Dissertation Abstract

Analysis of Molecular Mechanism of Plant and Fungal Pathogen Interaction

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Abstract

In this study, I tried to identify proteins and metabolites involved in the plant and pathogen interaction. The plant epidermis contains stomata which is one of the entry gates for phytopathogens. A phytopathogenic fungus, *Fusarium graminearum* can infect many plant species such as wheat, barley and Arabidopsis through stomata of leaves and lemma. The epidermal proteomics was established to find the specifically expressed plant and pathogenic proteins on the Arabidopsis leaf epidermis inoculated with *F. graminearum*. Shotgun proteomics using LC-MS/MS showed many upregulated Arabidopsis proteins such as transcription factors and protein kinases specifically expressed on the epidermis. Most of them were not upregulated in the whole leaves. Fungal proteins expressed on the epidermis contained effector-like proteins. On the other hand, I tried to identify useful metabolites to control wheat spike disease called as Fusarium head blight (FHB). I found that the NIM (nicotinamide)-pretreatment onto spikes effectively suppresses disease symptoms of FHB. Correspondingly, deoxynivalenol mycotoxin accumulation was also significantly decreased by NIM-pretreatment. Metabolome analysis showed that several antioxidant and antifungal compounds were significantly accumulated in the NIM-pretreated spikes after inoculation. Thus. my approach using proteomics and metabolomics is useful for understanding the molecular interaction between plant and pathogen.

1. The biomass of *Fusarium graminearum* was accumulated on the plant surface.

To investigate the development of *F. graminearum* strain H3 on the plant surface, we fixed and stained the inoculated leaves then observed them on the fluorescence microscopy. As shown in Figure 1a at high magnification, germinated conidia of *F. graminearum* can be observed at 4 hours post-inoculation (hpi) and a massive mycelial colonization on the plants surface can be seen at 24 hpi. Correspondingly, quantification of fungal genome DNA (gDNA) showed a significant elevation of fungal gDNA content in the epidermis from 4 hpi to 24 hpi (Figure 1b). The fungal gDNA was about 3-fold increased at 24 hpi compared to 4 hpi. Early development of *F. graminearum* is signed by the germination of macroconidia as an asexual growth. On the wheat spikelet, conidial germination is occurred on the glumes surface within 6 hours to 12 hours (Pritsch et al., 2000)

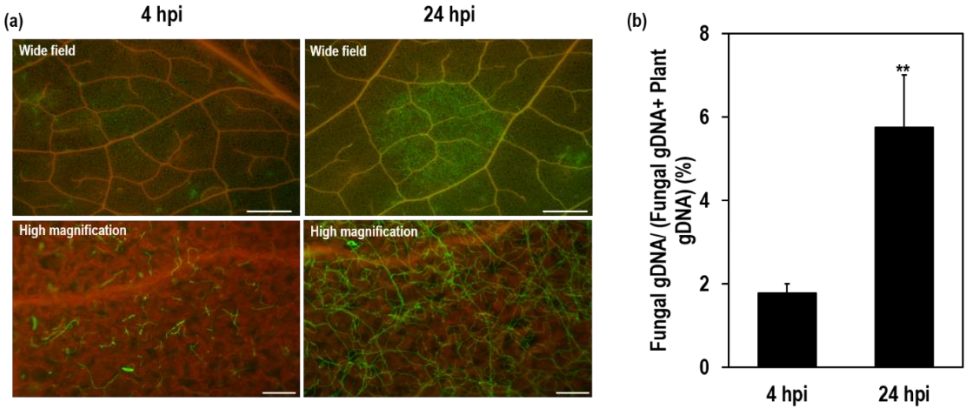

Figure 1. *Fusarium graminearum* colonization on the plant surface was initiated at 4 hpi and gradually increased at 24 hpi. (a) Visualization of Fusarium hyphae using WGA-Alexa488 (green) stain under fluorescent microscopy at 4 hours postinoculation (hpi) and 24 hpi. Red color is autofluorescence of host plant tissues. Bars = 1000 μm and 100 μm. (b) Fungal gDNA quantification at 4 hpi and 24 hpi in the leaf epidermis were conducted by qPCR, bars represent the standard deviation (n=3), while the asterisk indicates the result of Students' t-test ** *p-value* < 0.001.

