MoSET1-dependent transcription factors regulate different stages of infection-related morphogenesis in Pyricularia oryzae

Minh, Dang Ngoc
Tsukahara, Yusaku
Thach, Dang An
Ikeda, Ken-ichi
Nakayashiki, Hitoshi

(Citation)
Journal of General Plant Pathology,89(2):77-83

(Issue Date)
2023-03

(Resource Type)
journal article

(Version)
Accepted Manuscript

MoSET1-dependent transcription factors regulate different stages of infection-related morphogenesis in *Pyricularia oryzae*

Authors, Dang Ngoc Minh*, Yusaku Tsukahara*, Dang An Thach, Ken-ich Ikeda, Hitoshi Nakayashiki**

Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada, 657-8501 Kobe, Japan

*These two authors contributed equally to this work.

**Address for correspondence: Hitoshi Nakayashiki. Laboratory of cell function and structure, Graduate School of Agricultural Science, Kobe University, 1-1 Rokkodai-cho, Nada, 657-8501 Kobe, Japan. Phone & Fax : +81-78-803-5867 ; email : hnakaya@kobe-u.ac.jp

Total text pages:23

The numbers of tables and figures: 0 table and 5 figures

Abstract

MoSET1, an H3K4 histone methyltransferase in *Pyricularia oryzae* plays a key role in infection-related morphogenesis of the fungus. Our previous study identified approximately 400 genes whose expression were possibly regulated directly by MoSET1 during appressorium formation. In this study, we focused on five such MoSET1-dependent transcription factors (TF) whose mRNA expression was induced during infection. A gene deletion approach was used for three of the five TF genes (MGG_04699, MGG_06898, and MGG_07450) while a gene silencing technique was applied to the remaining two genes (MGG_00472 and MGG_07386) due to the difficulty in constructing a gene deletion mutant. The phenotypic characterization of the gene knock-out and -down mutants revealed that MGG_06898 played a crucial role in sporulation, MGG_04699 were involved in appressorium formation, and MGG_00472 and MGG_04699 were required for the full virulence of the fungus. These results demonstrated that MoSET1 contributes to the pathogenicity of the fungus by controlling transcription factors that further regulate different steps in the infection process of *P. oryzae*

Key words: Pyricularia oryzae, transcription factor, histone methylation

Introduction

The filamentous fungus *Pyricularia oryzae* (synonym: *Magnaporthe oryzae*) causes blast disease on various gramineous plants such as rice, wheat, oat and so on (Kato et al., 2000). The blast fungus shows drastic morphological changes during the infection cycle (Talbot, 2003). Following attachment on the host leaf surface, a spore germinates and forms an appressorium at the tip of the germ tube. At the base of the appressorium, a penetration pore and peg are formed to colonize a host cell with infection hyphae. These morphological changes are accompanied with remarkable changes in gene expression (Jeon et al., 2020) that may involve cell type-specific epigenetic control of chromatin structure.

Epigenetics describes heritable changes in gene expression caused by non-genetic mechanisms such as chemical modifications of DNA, RNA and histone protein as well as regulations by non-coding RNAs (Aristizabal et al., 2019). Histone methylation is a process that methyl groups are added to lysine or arginine in histone proteins. Both histone arginine methyltransferases (RMTs) and lysine methyltransferases (KMTs) catalyze the transfer of methyl groups from *S*-adenosyl methionine to core histone proteins. In *P. oryzae*, gene deletion analysis of eight possible KMTs revealed that MoSET1 responsible for H3K4 methylation plays a pivotal role in infection-related morphogenesis of the fungus (Pham et al., 2015a). Loss of MoSET1 led to a deficiency in cell growth, sporulation, appressorium formation, production of cell wall degradation enzymes, and pathogenicity (Pham et al., 2015a, b; Vu et al., 2013). RNA-seq analysis suggested that approximately 2,000 genes were up- or down-regulated during appressorium formation in a MoSET1-dependent manner. However, ChIP-seq analysis of MoSET1 protein revealed that only approximately 400 of the MoSET1-dependent genes were directly regulated by MoSET1 (Pham et al., 2015a), suggesting the involvement of a signaling cascade starting from MoSET1. In fact, the 400 genes contained possible signal mediators such as transcription factors (TFs) and kinases.

