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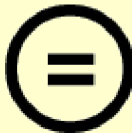

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농학석사학위논문

벼 도열병균의 Lignin Peroxidase 유전자에 대한 특성 규명

Characterization of Lignin Peroxidase Genes in the Rice Blast Fungus, *Magnaporthe oryzae*

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류 현 주

A THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Characterization of Lignin Peroxidase Genes in the Rice Blast Fungus, *Magnaporthe oryzae*

By

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February 2017

ABSTRACT

Characterization of Lignin Peroxidase Genes in the Rice Blast Fungus, *Magnaporthe oryzae*

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Magnaporthe oryzae is a causal agent of the rice blast which is one of the most devastating diseases of rice worldwide. The rice blast is considered as an important model for studying plant-fungal pathogen interactions. Plants induce a wide range of defense responses to cope with pathogen attacks. Lignification is one of the induced defense responses and involved in not only fortifying the plant cell wall but also inactivating fungal membranes and secretions. In addition, fungal hyphae may lose plasticity necessary for growth due to the lignification of a hyphal tip. In order to overcome this defense reactions, pathogens secrete lignin-degrading enzymes such as laccases and lignin peroxidases. Two of bZIP transcription factors of the rice blast fungus were found to regulate genes related to pathogenicity, including lignin-degrading enzyme genes. Among these lignin-degrading enzyme

genes, the expression levels of *MoLIP1* and *MoLIP3* were the most notably affected. Therefore, these two genes were chosen for in-depth analysis using gene deletion strategy. There were no significant differences in mycelial growth, conidiation, conidial germination, appressorium formation and resistance of oxidative stress between the wild-type and Δ*Molip1* and Δ*Molip3* mutants. These results indicated that *MoLIP* genes were not directly involved in mycelial growth, infection-related morphogenesis and detoxification of reactive oxygen species. However, pathogenicity of the mutants decreased and the invasive growth of the mutants was delayed when the mutants were inoculated on rice leaves and in sheath, respectively. These results suggest that lignin peroxidase is required for early infection stage of *M. oryzae*.

Keywords: Peroxidase, Lignin peroxidase, *Magnaporthe oryzae*, Rice blast fungus

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I. INTRODUCTION

Magnaporthe oryzae is a causal agent of the rice blast which is one of the most devastating diseases of rice worldwide. As the genome sequencing of both rice and the fungus has been finished, genome-wide functional analysis is available. In terms of molecular genetic approach, the fungus is the important model for studying plant-fungal pathogen interactions (Wojtaszek, 1997). Plants have evolved a wide range of defense responses to cope with their pathogens. Plants induce synthesis of jasmonic acid and ethylene inducing induced systemic resistance response and the expression of WRKY transcription factors related to pathogenesis (Pieterse et al., 1996), leading to antimicrobial protein synthesis, lignin synthesis, and reactive oxygen species (ROS) production (Dixon and Paiva, 1995; Mauch-Mani and Slusarenko, 1996).

Lignin is a class of complex organic polymer. As a final product of the phenylpropanoid pathway, it is important structural materials in plants, especially formation of cell wall. Also, it is first physical and nondegradable barrier for most microorganisms. Lignification is one of the induced defense responses and involved in not only fortifying the plant cell wall but also inactivating fungal membranes and secretions. In addition, fungal hyphae may lose plasticity necessary for growth due to the lignification of a hyphal tip (Vance et al., 1980). It was also confirmed that lignification was induced in rice as defense reactions when rice is infected with pathogen. Phenylalanine ammonia lyase (PAL) is involved in the early biosynthesis stage of the polyphenol compound such as flavonoid, lignin in plants. Giberti et al. (2012) reported that PAL specific activity increased when cell wall hydrolyastes from *M. oryzae.* This result indirectly confirmed that

lignification in rice was induced when *M. oryzae* infected the rice. In order to overcome this defense reactions, pathogens secrete lignin-degrading enzymes.

Ligninolytic enzymes consist of heme peroxidase family including lignin peroxidase, manganese peroxidase, and versatile peroxidase and phenol oxidase family including laccase. Laccases [EC 1.10.3.2, benzenediol:oxygen oxidoreductase] catalyze subtraction of one electron from phenolic hydroxyl groups of phenolic lignin model compounds, such as vanillyl glycol, 4,6-di(t-butyl)guaiacol, and syringaldehyde, to form phenoxy radicals. Lignin peroxidases [EC 1.11.1.14, 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen-peroxide oxidoreductase, LiP] catalyze the H_2O_2-dependent oxidative depolymerization of lignin. Lignin peroxidase interacts with the lignin polymer involving veratryl alcohol. Manganese peroxidase [EC 1.11.1.13, Mn(II):hydrogen-peroxide oxidoreductase, MnP] catalyzes the Mn-dependent reaction $2Mn(II)+2H^{+}+H_2O_2=2Mn(III)+2H_2O$ (Wong, 2009).

Studies on lignin-degrading enzymes have been performed in fungal pathogen which invades trees. *Heterobasidion irregular* in basidiomycetes is one of the most important fungal pathogen of conifers and other woody plants. The spores germinate and the mycelia of the fungus grows into the wood. The mycelia colonizes the wood by decomposing the lignin and cellulose, producing a stringy white rot. The major lignin-degrading enzymes are laccase and manganese peroxidase (Yakovlev et al., 2013). Lignin-degrading enzymes play an important role not only in trees but also in crops. *Fusarium solani* f. sp. *glycines* causes sudden death syndrome which is one of the most important disease of soybean. Lignin degradation may play a role in the infection, colonization, and survival of

the fungus in root tissue. Major fungal lignin-degrading enzymes are laccase and lignin peroxidase (Lozovaya et al., 2006). However, ligninolytic enzyme activity has not been determined for *M. oryzae*.

Therefore, the objective of this study is to identify the function of the fungal lignin-degrading enzymes as the key enzymes related to pathogenicity (peroxidase genes which are unknown function).