2. Differentially expressed proteins were enriched in the leaf epidermis by fungal inoculation.

We analyzed epidermal proteins through shotgun proteomic then generated the volcano plots. As shown in the Figure 2, the dots in the red or green areas represented the significantly up-regulated or down-regulated proteins, respectively. The quantification of abundance ratio was relative to the mock treated control. Our results showed the differentially regulated proteins were enriched in the epidermal tissues. Even though, the number of all identified proteins in epidermis and whole leaf was not so different (about 5000 proteins). The number of up-regulated proteins in the epidermal tissues were 192 and 280 proteins at 4 hpi and 24 hpi, respectively. Those numbers were decreased in the proteins samples from whole leaf which

were 77 proteins at 4 hpi and 127 proteins at 24 hpi. While the number of down-regulated proteins in the epidermis were 131 proteins at 4 hpi and 238 proteins at 24 hpi. Interestingly, the number of down-regulated proteins were not so different compared to whole leaf samples at 4 hpi. In the whole leaf samples, 135 proteins and 39 proteins were down-regulated at 4 hpi and 24 hpi, respectively. Thus,our approach of proteomic study clearly enriched the differentially expressed proteins in the leaf epidermis caused by *F. graminearum* infection. Therefore, it can be useful to study plant-pathogen interaction in the leaf epidermis.

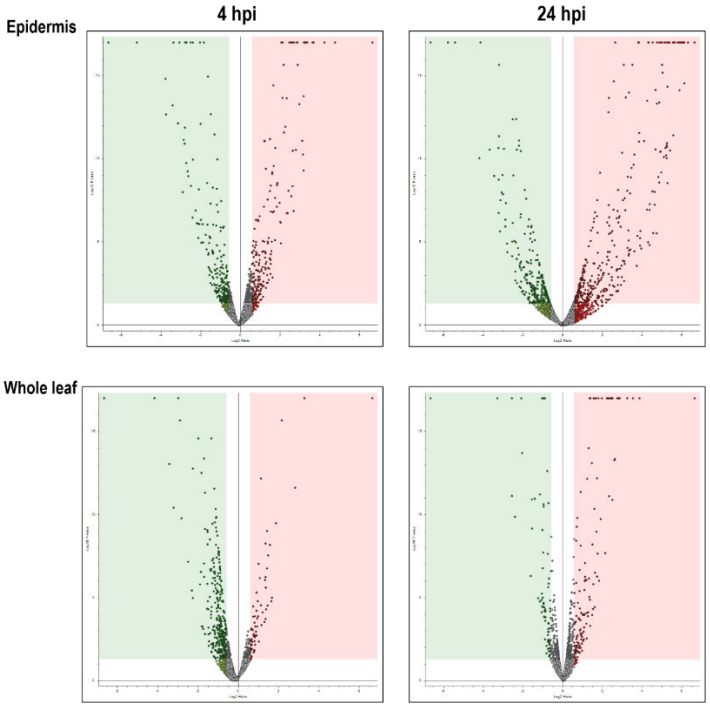

Figure 2. Volcano plots illustrate the spread of quantified proteins in the leaf epidermis inoculated by *F. graminearum*. The plots are showing the distributed proteins at 4 hpi and 24 hpi of leaf epidermis. The vertical axis is –log10 of the p-value (cutoff value 0.05) whereas the horizontal axis is log fold change (cutoff value 1.5 and 0.67). The dots indicate all the identified proteins in this experiment. Red and green square areas represent the significantly up-regulated and down-regulated proteins, respectively.

3. **Many plant proteins specifically up-regulated in the leaf epidermis.**

We 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 and created the venn diagrams (Figure 3). Based on the venn diagram, we can discriminate the proteins that specifically up-regulated proteins 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 more proteins, 264 (94.3% of significantly up-regulated epidermal proteins), were up-regulated at 24 hpi. Majority of highly up-regulated proteins were separated from epidermis and whole leaf.

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 also indicates the importance of leaf epidermis related to fungal infection. The further results and discussion will be about the specifically upregulated proteins in the leaf epidermis.