In this study, we focused on MoSET1-dependent TFs. In fungi, TFs are key players in the signal transduction pathways and regulatory mechanisms (Shelest, 2008). For instance, Gomi and his coworkers (2000) stated that the deletion of AmyR, a zinc binuclear cluster DNA-binding protein in the Gal4p TF family, led to a decrease in amylolytic enzyme activities and vegetative growth of *Aspergillus oryzae* on starch medium. In *Parastagonospora nodorum,* PnPf2, a TF belonging to the Zn2-Cys6 zinc finger subfamily positively regulated necrotrophic effector proteins and played an important role in the virulence on wheat (Jones et al., 2019). In

53 *P. oryzae*, systematic analyses of the Zn2-Cys6 TF family and the Cys2-His2 zinc finger TF
54 family were conducted (Cao et al., 2016; Lu et al., 2014) to reveal that 61 of 104 Zn2-Cys6
55 TFs and 44 of 47 Cys2-His2 TFs were involved fungal development and infection-related
56 morphogenesis such as growth, conidiation, appressorium formation and pathogenicity (Cao et
57 al., 2016; Lu et al., 2014). Among the 22 Cys2-His2 TFs required for the full virulence of the
58 fungus, 2 were MoSET1-dependent TFs.

59 Here we carried out the functional analysis of five MoSET1-dependent TFs to explore
60 their roles in the pathogenicity of *P. oryzae*. The results suggested that MoSET1 orchestrates
61 various TFs to achieve successful infection of host plants with *P. oryzae*.

62

Materials and methods

Fungal strains and host plants

The wheat-infecting *P. oryzae* strain Br48 was collected in Brazil (Urashima et al., 1999). Fungal strains were preserved on barley seed media at 4ºC for long-term storage, and cultured on potato dextrose agar (PDA) at 25ºC for working stock. "Norin 4" (N4), a cultivar of common wheat (*Triticum aestivum* L.) was used as a host plant of the Br48 strain.

Construction of gene knock-out and -down mutants

The split-maker disruption method (Catlett et al., 2003) was applied to construct deletion mutant strains (Fig. S1). First, up- and down-stream flanking regions of a target gene were amplified by PCR using KOD-Plus-Neo polymerase (TOYOBO, Osaka, Japan) and individually cloned by blunt-end ligation at PvuII and EcoRV sites of pSP72-hph containing a hygromycin B phosphotransferase gene (Hph) (Morita et al., 2013). Using the resulting plasmids, two DNA fragments containing a part of Hph and the flanking genomic region were amplified by PCR using KOD-Plus-Neo polymerase as shown in Fig. S1. The PCR products were purified using Wizard SV Gel and PCR Clean-up System (Promega, Madison, WI, USA) after agarose gel electrophoresis, and used for fungal transformation. A list of primers used to amplify the genomic fragments was given in Table S1.

A retrotransposon-based gene silencing vector, pSilent-MG (Vu et al., 2011) was used to generate gene knock-down mutants. A target fragment was amplified by PCR using KOD-Plus-Neo polymerase and a pair of specific primers (Table S1), and inserted at a BglII site in pSilent-MG.

To obtain fungal transformants with a gene disruption or silencing vector, a PEG-mediated transformation method was used as described previously (Nakayashiki et al., 1999). The resulting fungal transformants having a gene disruption at the desired genomic location or a silencing vector were screened by PCR with appropriate sets of primers for each target gene (Fig. S1, Table S1).

To construct a gene complementation strain, a genomic DNA fragment containing a target gene and its surrounding region was amplified by PCR with sets of specific primers (Table S1) and KOD One (TOYOBO). The PCR product was introduced into the corresponding gene deletion mutant through PEG-mediated co-transformation with the marker plasmid pII99

carrying a geneticin-resistant cassette. Presence or absence of a target gene in the transformants was checked by PCR using a pair of primers (Table S1) in the coding sequence (Fig. S1).

Phenotypic characterization of gene knock-out and -down mutants

Every phenotypic assay was performed with three biological replicates unless mentioned otherwise.

1. Vegetative growth

A mycelial plug was placed at the center of CM agar medium (0.3% casamino acids, 0.3% yeast extract, 0.5% sucrose, 0.5% agar) and cultured at 25ºC. The colony diameter was measured at 9 days after incubation.