II. MATERIALS AND METHODS

1. Selection of lignin peroxidase genes

Lignin-degrading enzymes of *M. oryzae* have 9 laccase genes and 3 lignin peroxidase genes (Choi et al., 2014). To select lignin-degrading enzyme genes used in this study, we referred to the previous study on bZIP transcription factor genes. Relative expression levels were analyzed using RNA sequence data of wild-type, *MobZIP14,* and *MobZIP22* deletion mutants.

2. Fungal strains, culture conditions and conditions of specialized treatments

M. oryzae strain KJ201 was offered by the Center of Fungal Genetic Resources (CFGR, http://genebank.snu.ac.kr) and was used a wild-type in this study. All strains including wild-type and mutants generated in this study were cultured on V8 juice agar plates (8% V8 juice (v/v), 1.5% agar powder (w/v), pH6.7) or minimal agar plate (MMA, 1% glucose, 0.6% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.1% trace element (v/v), 0.1% vitamin supplement (v/v) and 1.5% agar powder, pH 6.5; all w/v unless otherwise stated) at 25°C under the constant fluorescent light. Mycelia of wild-type and mutants were harvested from 3-day-old in liquid complete media (LCM, 0.6% yeast extract (w/v), 0.6% casamino acid (w/v) and 1% sucrose (w/v)) at 25°C under dark condition and used for genomic DNA and RNA.

3. Generation targeted disruption of the *MoLIP1* and *MoLIP3*

To generate targeted deletion mutants, homologous recombination strategy was used (homologous recombination). The original sequences of MGG_07790.8 and MGG_14940.8 (Comparative Fungal Genomics Platform, http://cfgp.riceblast.snu.ac.kr) were replaced with knock-out construct containing 1.4kb of hygromycin B phosphotransferase gene fused with about 1kb 5'- and 3'- flanking regions (Yu et al., 2004). Protoplast generation and fungal transformation of *M. oryzae* were performed using the standard polyethylene glycol (PEG) mediated protocol (Sweigard et al., 1992). Selection of hygromycin-resistant transformants were carried out using TB3 agar plates (0.3% yeast extract, 0.3% casamino acids, 1% glucose, 20% sucrose and 0.8% agar powder; all w/v) containing 200ppm hygromycin B and screened by PCR (Park et al., 2014). Additionally, Southern blot analysis was performed using genomic DNA to identify successful deletion mutants with single integration event.

4. Nucleic acid isolation and manipulation

Fungal genomic DNA extracted by standard method (Sambrook et al., 1989) was used to Southern hybridization analysis (Rogers and Bendich, 1985). DNA fragments were labeled with ^{32}P by using Rediprime™ II Random Prime Labeling System kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer's instructions for DNA hybridization probes.

5. Developmental phenotype assays – Mycelial growth, conidiation, germination and apprressorium formation

Mycelial growth rate was measured from 9-day-old with diameter of three replicates on Talbot modified complete agar plate (MCA, 0.2% peptone, 0.1% yeast extract, 0.1% casamino acid, 1% glucose, 0.6% $NaNO_3$, 0.05% KCl, 0.05% $MgSO_4$, 0.15% KH_2PO_4, 0.1% trace element (v/v), 0.1% vitamin supplement (v/v) and 1.5% agar powder, pH 6.5; all w/v unless otherwise stated) and minimal agar plate (Talbot et al., 1993). Conidiation was measured by counting number of asexual spores in 10μl of conidia suspension on hemocytometer under a light microscope. Conidia were harvested from 7-day-old on V8 juice agar media with 5ml of sterilized distilled water. Germination and appressorium formation were observed on hydrophobic coverslips. For conidial germination and appressorium formation rate assay, conidia were harvested from 7-day-old on V8 juice agar media with sterilized distilled water and 30μl of conidial suspension was dropped onto coverslips with three replicates in $2X10^4$ conidia per milliliter concentration after filtering with 1 layer of mira cloth. After incubation droplets for 2 hours in moistened box at 25 ^oC, both germinated and non-germinated conidia were counted and this was converted into a percentage under a microscope in order to identify spore germination rate. To identify appressorium formation rate, conidia were harvested and incubated in same manner as germination assay. After 8 hours of incubation, appressorium formation rate was determined within the percentage of germinated conidia.

6. Pathogenicity assessment

For spray inoculation, conidia were harvested from 7-day-old V8 juice agar media, suspended sterilized distilled water and filtered with 1 layer mira cloth as $1X10^5$ conidia per milliliter concentration in 10ml mixture containing Tween 20 (250 ppm final concentration). Conidial suspension was sprayed to the 4-week-old susceptible rice seedlings (*Oryzae sativa* cv. Nakdongbyeo). Inoculated rice seedlings were incubated in a dark, humid dew chamber for 24 hours at 25°C. Next, they were transferred to a growth chamber maintained at 25°C with 80% humidity (Valent et al., 1991). Symptoms of infection were observed at 6 days post inoculation. The disease severity of each strain was assessed from the percentage diseased leaf area as calculated using the Axiovision image analyzer (Chi et al., 2009). Values are the mean±SD from five rice leaves inoculated by each strain. For sheath inoculation, conidial suspension of $2X10^4$ spores/ml inoculated sheath of 4-week-old rice plant. Infected sheath were incubated for 48 hours in moistened box at 25 °C. Fungal growth inside the rice sheath epidermal cells was examined under a light microscope.

7. Oxidative stress resistance assessment

To identify oxidative stress resistance, each strain incubated minimal agar plate. And then, 5mm radius size of agar block respectively inoculation on modified complete agar plate (MCA) containing 5mM hydrogen peroxide, and 200ppm congo red. Mycelial growth rate was measured from 9-day-old with diameter of three replicates on MCA plate containing 5mM hydrogen peroxide. In the case of the MCA supplemented with congo red, it is confirmed by the presence of the halo region.