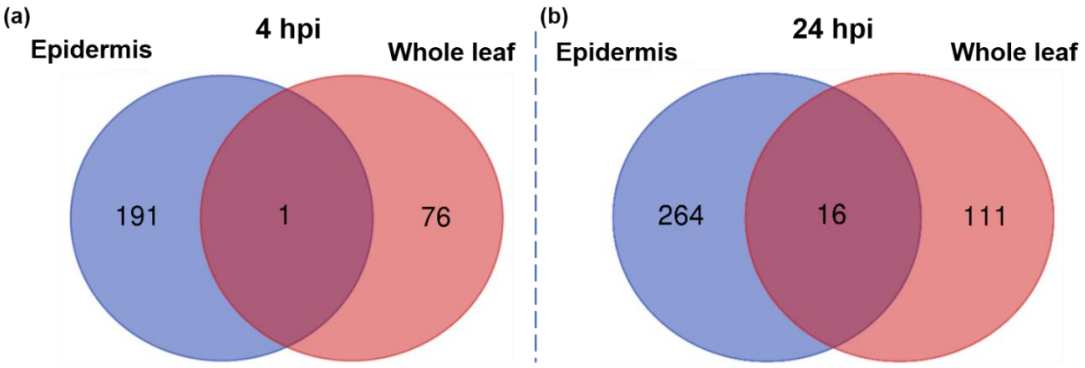

Figure 3. Many plant proteins specifically up-regulated in the leaf epidermis caused by the *F. graminearum* inoculation. Abundance ratio between inoculated and mock treated samples was compared and the cutoff values were determined as FC>1.5 anf p-value<0.05. The up-regulated proteins were compared between epidermal and whole leaf proteins (a) at 4 hpi and (b) 24 hpi.

4. **Plant proteins in the leaf epidermis were related to biotic stress.**

To reveal the effect of fungal inoculation in the leaf epidermis we subjected identified proteins into MapMan bin codes of isoform model from TAIR10 database. The mapped proteins are presented onto a diagram of metabolic pathway or other events (Usadel et al., 2005). The log base 10 values of abundance ratio of each protein were used for this analysis. Figure 4 clearly showed the different mapped proteins between epidermis and whole leaf. Epidermal proteins present more different changes than proteins of whole leaf. Among the epidermal proteins with significant different regulation at 4 hpi, many proteins were related to hormone signalling (9 proteins), redox state (5 proteins), pathogen-related (PR) (9 proteins), signalling pathway (26 proteins), and proteolysis (13 proteins). One protein related to MAPK signalling was up-regulated at 4 hpi in the leaf epidermis.

Eight out of 26 plant proteins related to defence signalling were up-regulated by *F. graminearum* infection specifically at 4 hpi. They were not expressed at 24 hpi. Six of Eight proteins are kinase family proteins included Mitogen-activated protein kinase kinase 5 (MKK5) protein (AT3g21220.1). MKK5 has been reported to be involved in stomatal response and root growth through the MAPKKK20-MKK5-MPK6 cascade (Li et al., 2017). Also, MKK5 and MKK4 are the key regulator of stomatal development and patterning (Wang et al., 2007). In addition, MKK5 involved in plant innate immune response against bacterial flagellin (flg22) through the MKK1-MKK4/MKK5-MPK3/MPK6 cascade. It involves in MAPKs cascades as

downstream of flg22 receptor kinase (FLS2) and upstream of WRKY29 transcription factor which leads to activation of plants disease resistance (Asai et al., 2002). Recently, we revealed that MAPKKK δ-1 (MKD1) which is required for the full immunity against bacterial and fungal pathogen interacts with MKK5 in the cytoplasm of plant cells and phosphorylates MKK5 as of invitro analysis (Asano et al., 2020).

On the other hand, up-regulation of proteins related to pathogen infection became more severe at 24 hpi. Some striking differences are the upregulation of proteins related to hormone signalling, transcription factors, pathogen, and cell wall. Figure 4 surely showed the up-regulation of transcription factor proteins. It is reported that during microbial infection, defence genes are expressed and some transcription factors are involved (Asai et al., 2002). BAK1 protein was up-regulated. BAK1 associates to PTI and interacts to FLS receptor during pathogen perceptions by plants. Other up-regulated proteins were plant defensin 1.3 (PDF1.3, AT2G26010.1), disease resistance protein TIR-NBS-LRR class (AT4G09430.1), disease resistance protein CC-NBS-LRR class (AT3G46730.1), and Defender Against Apoptotic Death 1 (DAD1) protein (AT1G32210.1). We have described the plant defensin 1.3 proteins in the previous discussion. Here, we showed the upregulation of disease resistance proteins from two nucleotide binding side with leucin rich repeat domain (NBS-LRR) classes based on N-terminal feature and role. First class is NBS-LRR with Toll/interleukin-1 receptor (TIR) domain in the N-terminal (TIR-NBS-LRR) and the second class is NBS-LRR with coiled-coil (CC-NBS-LRR) (Meyers et al., 2003). CC domain is also known as resistance to powdery mildew domain (RPW8) in Arabidopsis plant (Xiao et al., 2001). The majority genes which are involved in Effector-triggered immunity in plant contain NBS-LRR domain. This domain has crucial role for the recognition against broad range pathogen molecules then subsequently triggered the plant ETI.