2. Conidiation, conidial germination, and appressorium formation

Fungal strains were cultured on oatmeal agar media and incubated at 25ºC for 7 days, and then, aerial mycelia were removed using the tip of a 1.5 ml microtube. The fungal strains were further cultured under blacklight blue (BLB) light for 3 days. Conidial suspension was prepared by adding 10 ml of distilled water per plate to fungal culture as described previously (Hyon et al., 2012). For germination and appressorium formation assay, 10 µl conidial suspension (12×10^5 conidia/ml) were dropped on a cover glass and incubated at 25ºC in the dark under a high humidity condition. Conidial germination was observed after 5 h incubation, and the rate of appressorium formation was counted after 8, 12, 24 h incubation. At least 100 conidia were observed to calculate the rates of conidial germination and appressorium formation in each replicate.

3. Plant infection assay

Seeds of the wheat cultivar Norin 4 were sown in vermiculite supplied with Hyponex (Hyponex Japan, Osaka, Japan) in plastic seedling pots (5.5 cm × 15 cm × 10 cm), and grown in a plant growth chamber at 23ºC with a 12 h-photoperiod for 8 days. Tween 20 (0.01%) was added to conidia suspension, and sprayed onto the adaxial side of the 8-day-old primary wheat leaves. The inoculated seedlings were incubated in a dark and high humidity box for 24 h, and then moved to a plant growth chamber at 23ºC with a 12 h-photoperiod for 4-5 days. The disease symptoms were graded by a combination of lesions size (0 to 5) and color (brown [B] or green [G]) as described previously (Hyon et al., 2012). 0, no visible lesion; 1, pinpoints spots; 2, small size (< 1.5 mm); 3, intermediate size (< 3 mm); 4, large and typical blast lesion; 5, whole blighting of leaf blades.

125 **Cytological assay**

126 Cytological observation was performed as described by Hyon et al. (2012) with a slight
127 modification. The inoculated leaves were collected for observation of appressorium formation
128 at 12 hpi and of infection hyphae in host cells at 24 hpi. The samples were bleached by deeply
129 boiled in alcoholic lactophenol (lactic acid/phenol/glycerol/distilled water/ethanol, 1:1:1:1:8 in
130 volume) at 98°C for 5 min. Then, microscopic observation was performed using an
131 epifluorescent microscope under bright and fluorescent fields.

132 **RNA isolation and qRT-PCR analysis**

133 For RNA extraction, approximately, 50-120 mg of vegetative mycelia, spores or
134 infected leaves were ground to a fine powder in liquid nitrogen with a mortar and pestle. Total
135 RNA was isolated from frozen samples using Sepasol RNA I Super G (Nacalai Tesque, Kyoto,
136 Japan). Total RNA was further cleaned up using the NucleoSpin RNA Clean-up Kit (Macherey-
137 Nagel, Düren, Germany) following manufacture's instruction.

138 For RT-qPCR analysis, 1 μg of total RNA was subjected to cDNA synthesis using the
139 ReverTra Ace qPCR RT Master Mix with gDNA Remover kit (TOYOBO). RT-qPCR assay
140 was carried out with GeneAce SYBR qPCR Mix α (Nippon Gene, Tokyo, Japan) using pairs
141 of primers listed in Table S1. The actin gene (MGG_03982) was used as an internal control.
142 The level of target mRNA, relative to the mean of the internal control was calculated by the
143 comparative Ct method.

144

Results

Transcriptional analysis of MoSET1-dependent TFs during plant infection by *P. oryzae*

Among approximately 400 genes that were potentially regulated directly by MoSET1 (Pham et al., 2015a), 18 genes exhibited a typical characteristic of TFs. In this study, we chose 5 of the 18 putative TFs, MGG_00472, MGG_04699, MGG_06898, MGG_07386 and MGG_07450 for functional analysis. First, to gain insight into the functions of these TFs, their gene expression profile at hyphal, conidial and infectious stages was examined by qRT-PCR. At the conidial stage, the mRNA abundance of MGG_04699, MGG_07386 and MGG_07450 was decreased while that of MGG_06898 was significantly increased compared to the hyphal stage (Fig. 1). Interestingly, the expression of the MoSET1-dependent TFs was generally increased in infection stages relative to the hyphal stage, especially at an early stage (5 hpi). The abundance of MGG_04699 and MGG_06898 mRNA was increased by approximately 8-fold and 16-fold, respectively, at 5 hpi (Fig. 1). In contrast, the mRNA abundance of MGG_07386 was significantly decreased at 5 hpi but increased later at 12 hpi (Fig. 1). These results were consistent with the idea that these TFs play roles in the infection process of *P. oryzae*.

