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学　位　論　文　概　要

Dissertation Summary

学位請求論文（Dissertation）
題名 Analysis of Molecular Mechanism of Plant and Fungal Pathogen Interaction
（邦訳又は英訳）植物と病原性糸状菌の相互作用における分子機構の解析

専攻（Division）: Natural System
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学位論文概要（Dissertation Summary）

Plant-fungal pathogen interactions involved several molecules including proteins and metabolites. Pathogen-associated molecular pattern (PAMPs)-triggered immunity (PTI) and Effector-triggered immunity (ETI) are two stages of plant-pathogen interactions that involved various important proteins. Understanding of molecular mechanism of plant-fungal pathogen interaction will greatly contribute toward effective disease control. *Fusarium graminearum* is the fungal pathogen causes Fusarium head blight (FHB) in wheat plant. FHB disease caused significant loss of yield and contaminated the grains with the harmful mycotoxins. The commercial cultivar with resistant phenotype is unavailable, therefore, farmers frequently use fungicide which is unsafe for their health and may contaminate the grains. New candidates of natural compound are necessary to control FHB disease as well as safe for the farmer and human food.

In this study, we screened the candidates of important factors from both plant and fungal proteins involved in plant-fungal pathogen interaction in the leaf epidermis of *Arabidopsis thaliana*. Also, natural compound to control the FHB disease was also examined. We peeled the epidermis of inoculated Arabidopsis leaves and extracted the proteins. Plant proteins from whole part of inoculated leaves were also prepared. We performed free-label shotgun proteomics utilizing Nano-liquid chromatography coupled with Orbitrap QE Plus mass spectrometry using the prepared proteins. Figure 1 showed the illustration of shotgun proteomics in this experiment. Fungal proteins expressed on the leaf epidermis were also identified and compared to proteins from fungal conidia before inoculation (0h).

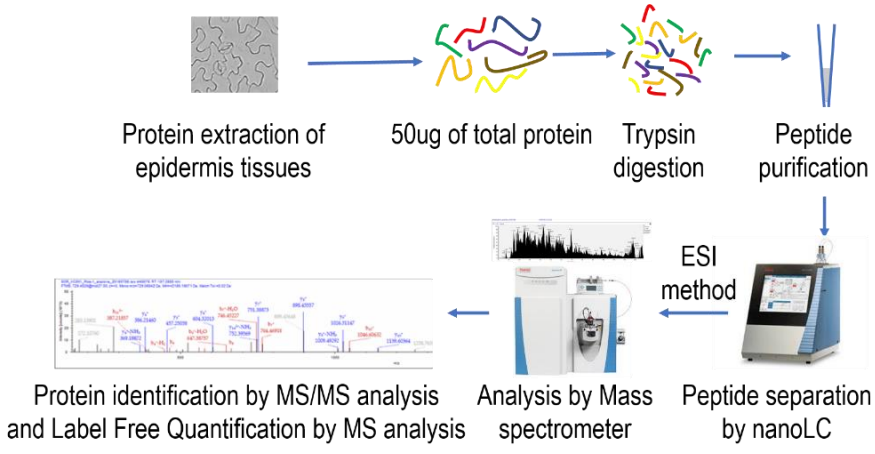

Figure 1. Schematic showing the workflow of shotgun proteomics in this experiment (Nishiuchi, 2021).

We identified about 5000 plant proteins from epidermis then considered the cut off value to determine differentially expressed proteins. The cut off value of abundance ratio was 1.5-fold with p-value<0.05. Then, we compared between epidermal proteins and whole leaf proteins then discriminated the proteins that specifically up-regulated in the epidermal tissues after fungal inoculation. At 4 hpi, 191 (99.4% of highly up-regulated epidermal proteins) proteins were uniquely up-regulated in the leaf epidermis, while 264 (94.3% of significantly up-regulated epidermal proteins) were up-regulated at 24 hpi. There were only small numbers of overlapped proteins in epidermis and whole leaf. This result showed some specific proteins in the leaf epidermis were up-regulated by *F. graminearum* inoculations in our established approach. It indicates the importance of leaf epidermis related to fungal infection.