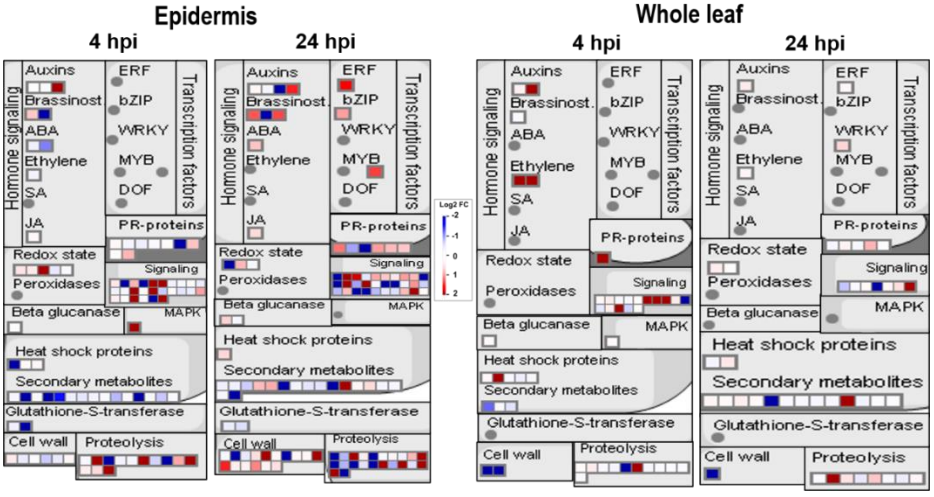

Figure 4. Mapping of identified proteins of leaf epidermis and whole leaf based on Mapman bin codes of Isoform Model TAIR10 database (Aug2012) in the category of pathogen/pest attack. Identified proteins in the leaf epidermis and whole leaf were listed up with the fold-change value and converted the values into log2 fold-change. The list of proteins and the values were mapped to the Mapman database.

5. Fungal proteins specifically expressed on the leaf surface.

We identified many Arabidopsis proteins in the inoculated epidermal tissues. In contrast, the number of identified fungal proteins was relatively low in plant epidermis (Figure 5). Proteins prepared from epidermal tissues contained many Arabidopsis proteins including high molecular weight proteins. After trypsin digestions, large amounts of peptides derived from Arabidopsis proteins likely affected the detection of fungal proteins. Even in this situation, we tried to identify the fungal proteins specifically expressed on the leaf surface compared with conidial proteins (0h) before inoculations. Figure 5 showed 61 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. The 61 proteins consisted of 55 proteins were commonly expressed at 4 hpi and 24 hpi and six proteins were specifically expressed at 24 hpi.

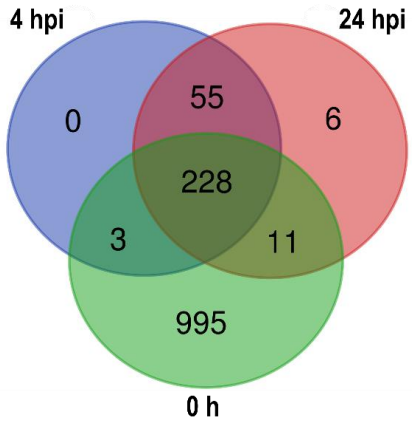

Figure 5. The number of identified fungal proteins expressed on the leaf surface at 4 hpi and 24 hpi compared to 0 h proteins from fungal conidia.