Table 1. List of primers used in this study

Primer	Sequences (5'→3')
For generation and confirmation of deletion mutants:	
HyR_F	GGCTTGGCTGGAGCTAGTGGAGG
HyR_R	CTCCGGAGCTGACATCGACACCAAC
MGG_07790T0 5'Flank F	GACTCTTGGAGATTACGCTG
MGG_07790T0 5'Flank R	CCTCCACTAGCTCCAGCCAAGCCTGTAAGTGGACTCCTATGAG
MGG_07790T0 3'Flank F	TGGTGTCGATGTCAGCTCCGGAGGACCACTTGCCTTGAAAG
MGG_07790T0 3'Flank R	GTCCCGACCCAATCAATG
MGG_07790T0 Nested F	AGCCTCCGTAGCTATGCG
MGG_07790T0 Nested R	ATAGCGTGGGCTGGCTCT
MGG_14940T0 5'Flank F	GACGACCGTTGCACAGGAAGAA
MGG_14940T0 5'Flank R	CTCGAGGGGGTATATGGTAGTAAGAC
MGG_14940T0 3'Flank F	CCTGGGCTTGATTTCTTTAGGGTA
MGG_14940T0 3'Flank R	CCTTGATGTGGCGGTCGTTGAT
MGG_14940T0 Nested F	GTCCGTGGCCCAGGCAAATAAT
MGG_14940T0 Nested R	CGTGCTCAGGATCTCGATGCA
For confirmation of deletion mutants:	
MGG_07790 qRT_F	CAGCGGGTGTTCAAGTTC
MGG_07790 qRT_R	GCGTAGTCAAAGTTCCAGT
MGG_14940 qRT_F	AAAGGCACGGCACCGATC
MGG_14940 qRT_R	GCCATACCGACGCCGTAT

III. RESULTS

1. Selection of genes encoding lignin-degrading enzymes in *M. oryzae*.

Relative expression of genes encoding lignin-degrading enzymes in bZIP deletion mutants against wild-type was observed. Commonly, *MoLIP1* gene is downregulated and *MoLIP3* gene is upregulated in two bZIP deletion mutants (Figure 1). Since these two genes are most differentially expressed in the bZIP mutants than the wild-type, the functional analysis was performed with these two genes.

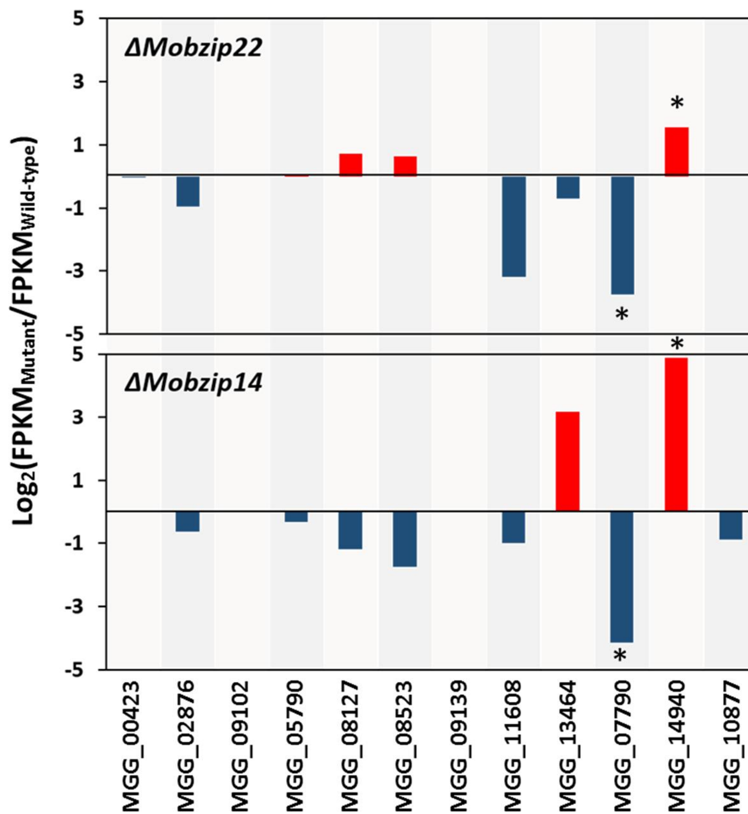


Figure 1. Relative expression level of transcript encoding lignin-degrading enzyme.

2. Domain architecture of *MoLIP1* and *MoLIP3* in *M. oryzae*

MoLIP1 and MoLIP3 commonly have heme peroxidase domain (IPR010255), fungal ligninase (IPR001621), and heme peroxidase (plant/fungal/bacterial) domain (IPR002016) predicted by InterPro analysis. The InterPro analysis predicted *MoLIP1* and *MoLIP3* genes would function as a lignin-degrading enzyme.

- ***MoLIP1* (MGG_07790)**

- ***MoLIP3* (MGG_14940)**

30 a.a.

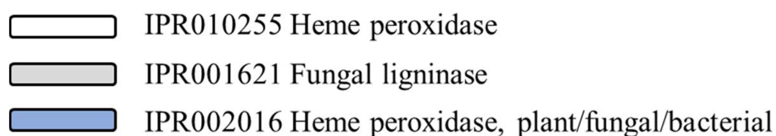


Figure 2. Domain structure of MoLIP1 and MoLIP3 predicted by InterPro analysis.