Figure 2 showed the mapping analysis of up-regulated and down-regulated plant proteins to the Mapman database in category of pathogen/pest attack. Specific up-regulated proteins in the leaf epidermis were related to activation of plant immunity and defense response such as MKK5, BAK1, and NBS-LRR disease resistance protein. At 4 hpi MKK5 was up-regulated in the leaf epidermis. MKK5 and BAK1 are involved in PTI which are important for PAMPs perception and activate signal transduction to stimulate the transcription of resistance genes. At 24 hpi proteins related to ETI were upregulated specifically in the leaf epidermis. Disease resistance protein from two different classes, TIR-NBS-LRR class and CC-NBS-LRR class, were upregulated in the leaf epidermis and likely act as fungal effector-targeted proteins and trigger ETI system. Upregulated proteins at 24 hpi were also related to hormone signalling, transcription factors, pathogen, and cell wall.

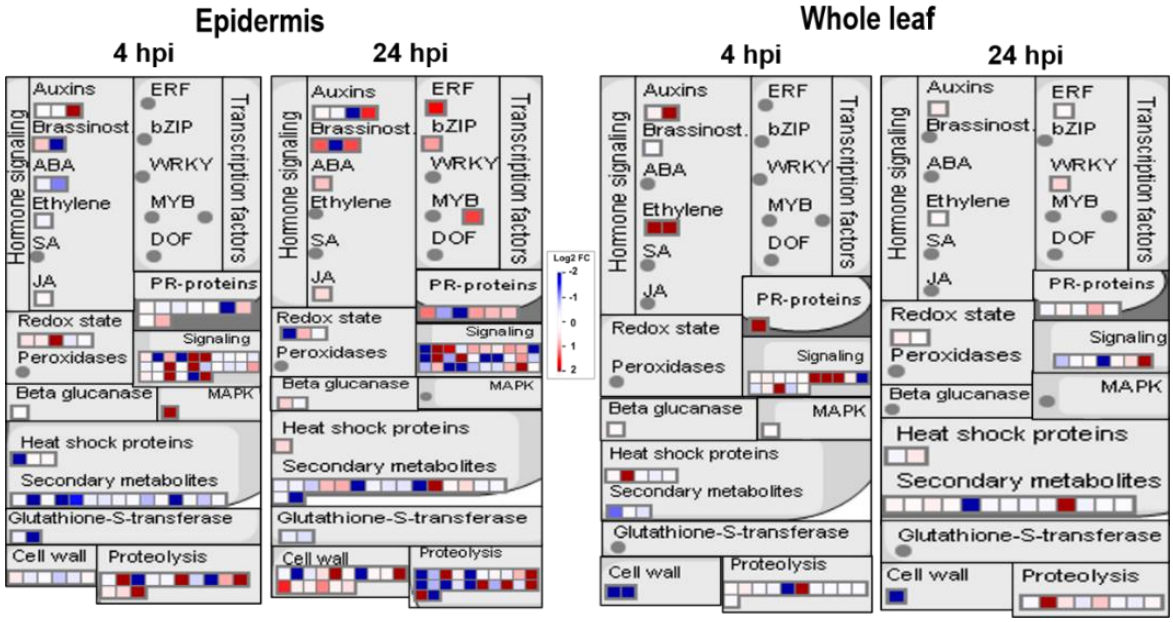

Figure 2. Mapping of identified proteins of leaf epidermis and whole leaf based on Mapman bin codes of Isoform Model TAIR10 database (Aug2012) with pathogen/pest attack. Identified proteins in the leaf epidermis and whole leaf were listed up with the fold-change value and converted the values into log2 fold-change. The list of proteins and the values were mapped to the Mapman database.