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. Protein 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. Overall, epidermal proteomics can be a beneficial approach to reveal the candidate of important proteins involved in plant-fungal pathogen interactions. Thus, these candidates of important proteins can be further analysis and may be a useful contribution in the effort to tackle the FHB disease in crops. We also examined some natural compounds that can be a beneficial chemical treatment to decrease the FHB disease incidence. We previously

reported that nicotinamide mononucleotide (NMN) enhanced disease resistance in Arabidopsis and barley. In this study, we examined nicotinamide (NIM) to know its effect to control FHB disease.

6. Application of NIM elevated FHB resistance in wheat

We investigated if Nicotinamide mononucleotide (NMN) and Nicotinamide (NIM) are beneficial for enhancing resistance to FHB in wheat. The disease incidence rate gradually increased over time, reaching approximately 26%, 12%, and 8% in the control, NMN-, and NIM-pretreated spikes, respectively, at 7 dpi. Additionally, the disease symptoms were significantly less in NMN- and NIM-pretreated spikes than in control spikes. These results demonstrate that NMN and NIM suppress FHB disease development and enhance disease resistance in wheat plants. In addition, the amount of fungal gDNA was lower in NMN-pretreated spikes (0.06%) and NIM-pretreated spikes (0.02%) than in control spikes (~0.13%) (Figure 6). This result is consistent with the suppression of disease symptoms and indicates that NMN- and NIM-pretreatment block the growth of fungal cells in wheat spikes. Pre-treatment of wheat spikes with NMN and NIM also significantly decreased the contamination with deoxynivalenol (DON) mycotoxin.

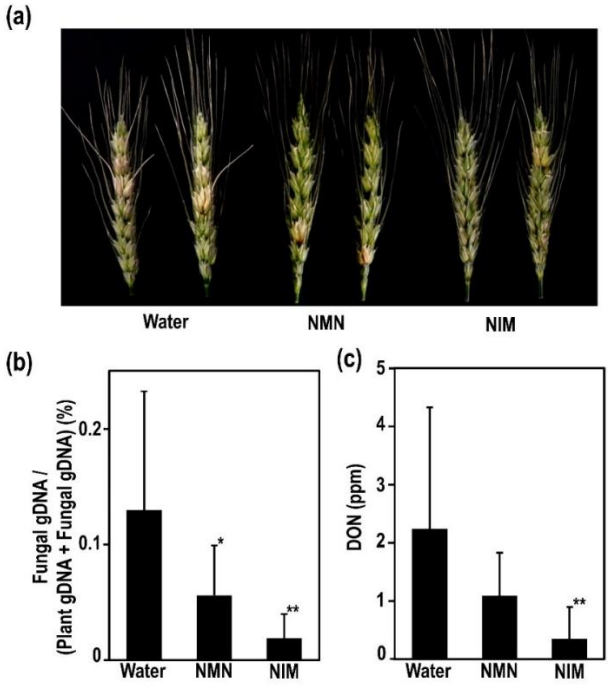

Figure 6. NMN- and NIM-pretreatment enhances the resistance against *F. graminearum* in wheat spikes. The spikes were treated with NMN, NIM, or water (control) by spraying before inoculation with *F. graminearum* (1×10^4 conidia/mL). (a) Representative photographs of spikes showing disease symptoms at 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).

7. NMN and NIM affect the level of pyridine metabolites in the inoculated spikes

To determine the effect of NMN and NIM on the metabolomics of wheat plants, we measured the contents of NMN, NIM, and related metabolites in spikes pre-treated using LC-MS/MS. The total metabolites were extracted from NMN-pretreated, NIM-pretreated, and control spikes at 7 dpi. The extracted metabolites were concentrated by UPLC, and identified by Orbitrap QE plus mass spectrometer. NMN and NIM were highly accumulated in NMN- and NIM-pretreated spikes, respectively, at 7 dpi (Figure 7). This suggests that both the sprayed metabolites were incorporated into and subsequently retained by the spikes. Interestingly, the level of NMN was significantly higher in NIM-pretreated spikes than in control spikes. By contrast, the level of NIM was very low in NMN-pretreated spikes. Both NMN- and NIM-pretreatment significantly influenced the accumulation of TRG. NIM increased TRG accumulation to a greater extent than NMN. On the other hand, the accumulation of nicotinic acid (NA) and NAD were only slightly affected by NMN and NIM application compared with the water pre-treatment. Thus, pre-treatment of spikes with NMN and NIM significantly increased the contents of NMN and NIM, respectively. Both pre-treatments also increased the content of TRG, which acts as an antimicrobial compound in plants.