3. Phylogenetic analysis of *MoLIP1* and *MoLIP3*

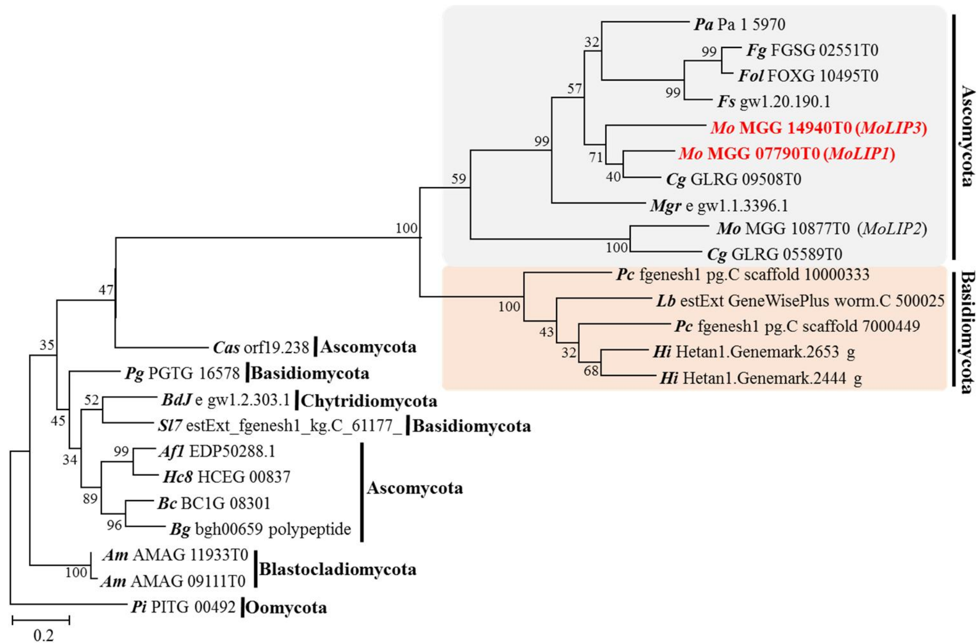


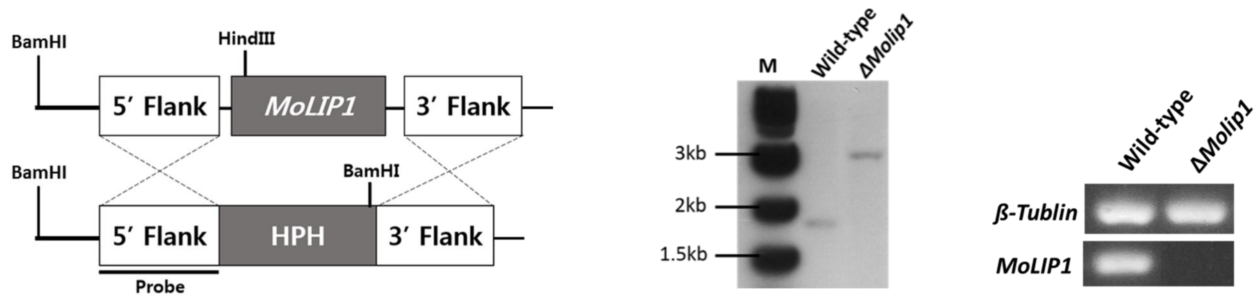
Figure 3. Phylogenetic analysis of *MoLIP1*, *MoLIP3* genes and genes homologous to the two *MoLIP* genes in fungi.

A neighbor-joining tree was constructed based on the amino acid sequences of lignin peroxidase genes in *M. oryzae* and genes homologous to the two genes in fungi. The numbers at the nodes indicate bootstrap values (%) in 10,000 bootstrap replicates. Gray box is the phylogenetic positions of Ascomycota. Red box is the phylogenetic positions of Basidiomycota.

4. Targeted genes replacement of *MoLIP1* and *MoLIP3* in *M. oryzae*

Gene deletion mutants were manufactured by targeted gene replacement with a resistance marker cassette. It was used by homologous recombination strategy and knockout constructs double jointed with up and downstream 1.2 kb~1.5kb of each genes and *HPH* cassette. Protoplasts were transformed with these constructs. Gene deletion was confirmed by Southern blot analysis and RT-PCR of transcript. (Figure 4)

(A)



(B)

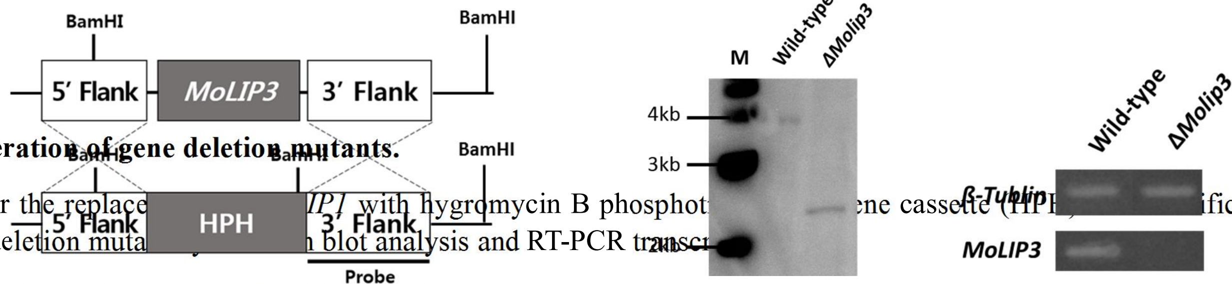


Figure 4. Generation of gene deletion mutants.

(A) Strategy for the replace[illegible]IP1 with hygromycin B phosphot[illegible]ene cassette (HP[illegible]ification of *MoLIP1* gene deletion muta[illegible] blot analysis and RT-PCR transcr[illegible]

(B) Strategy for the replacement of *MoLIP3* with hygromycin B phosphotransferase gene cassette (HPH) and identification of *MoLIP3* gene deletion mutant by southern blot analysis and RT-PCR transcript.

5. Developmental phenotypes and resistance of oxidative stress of Δ*Molip1*, Δ*Molip3* mutants

Conidiation, mycelial growth, and resistance of oxidative stress of mutants were not different from wild-type. Therefore, *MoLIP1* and *MoLIP3* were not involved in conidiation (Table 2) and mycelial growth (Figure 5). Also, *MoLIP* genes were not involved in general functions of peroxidases including detoxification of reactive oxygen species (Figure 6).