Additionally, 61 fungal proteins at 4 and 24 hpi were specifically expressed on the plant surface. Since these proteins were not expressed in 0 h protein of fungal conidia, we assured their expression was caused by contacting the plant surface and incubation for 4 h or 24 h. Therefore, these proteins indicate the important factor for fungal infection on leaf epidermis. Interestingly, fungal effector-like and kinase-domain proteins which have crucial role in fungal pathogenesis were identified in the leaf epidermis only. One of

fungal effector-like proteins was predicted as secretory protein. Rab7 protein has been reported as key regulator of FgAtg9 (ATG9 protein). It has been confirmed that ATG9 is essential for hyphal development and pathogenicity of *F. graminearum* (Zheng et al., 2018). Two kinase domain proteins were specifically identified in leaf epidermis. Kinase domain-containing protein (I1RW1, FGSG_08468) and Mitogen-activated protein kinase (I1RQN9, FGSG_06385) are essential for vegetative growth and pathogenicity of *F. graminearum* on wheat spikes (Wang et al., 2011). Overall, epidermal proteomics can be a beneficial approach to reveal the candidate of important proteins involved in plant-fungal pathogen interactions.

Also, we examined the effects of two natural compounds, nicotinamide mononucleotide (NMN) and nicotinamide (NIM), toward wheat plant against FHB. NMN or NIM was applied onto wheat spikes by spraying prior to fungal inoculation. We employed UHPLC-MS/MS for metabolome analysis to evaluate the metabolites accumulation caused by compounds pre-treatments. NIM-pretreatment significantly decreased the FHB disease, fungal gDNA, and mycotoxin contamination. Figure 3 showed the effect of NMN- and NIM-pretreatments on wheat spikes.

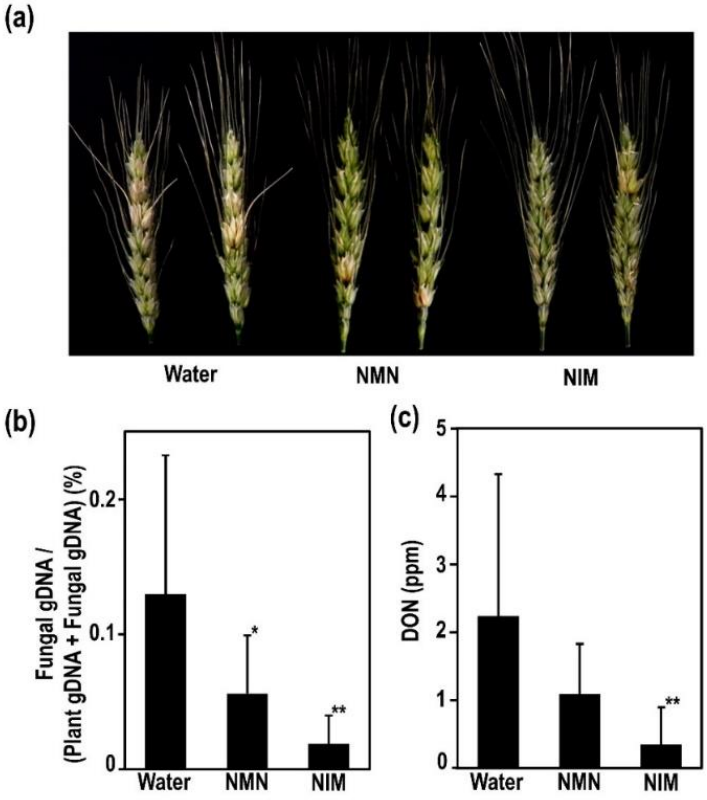

Figure 3. NMN- and NIM-pretreatment enhances the resistance against *F. graminearum* in wheat spikes. The spikes were treated with NMN, NIM, or water (control) by spraying before inoculation with *F. graminearum* (1×10^4 conidia/mL). (a) Representative photographs of spikes showing disease symptoms at 7 dpi. (b) Quantification of the relative fungal gDNA content of spikes by qPCR. (c) Accumulation of DON mycotoxin in spikes. In (b, c), error bars represent standard deviation (SD; $n = 12$). Asterisks indicate significant differences compared with the control (*$p < 0.05$, **$p < 0.01$; Student's *t*-test) (Sidiq, et al., 2021).