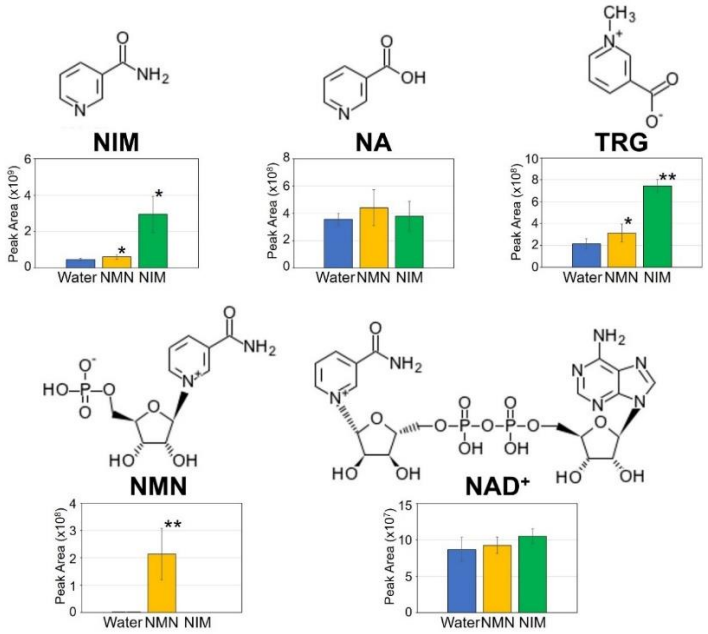

Figure 7. NMN and NIM-pretreatment affect pyridine metabolism in the inoculated spikes. The relative contents of NMN, NIM, NAD, NA, and TRG were quantified in NMN- pre-treated, NIM-pretreated and control spikes, based on the peak area. Error bars represent SD (n = 4–5). Asterisks indicate significant differences compared with the control (*p < 0.05, **p < 0.01; Student's t-test).

8. Differential accumulation of metabolites in NMN- and NIM-pretreated spikes

All differentially regulated metabolites were divided into three groups: highly accumulated by the NIM-pretreatment (Figure 8a), highly accumulated by the NMN-pretreatment (Figure

8b), and significantly reduced by both NMN- and NIM-pretreatments (Figure 8c). Among these metabolites, bacancosin, a plant saponin and a natural detergent that degrades the microbial membranes (Osbourn, 1996), was accumulated in the NIM-pretreated spikes. Alkaloids such as debromohymedialdisine and buchananine also accumulated in the NIM-pretreated spikes. In addition, buchananine was reported as an antifungal metabolite (Lemaitre-Guillier et al., 2020). Buchananine was highly accumulated in NIM-pretreated spikes but without statistical significance compared with the control. Additionally, sulfamethazine exhibits antimicrobial activities (Peng et al., 2015), while cyclo-Dopa 5-O-glucoside acts as a reactive oxygen species (ROS) scrounger (Nakagawa et al., 2018). DIMBOA-glucoside, which is likely related to defense signaling (Ahmad et al., 2011), was also accumulated in NIM-pretreated spikes. Additionally, cystothiazole A and picolinic acid were accumulated in NMN-pretreated spikes (Figure 5b). Cystothiazole A exhibits antifungal activity against Phytophthora capsici (Ojika et al., 1998). Although the accumulation of picolinic acid in NMN-pretreated spikes was not strikingly different from that in the control, it was statistically significant. Spray treatment of picolinic acid was reported to activate defense against *Magnaporthe oryzae* in wheat plants (Aucique-Pérez et al., 2019). Two fungal metabolites, deoxynivalenol and ergosterol peroxide, were reduced by NMN-pretreatment and significantly reduced by NIM-pretreatment (Figure 8c). These results further support the hypothesis that NIM significantly reduces the FHB disease in wheat plants.