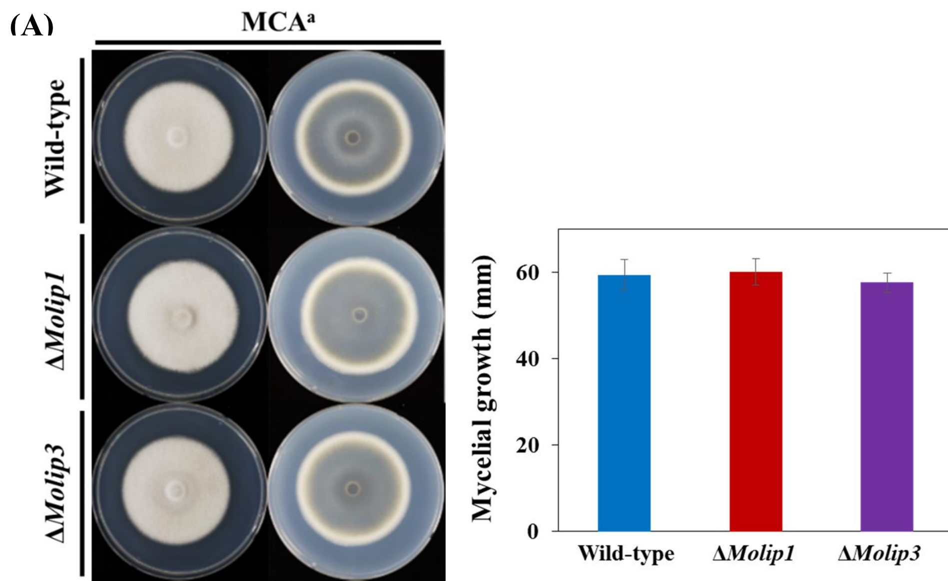
Table 2. Developmental phenotypes of Δ*Molip1* and Δ*Molip3* mutant

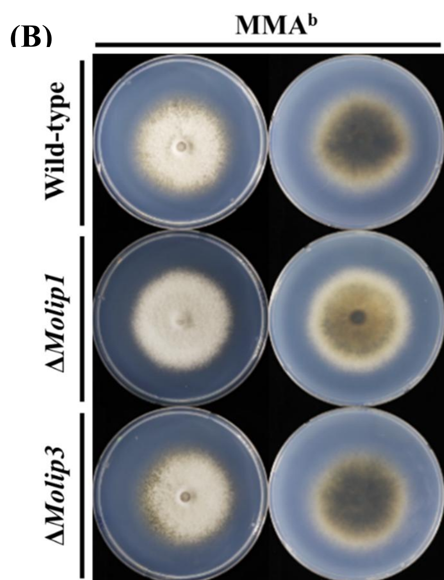
Strain	Conidiation[a] (10^4/ml)	Germination[b] (%)	Appressoria formation[c] (%)
Wild-type	38.11±12.32	82.67±3.21	79.24±1.37
Δ*Molip1*	38.56±1.26	81.00±0.00	71.67±2.08
Δ*Molip3*	37.78±4.81	86.00±1.73	85.17±1.04

[a] Conidia were harvested from 7-day-old on V8 juice agar media with 5ml of sterilized distilled water, the number of conidia was counted.

[b] The percentage of germinated conidia was counted the numeric at 2 hours after being placed on hydrophobic coverslips in the humid condition.

[c] The percentage of appressoria formation counted by rate of conidia which have formed appressorium by conidia was confirmed at 8 hours after being placed on hydrophobic coverslips in the humid condition.

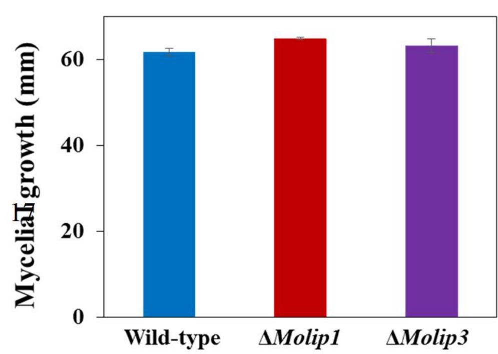




(A) Mycelial growth on [a]modified complete media plate (MCA) on day 9.

(B) Mycelial growth on [b]minimal agar plate (MMA) on day 9.

Figure 5. Vegetative growth of Δ*Molip1* and Δ*Molip3* mutants.