Gene silencing of MGG_00472 and MGG_07386

Despite several trials, we were not able to obtain a gene deletion mutant of MGG_00472 or MGG_07386. This might be due to a lethality of the gene deletion mutants. Indeed, the knock-out mutant of NCU00116, the ortholog of MGG_00472 in *Neurospora crassa*, is maintained only in the form of a heterokaryon (Chen et al., 1998; https://www.fgsc.net/fgsc/strain_detail.php?OrgID=22094). Thus, we decided to use pSilent-MG to analyze the function of MGG_00472 and MGG_07386. pSilent-MG is a gene silencing vector that triggers retrotransposon-induced gene silencing. In this system, a target gene fragment inserted at a cloning site in the LTR-retrotransposon MAGGY induces both transcriptional and post-transcriptional gene silencing together with the element in *P. oryzae* (Vu et al., 2011).

After initial screening, two candidate transformants each for MGG_00472 or MGG_07386 were subjected to qRT-PCR analysis to assess levels of gene silencing. The candidates showed silencing of the target gene at varying degrees in vegetative mycelia (Fig.

2). Consequently, the transformants, KD_mgg00472-12 and KD_mgg07386-13, were selected for further phenotypic analyses.

Phenotypic characterization of gene knock-out and -down mutants of MoSET1-dependent TFs

Three gene knock-out mutants, Δmgg_04699, Δmgg_06898 and Δmgg_07450, and two knock-down mutants, KD_mgg00472-12 and KD_mgg07386-13 were subjected to phenotypic analyses with respect to growth, sporulation, germination, appressorium formation, and pathogenicity on the host plant.

First, the growth rates of the mutants on rich media (CM agar media) were assessed. Δmgg_04699, Δmgg_06898, and KD_mgg00472-12 showed a slower growth while, interestingly, the growth rate of KD_mgg07386-13 was a little faster than the WT strain (Fig. 3a).

With an exception of Δmgg_07450, all the mutant examined displayed a decrease in conidiation compared to WT at varying degrees (Fig. 3b). Especially, Δmgg_06898 produced almost no conidia, suggesting that MGG_06898 plays a crucial role in conidiogenesis in *P. oryzae*. Meanwhile, due to this, further phenotypic assays that use conidia were not applicable to Δmgg_06898.

The rates of germination and appressorium formation were assessed using conidial suspension dropped on a cover glass. The conidia were incubated in the dark at 25ºC up to 24 h. The germination rates of the mutants did not differ much from that of WT except that Δmgg_04699 exhibited a rate less than 60% relative to WT (Fig 3c). In addition, only Δmgg_04699 showed a deficiency in appressorium formation. At 24 hpi, approximately 50% of conidia formed an appressorium in Δmgg_04699 while more than 90% of conidia did it in the WT and other mutant strains (Fig. 3d). The deficiency of Δmgg_04699 in germination and appressorium formation was restored to the WT levels in the gene complementation strain, indicating that MGG_04699 is responsible for the phenotypic alterations in the mutant.

In infection assay, KD_mgg00472-12 and Δmgg_04699 showed significantly reduced virulence on the host plant (Fig. 4a). The infection types of KD_mgg00472-12 and Δmgg_04699 were 3-4BG and 23BG, respectively. Cytological observation of infected leaves indicated that both KD_mgg00472-12 and Δmgg_04699 exhibited a delay in invasion to plant cells compared to WT. At 24 hpi, the rates of appressoria that successfully formed infection hyphae in the host cell were 45% and 31% with KD_mgg00472-12 and Δmgg_04699,

respectively, while that with WT were approximately 80% (Fig. 4b). These results suggested that MGG_00472 and MGG_04699 contributed to the full virulence of *P. oryzae* by regulating their downstream genes.

The infection type of KD_mgg07386-13 was 5B. Lesions appeared in almost the whole leave (Fig. 4a). However, in contrast to leaves infected with the WT strain, the color of lesions was mostly brown, indicating that this mutant induced more resistant reaction than did the WT strain. Thus, MGG_07386 may be required for suppressing a part of resistant reactions by the host plant.

