserting the retrotransposon Tos17 (Tos17 triggered mutational) into the genome of Nipponbare (Takahashi et al., 2007). About 30 days after
ttm1 mutants were planted in the field, fuzzy brown spots spontaneously appeared on both sides of the leaves. The resistance of *Xanthomonas oryzae* and *Magnaporthe grisea* increased after the appearance of the lesion mimic. *OSPT11A* is a plasma membrane protein kinase-related gene. The expression of resistance and the signal transduction of defense response play a negative regulatory role.

A mutation caused by transposon insertion of the *hpl3-1* mutant on chromosome 2. The seedlings of *hpl3-1* mutant began to appear lesion in 2 weeks. With the growth of the plant, the lesion extended to the whole leaf until the leaf necrosis. In addition, the mutant showed dwarfing, which affected the main agronomic shape (Tong et al., 2012). *OsHPL3* gene encodes fatty acid hydroperoxide lyase, which affects lipoxygenase metabolism. The mutation caused damage to HPL, accumulation of SA, and activation of plant stress defense response.

### 4 Resistance Mechanism of Rice Lesion Mimic Mutants

Many lesion mimic mutants would changes in physiological indicators at the cellular level, such as the initiation of anaphylaxis in the plant, thereby showing increased resistance in the plant. A large number of studies also showed that most of the rice lesion mimic mutants were resistant to *Xanthomonas oryzae* or *Magnaporthe grisea* (Chen et al., 2011). Luo et al. (2012) also confirmed that *AtNPR1* could enhance the resistance to *Magnaporthe grisea* and reduce yield loss. During the process from the emergence of lesion mimic to leaf wilt and necrosis, intermediate metabolites of reactive oxygen species accumulate rapidly, which directly leads to allergic reactions and stress reactions that kill pathogenic bacteria (Wang et al., 2006). Takahashi et al. (1999) showed that during the onset of mutants cdr1~2 and cdr3, in addition to the accumulation of intermediate metabolites of reactive oxygen species, a large amount of callose and phenolic substances were also accumulated in cells, and the production of these substances could enhance the defense system of plant. Summarized the resistance mechanism of some rice lesion mimic mutants (Figure 1), it was found that ethylene, green leaf volatiles, jasmonic acid and salicylic acid were also involved in plant resistance and stress response during the PCD process of rice. Reactive oxygen species (ROS) and their intermediate metabolites are the first type of signal molecules. Most of them cause the production rate of reactive oxygen species to exceed the decomposition rate, resulting in the accumulation of reactive oxygen species, thus activating the intracellular antioxidant enzyme system and maintaining the balance system of intracellular reactive oxygen species. When the reactive oxygen species reached a certain level, it would activate the programmed cell death pathway in the cells, and finally the lesion mimic appeared on the leaves.

### 5 Progress

The formation of rice lesion mimic is affected by many factors, and rice resistance is also related to the lesion mimic process. The lesion mimic mutants usually induce immune response in the process of lesion, and induce defense system and show certain resistance to rice diseases. Meanwhile, the plant height, effective panicle number, grain number per spike, seed setting rate and other agronomic traits were also affected. Therefore, the cloning and functional analysis of rice lesion mimic mutation genes are becoming more and more important, and the molecular regulation mechanism of rice lesion mimic mutation needs to be further studied.

![Figure 1 Mechanism of PCD in some rice lesion mimic mutation](image-url)
At present, most of the cloned lesion mimics mutation genes showed good resistance to *Xanthomonas oryzae* and *Magnaporthe grisea*. Therefore, how to use this type of gene, combined with the major functional genes of *Xanthomonas oryzae* and *Magnaporthe grisea*, to improve yield, and quality, and to play a synergistic role in resistance, will be worthy of further research. Especially for *Xanthomonas oryzae* resistance breeding, it is urgent to improve the horizontal resistance, aggregate different main effect functional genes, and enhance the broad-spectrum and long-lasting disease resistance in rice breeding and production.

**Authors’ contributions**

QJY completed data collection and analysis, drafted the manuscript, and proofread. LF participated in the design and revision of the study. QC conceived and directed of this study, helped to draft and revises the manuscript. All authors read and approved the final manuscript.

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