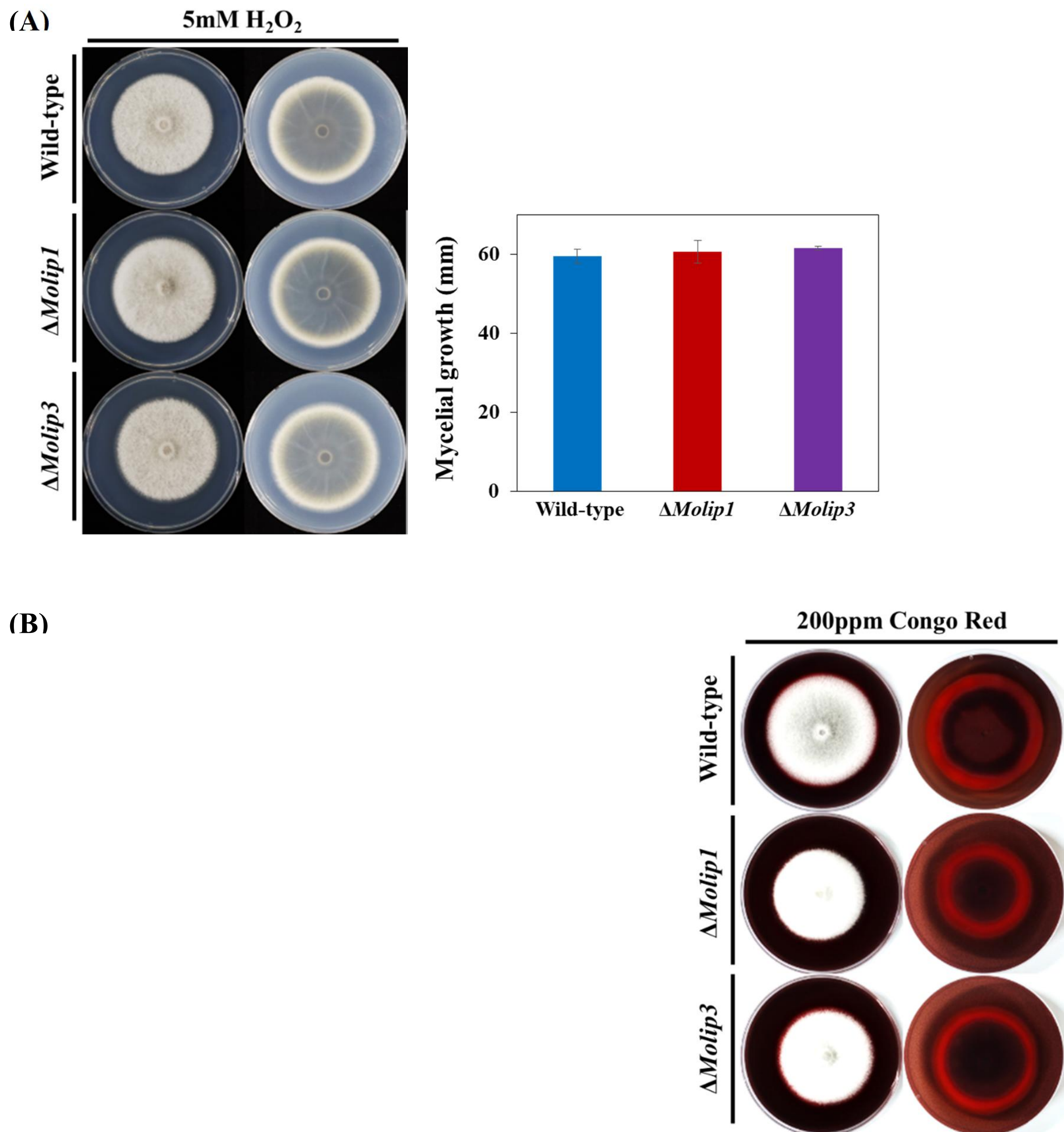


Figure 6. Oxidative stress resistance of Δ*Molip1* and Δ*Molip3* mutants.

(A) Mycelial growth on modified complete agar plate containing 5mM hydrogen-peroxide on day 9.

(B) Halo region on modified complete agar plate containing 200ppm congo red on day 9.

6. Pathogenicity of Δ*Molip1* and Δ*Molip3* mutants

Pathogenicity tests were performed to determine the role of lignin peroxidase involving the two genes during infection. Conidial suspension was sprayed on 4-week aged leaves of Nakdongbyeo cultivar that do not have any known R genes and make it a susceptible cultivar to all strains. Diseased leaves were harvested 6 days after inoculation. As a result, mutants showed a reduction of pathogenicity (Figure 7). When the diseased leaf area (%DLA) was measured, difference between wild-type and Δ*Molip1* mutant was statistically significant. However, in the case of Δ*Molip3* mutant, there is a difference but not statistically significant.