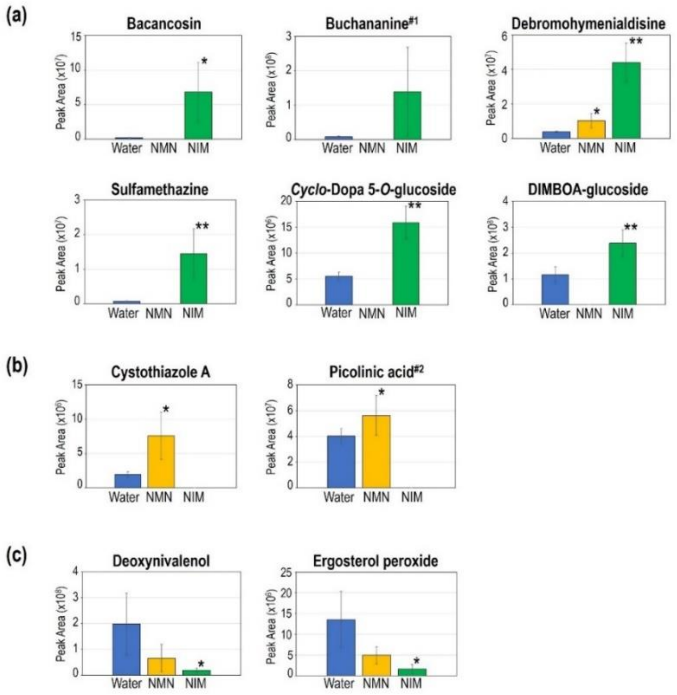

Figure 8. Quantification of differentially regulated metabolites in NMN- or NIM-pretreated and *F. graminearum*-inoculated spikes at 7 dpi. (ac) Metabolites significantly accumulated in NIM-pretreated spikes (a), significantly accumulated in NMN-pretreated spikes (b), and significantly reduced in NIM-pretreated spikes (c). 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).

References:

Ahmad, S., Veyrat, N., Gordon-Weeks, R., Zhang, Y., Martin, J., Smart, L., Glauser, G., Erb, M., Flors, V., Frey, M., & Ton, J. (2011). Benzoxazinoid metabolites regulate innate immunity against aphids and fungi in maize. *Plant Physiology*, *157*(1), 317–327.

Asai, T., Tena, G., Plotnikova, J., Willmann, M. R., Chiu, W. L., Gomez-Gomez, L., Boller, T., Ausubel, F. M., & Sheen, J. (2002). Map kinase signalling cascade in Arabidopsis innate immunity. *Nature*, *415*(6875), 977–983.

Asano, T., Nguyen, T. H. N., Yasuda, M., Sidiq, Y., Nishimura, K., Nakashita, H., & Nishiuchi, T. (2020). Arabidopsis MAPKKK δ-1 is required for full immunity against bacterial and fungal infection. *Journal of Experimental Botany*, *71*(6), 2085–2097.

Aucique-Pérez, C. E., Resende, R. S., Neto, L. B. C., Dornelas, F., DaMatta, F. M., & Rodrigues, F. Á. (2019). Picolinic acid spray stimulates the antioxidative metabolism and minimizes impairments on photosynthesis on wheat leaves infected by *Pyricularia oryzae*. *Physiologia Plantarum*, *167*(4), 628–644.

Lemaitre-Guillier, C., Fontaine, F., Roullier-Gall, C., Harir, M., Magnin-Robert, M., Christophe Clément, Trouvelot, S., Gougeon, R. D., Schmitt-Kopplin, P., & Adrian, M. (2020). Cultivar- And wood area-dependent metabolomic fingerprints of grapevine infected by *botryosphaeria dieback*. *Phytopathology*, *110*(11), 1821–1837.

Li, K., Yang, F., Zhang, G., Song, S., Li, Y., Ren, D., Miao, Y., & Song, C. P. (2017). AIK1, a mitogen-activated protein Kinase, modulates abscisic acid responses through the MKK5-MPK6 kinase cascade. *Plant Physiology*, *173*(2), 1391–1408.

Meyers, B. C., Kozik, A., Griego, A., Kuang, H., & Michelmore, R. W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell*, *15*(4), 809–834.

Nakagawa, S., Tachrim, Z. P., Kurokawa, N., Ohashi, F., Sakihama, Y., Suzuki, T., Hashidoko, Y., & Hashimoto, M. (2018). pH stability and antioxidant power of CycloDOPA and its derivatives. *Molecules*, *23*(8), 1–9.

Ojika, M., Suzuki, Y., Tsukamoto, A., Sakagami, Y., Fudou, R., Yoshimura, T., & Yamanaka, S. (1998). Cystothiazoles A and B, new bithiazole-type antibiotics from the myxobacterium *Cystobacter fuscus*. *Journal of Antibiotics*, *51*(3), 275–281.