Metabolome analysis revealed that NIM-pretreatment induced the accumulation of numerous metabolites related to antifungal activity, antimicrobial and antioxidative. Figure 4a showed plant metabolites that were significantly accumulated in the NIM-pretreated spikes. Among these metabolites, it was bacancosin, a plant saponin and a natural detergent that degrades the microbial membranes (Osbourn, 1996). In addition, buchananine was reported as an antifungal metabolite (Lemaitre-Guillier et al., 2020). Additionally, sulfamethazine exhibits antimicrobial activities (Peng et al., 2015), while *cyclo*-Dopa 5-*O*-glucoside acts as a reactive oxygen species (ROS) scavenger (Nakagawa et al., 2018). Buchananine was

highly accumulated in NIM-pretreated spikes but without statistical significance compared with the control. Trigonelline as a plant alkaloid was likely contributed to the suppression of *F. graminearum* growth. Application of trigonelline onto barley leaves at 2 dpi enhanced disease resistance against powdery mildew by 56% (Kraska & Schönbeck, 1993). Figure 4b showed DON and ergosterol peroxide, metabolites derived from the fungal pathogen, were significantly reduced by NIM-pretreatment. Ergosterol is specifically found in fungal membranes (Alvarez et al., 2007). These results further support the hypothesis that NIM significantly reduces the FHB disease in wheat plants.

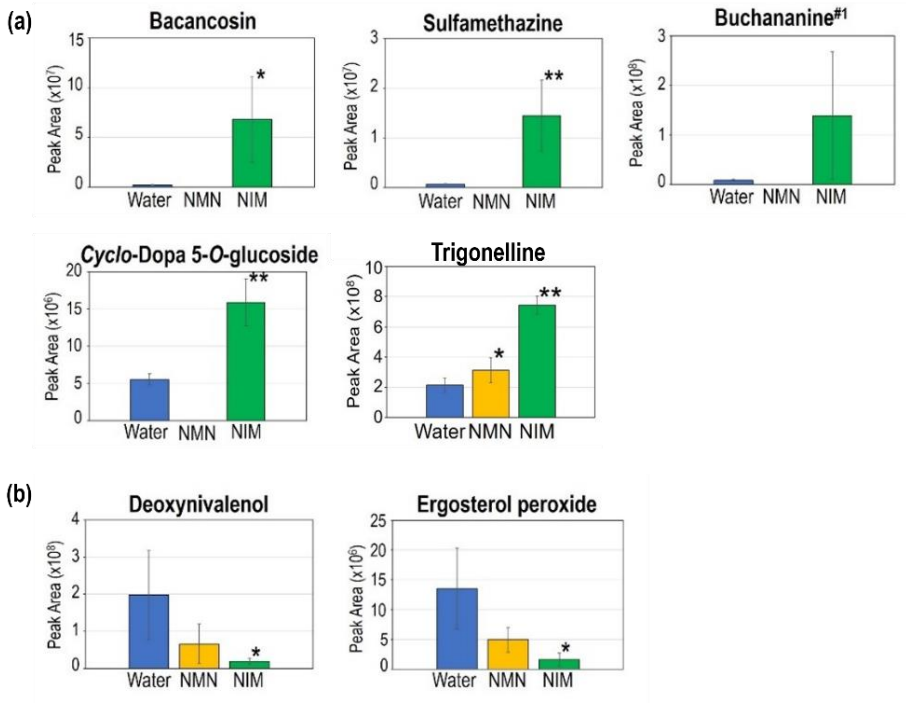

Figure 4. Quantification of differentially regulated metabolites in NMN- or NIM-pretreated and *F. graminearum*-inoculated spikes at 7 dpi. (a) Accumulation of plant metabolites, while (b) reduction of fungal metabolites. Error bars represent SD (n = 4–5). Asterisks indicate significant differences compared with the control (*$p < 0.05$, **$p < 0.01$; Student's t-test) (Sidiq, et al., 2021)