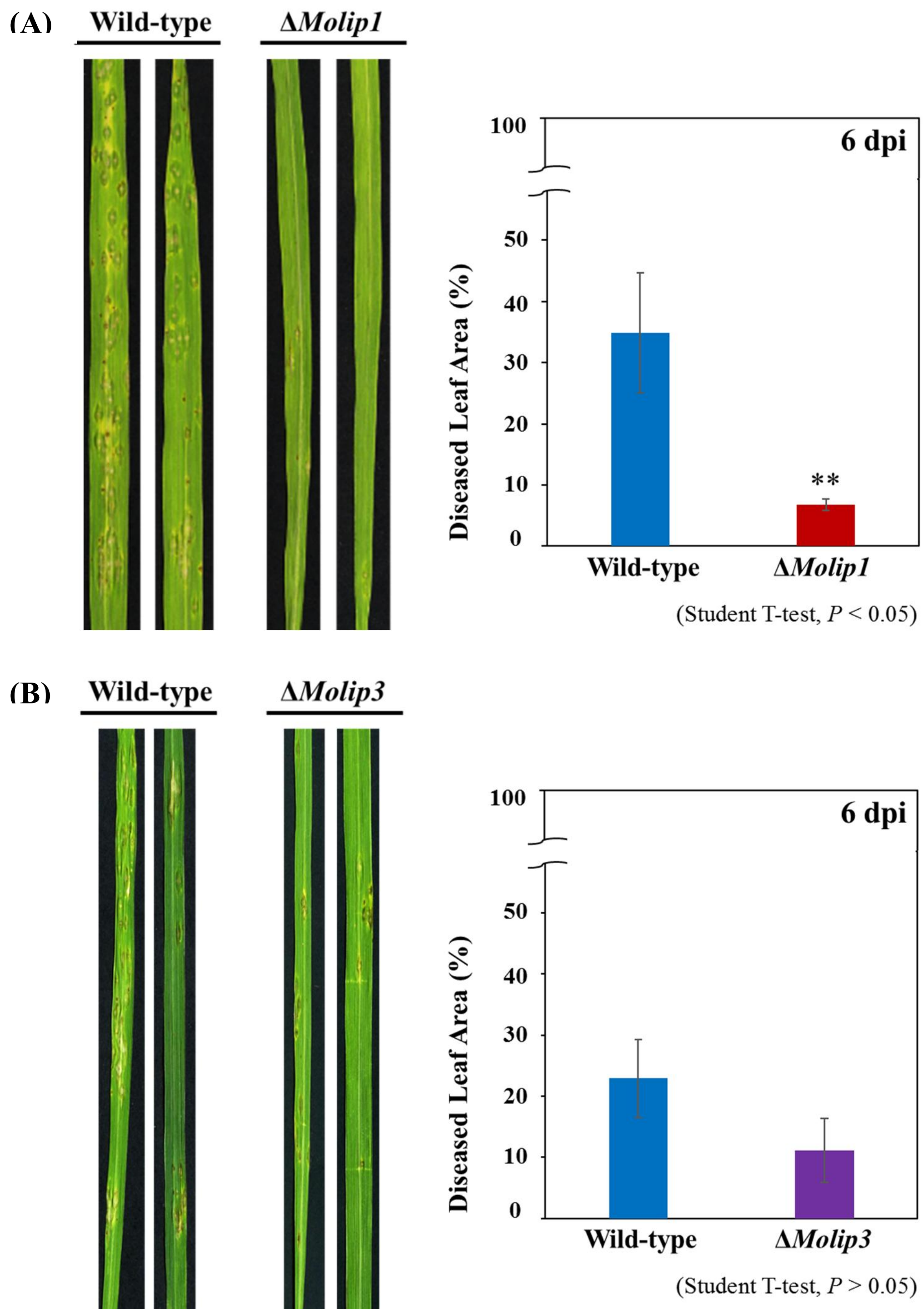


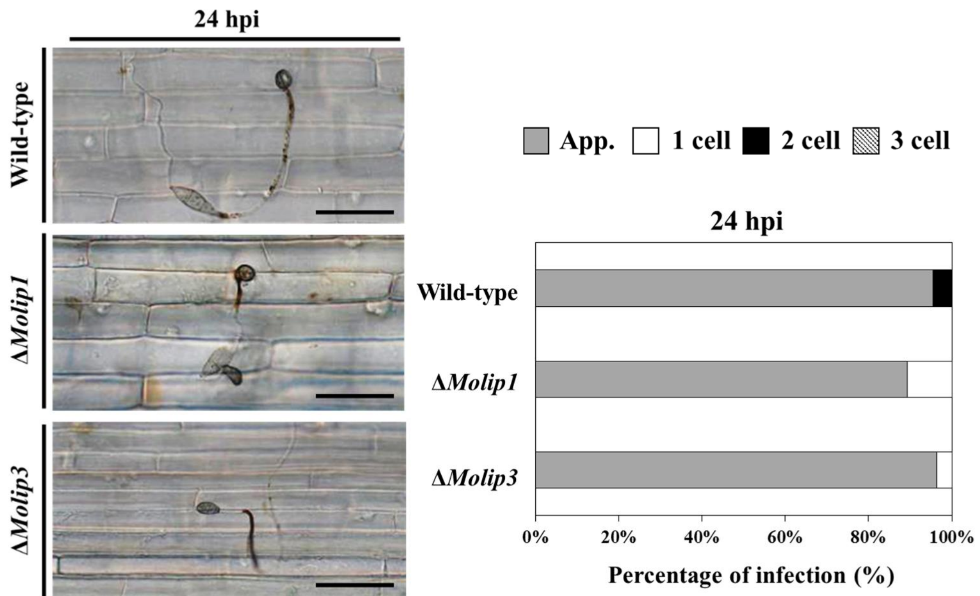
Figure 7. Effect of Δ*Molip1* and Δ*Molip3* mutants on pathogenicity.

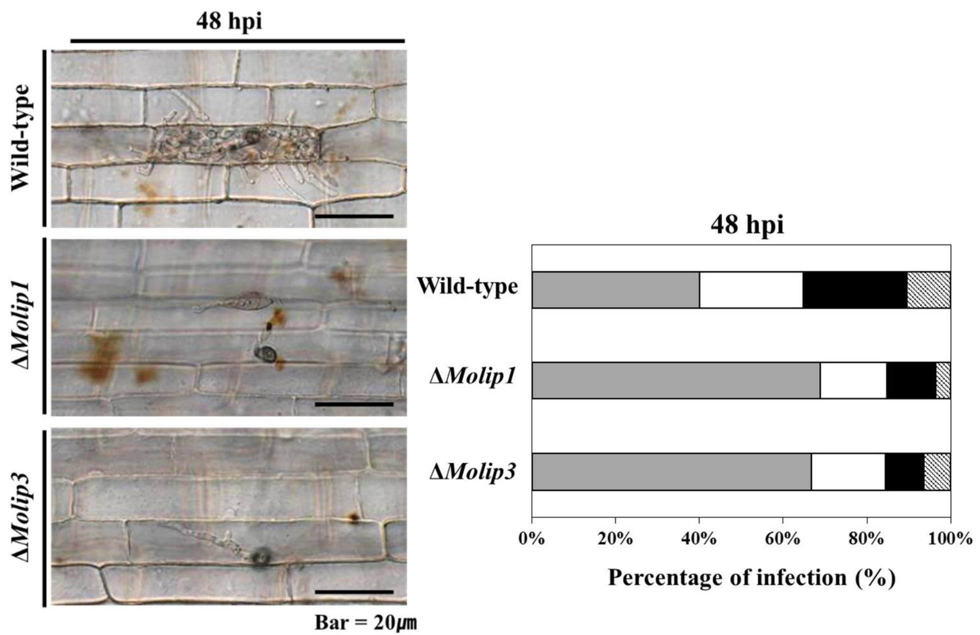
(A) Diseased leaf area of Δ*Molip1* mutant was significantly different from wild-type.

(B) Diseased leaf area of Δ*Molip3* mutant was different from wild-type but not significant.

7. Retardation of invasive growth

In order to identify the reduced pathogenesis, invasive growth in rice sheath was experimented. Twenty-four hours after inoculation, the individuals were found to appressorium formation stage or early infection stage. However, forty-eight hours after inoculation, 60 percentage of spores of wild-type was able to successfully invade rice sheath cells and invasive growth progressed from one cell to three. Respectively 68.8 and 66.8 percentage of spores of Δ*Molip1* and Δ*Molip3* mutants were found to stay in the appressorium formation stage (Figure 9). Through this results, *MoLIP* genes are involved in normal invasive growth.


(A)
24 hpi
Wild-type
ΔMolip1
ΔMolip3
App.
1 cell
2 cell
3 cell
24 hpi
Wild-type
ΔMolip1
ΔMolip3
0%
20%
40%
60%
80%
100%
Percentage of infection (%)


(B)
48 hpi
Wild-type
ΔMolip1
ΔMolip3
Bar = 20μm
48 hpi
Wild-type
ΔMolip1
ΔMolip3
0%
20%
40%
60%
80%
100%
Percentage of infection (%)

IV. DISCUSSION

Plants have evolved a wide range of defense responses to cope with their pathogens. Reactive oxygen species (ROS) in plant cells are one of the most rapid and dramatic defense reactions activated following pathogen attack (Wojtaszek, 1997). Accordingly, fungi have to overcome plant-derived ROS in order to

Figure 8. Invasive growth of Δ*Molip1* and Δ*Molip3* mutants *in planta*.
Quantitative measurement of rice sheath assay by Δ*Molip1* and Δ*Molip3*. Frequency was measured by counting five replicates of 50 conidia.
(A) 24 hours post inoculation (B) 48 hours post inoculation

successfully infect and colonize plant cells. Peroxidases are one of the enzymes integral to this process. There are twenty eight peroxidase genes in seventeen classes of *M. oryzae* (Choi et al., 2014). Previous studies in *M. oryzae* indicated that peroxidases are involved in not only detoxification of plant-derived ROS and increase in cell wall integrity but also production of internal ROS for development (Egan et al., 2007; Fernandez and Wilson, 2014; Huang et al., 2011; Skamnioti et al., 2007; Tanabe et al., 2011). These are the common functions of peroxidases. However, MoLIP1 and MoLIP3 have the fungal lignin peroxidase domain and the heme peroxidase domain through the InterPro analysis (Figure 2). Also, the peroxidases which are highly similar to *MoLIP1* and *MoLIP3* were found to be fungal manganese peroxidases in Basidiomycota (Figure 3). It was expected that two lignin peroxidases of *M. oryzae* would show lignin degradation activity.