Osbourn, A. (1996). Saponins and plant defence-a soap story. *Trend in Plant Science*, *1*(1), 4–9.

Peng, F. J., Ying, G. G., Liu, Y. S., Su, H. C., & He, L. Y. (2015). Joint antibacterial activity of soil-adsorbed antibiotics trimethoprim and sulfamethazine. *Science of the Total Environment*, *506–507*, 58–65.

Pritsch, C., Muehlbauer, G. J., Bushnell, W. R., Somers, D. A., & Vance, C. P. (2000). Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Molecular Plant-Microbe Interactions*, *13*(2), 159–169.

Usadel, B., Nagel, A., Thimm, O., Redestig, H., Blaesing, O. E., Palacios-Rojas, N., Selbig, J., Hannemann, J., Piques, M. C., Steinhauser, D., Scheible, W. R., Gibon, Y., Morcuende, R., Weicht, D., Meyer, S., & Stitt, M. (2005). Extension of the visualization tool MapMan to allow statistical analysis of arrays, display of coresponding genes, and comparison with known responses. *Plant Physiology*, *138*(3), 1195–1204.

Wang, H., Ngwenyama, N., Liu, Y., Walker, J. C., & Zhang, S. (2007). Stomatal development and patterning are regulated by environmentally responsive mitogen-activated protein kinases in Arabidopsis. *Plant Cell*, *19*(1), 63–73.

Xiao, S., Ellwood, S., Calis, O., Patrick, E., Li, T., Coleman, M., & Turner, J. G. (2001). Broad-spectrum mildew resistance in *Arabidopsis thaliana* mediated by RPW8. *Science*, *291*(5501), 118–120.

Zheng, H., Miao, P., Lin, X., Li, L., Wu, C., Chen, X., Abubakar, Y. S., Norvienyeku, J., Li, G., Zhou, J., Wang, Z., & Zheng, W. (2018). Small GTPase Rab7-mediated FgAtg9 trafficking is essential for autophagy-dependent development and pathogenicity in *Fusarium graminearum*. *PLoS Genetics*, *14*(7), 1–22.

令和 3 年 8 月 5 日

学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

Analysis of Molecular Mechanism of Plant and Fungal Pathogen Interaction

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

2．論文提出者 (1) 所　　属 自然システム学　　専攻

(2) 氏（ふり）　名（がな） Yasir Sidiq（やしろ　しでぃく）

3．審査結果の要旨（600～650 字）

Yasir Sidiq 氏は、本学入学後に、植物病原糸状菌であるムギ類赤かび病菌と植物との相互作用の研究に着手した。植物物表層において病原菌との相互作用に関与しているタンパク質を明らかにするため、病原糸状菌を接種した植物の表皮組織を用いたプロテオーム解析の実験系の構築し、葉全体を用いた解析では捉えられなったタンパク質の発現変動を明らかにし、これらに植物免疫や病原菌の病原性に関わるタンパク質が多く含まれることを見出した。一連の実験の過程で、自分自身で工夫しながら実験系を改善し、植物表皮を用いたプロテオミクスの手法を確立したことは関連分野への波及効果も大きく、評価すべき点である。また、コムギにおける赤かび病の被害を抑制するため、植物由来の防除剤の開発にも取り組んだ。ニコチンアミドモノヌクレオチド(NMN)をコムギやオオムギの開花期の穂に散布すると、赤かび病に対する抵抗性が強化されることを明らかにしていたが、ニコチンアミドモノヌクレオチドが不安定で高価なことが問題となっていた。Yasir Sidiq 氏は、類似した構造を持つ化合物の中から、ニコチンアミド(NIM)が穂における赤かび病菌の増殖及びかび毒汚染を顕著に抑制することを見出し、作用機構も明らかにした。世界的にも被害の大きいムギ類赤かび病に対して、ビタミンの１種でもある安全性の高いニコチンアミドに顕著な防除効果を示したことは評価すべき点である。以上の研究成果は当該学問分野における新知見を含むものであり、審査委員会として合格に値すると判断した。

4．審査結果 (1) 判　　定（いずれかに○印） （合　格） ・ 不合格

(2) 授与学位 博　士（学術　）