Plant cell wall is composed of cellulose, callose, pectin, and lignin. Damage in cell wall induces the expression of genes related to defense responses in order to

fortify cell wall structure. The more evident defense strategies are to deposit callose and lignin and to induce peroxidase/ROS-mediated crosslinks between cell wall structural proteins and polysaccharides in necrotrophic fungi (Bellincampi et al., 2015). In the case of powdery mildew which is biotrophic fungus, a papillae structure was formed at the penetration sites. As lignin was accumulated in a papillae, invasive growth of the fungus was delayed by the lignified papillae although pathogens invade plant cells. Lignification makes the cell wall more resistant to mechanical pressure given by fungal appressorium during penetration as well as less accessible to cell wall-degrading enzymes. Accordingly, lignification is considered first defense response against successful penetration of pathogens (Bhuiyan et al., 2009).

The infection mechanism of *M. oryzae* is that appressorium is formed and used to penetrate into host cell by physical force in the same manner with powdery mildew (Wilson and Talbot, 2009). However, it is not clear whether *M. oryzae* invading through physical force is disturbed by lignification which is plant defense response. When *M. oryzae* was infected, papillae-like cell was formed in non-host Arabidopsis although papillae structure was not formed in host rice (Giraldo and Valent, 2013; Park et al., 2009). The previous studies suggested that *M. oryzae* could induce papillae-like cell structure.

When two *MoLIP* genes were deleted, there were no significant differences in mycelial growth, conidiation, conidial germination, appressorium formation and resistance of oxidative stress between the wild-type and mutants. These results indicated that *MoLIP* genes were not directly involved in mycelial growth, infection-related morphogenesis and detoxification of reactive oxygen species.

However, pathogenicity of the mutants decreased and the invasive growth of the mutants was delayed when the mutants were inoculated on rice leaves and in sheath, respectively. These results suggest that lignin peroxidase is required for early infection stage of *M. oryzae*. In order to confirm lignin peroxidase activity, enzyme activity test was examined using veratryl alcohol (Arora and Gill, 2001). But, enzyme activity did not appear in not only wild-type but also deletion mutants (Data not shown). The reason for this result is that the expression level of *MoLIP1* gene is only expressed in biotrophic stage, and that of *MoLIP3* gene is totally low in mycelial and total infection stages (Unpublished). According to the expression data, the enzymatic assay was conducted with the strains incubated in CM broth medium supplemented with rice leaves to enhance the expression of the *MoLIP* genes. Contrary to the expectation, lignin peroxidase activity did not appear in all strains. These results suggest that the *MoLIP* genes are only expressed when *M. oryzae* interacts with physiologically active host plants. Therefore, further experiments are required to investigate conditions in which *MoLIP1* and *MoLIP3* genes are expressed *in vitro*. After that, it is essential to confirm enzyme activity in order to identify the reduction of full virulence caused by the lignin peroxidases. However, this study suggests that lignin peroxidase is vital to not directly attack the plant but overcome plant defense response in early infection stage.

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벼 도열병균의 Lignin peroxidase 유전자에 대한 특성 규명

류현주

초록

벼 도열병균 *Magnaporthe oryzae*는 벼를 기주로 하는 식물병원균으로 벼 수확량을 감소시키는 주요 병원균 중 하나이다. 또한 벼 도열병은 식물병원균 상호작용의 중요한 모델로 알려져 있다. 벼는 벼 도열병균 침입 시, 리그닌화를 유도한다. 식물의 리그닌화는 식물의 세포벽을 강화할 뿐만 아니라 곰팡이의 세포막과 분비물을 비활성화 시키며 곰팡이 균사 끝을 리그닌화 시킴으로써 유연성을 잃게 한다. 벼 도열병균은 이러한 식물 방어반응을 극복하기 위해 리그닌 분해효소를 분비한다. 병원성 관련한 리그닌 분해효소의 역할은 수목병원균인 *Heterobasidion irregular*와 식물병원균인 *Fusarium solani* f. sp. *glycines*에서는 연구가 이루어져 있지만 그 기능을 확인함에 있어 리그닌 분해효소를 암호화 하는 유전자의 기능에 관한 연구는 이루어지지 않았다. 본 연구에서는 리그닌 분해효소 중 병원성에 관여하는 bZIP 전사조절인자에 의해 조절되는 Lignin peroxidase

유전자인 *MoLIP1*, *MoLIP3* 의 기능을 밝히기 위해 유전자 삭제 돌연변이체를 제작하였다. 이 두 유전자의 삭제 돌연변이체는 야생형 균주와 비교 하였을 때, 균사 생장과 분생포자형성, 포자 발아율, 부착기 형성률, 산화스트레스에 대한 저항성에 유의적인 차이가 없었다. 이 결과는 *MoLIP* 유전자들이 균사 생장과 침입과 관련된 형태형성, 활성 산소종 분해에 직접적으로 관여하지 않는 것을 확인할 수 있었다. 그러나, 벼에 분무접종을 실행하였을 때, 삭제돌연변이체의 잎 병반 면적률이 야생형 균주에 비해 유의적으로 감소하였다. 또한, 벼 줄기세포에 분생포자를 접종했을 때 병원균의 침입률이 감소하였다. 이 결과를 통해 Lignin peroxidase가 벼 도열병균의 초기 침입 과정에 관여하여 병원성에 영향을 준다는 것을 보여준다.

주요어 : 과산화수소 분해효소, Lignin peroxidase, *Magnaporthe oryzae*, 벼 도열병균

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