Discussion

Among the MoSET1-dependent TFs examined in this study, MGG_00472 and MGG_04699 considerably contributed to the virulence of *P. oryzae*. MGG_04699 named as MoFLBC (Cao et al., 2016) is a homolog of FlbC, C2H2 transcription factor in *Aspergillus nidulans* (Kwon et al., 2010). FlbC is necessary for conidiation, conidial germination and, proper development in *A. nidulans*. Possible orthologs of FlbC were present in a wide range of fungal species. In *Fusarium verticillioides*, the FlbC ortholog negatively regulated the production of conidia (Malapi-Wight et al., 2014). More recently, Boni et al. (2018) reported that FLB-3, an ortholog of FlbC in *Neurospora crassa*, was essential for fungal development. Thus, FlbC homologs play roles in regulating various stages of fungal development in a wide range of ascomycetes.

In *P. oryzae*, Cao et al. (2016) showed that a deletion mutant of MGG_04699 had a severe defect in sporulation and its virulence to the host plant as shown in this study. However, contrary to our study, the rates of germination and appressorium formation were 96.9% and 94.9%, respectively, relative to WT in their study. This apparent discrepancy may be due to a difference in the strains and/or experimental conditions.

To date, MGG_00472 as well as MGG_07386 and MGG_07450, have not been well-characterized in *P. oryzae*. MGG_00472 is an ortholog of the *Aab-1* gene of *N. crassa* (NCU00116) encoding a CCAAT-binding TF subunit. *Aab-1* was reported to regulate a glutamate dehydrogenase gene and have pleiotropic effects on growth and development (Chen et al., 1998). Consistently, KD_mgg00472-12 showed defects in growth, sporulation, and virulence to the host plant. Thus, MGG_00472 may also have pleiotropic effects on growth and development in *P. oryzae*. Possible orthologs of MGG_07386 and MGG_07450 were conserved in ascomycete fungi but their biological functions were so far not well-understood.

MGG_06898, namely MoMyb1, encodes a TF belonging to the Myb protein family. Consistent with our results, Dong et al. (2015) previously revealed that a deletion mutant of MoMyb1 showed defects in vegetative growth, conidiation and conidiophore development. In the MoMyb1 deletion mutant, several conidiogenesis-related genes such as MoMSN2, MoFlbC (MGG_04699), MoGLUS, MoSTUA, and MoCON8 were significantly down-regulated (Dong et al., 2015). Thus, MoSET1-dependent MoMyb1 plays a key role in conidiogenesis.

Based on the literatures and this work, at least fifteen MoSET1-dependent TF genes in *P. oryzae* have been characterized to date, which includes MGG_00472, MGG_07386, MGG_07450, MGG_01414 (Xlr1), MGG_01518 (MoNIT4), MGG_01486 (FZC2),

MGG_02880 (FZC9), MGG_04699 (MoFLBC), MGG_06898 (MoMyb1), MGG_08199 (FAR2), MGG_09200 (TDG1), MGG_09950 (FZC54), MGG_00617 (MoVOSA), MGG_01734, and MGG_13778 (MoGIS2) (Cao et al., 2016; Dong et al., 2015; Kim et al., 2014; Lu et al., 2014). Among those, three (MGG_00472, MGG_06898, MGG_09950), ten (MGG_00472, MGG_01518, MGG_01486, MGG_02880, MGG_04699, MGG_06898, MGG_08199, MGG_09200, MGG_01734, MGG_07386), two (MGG_01486, MGG_04699), and four (MGG_00472, MGG_04699, MGG_09200, MGG_13778) TFs were shown to play a role in vegetative growth, sporulation, appressorium formation and virulence to the host plant, respectively (Fig. 5). Thus, MoSET1 may function as a key regulator of the pathogenicity of *P. oryzae* by controlling TFs that further regulate various steps in the infection process such as sporulation, appressorium formation, and invasion to the host cell.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science (#25292028 and 16H04883).

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

References

Aristizabal MJ, Anreiter I, Halldorsdottird T, Odgersc CL, McDadec TW, Goldenberg A, Mostafavi S, Kobor MS, Binder EB, Sokolowski MB, O'Donnell KJ (2019) Biological embedding of experience: A primer on epigenetics. Proc Natl Acad Sci USA 117:23261-23269

Boni AC, Ambrosio DL, Cupertino FB, Montenegro-Montero A, Freitas FZ, Corrocher FA, Goncalves RD, Yang A, Weirauch MT, Hughes TR, Larrondo LF, Bertolini MC (2018) *Neurospora crassa* developmental control mediated by the FLB-3 transcription factor. Fungal Biol 122:570-582

Cao H, Huang P, Zhang L, Shi Y, Sun D, Yan Y, Liu X, Dong B, Chen G, Snyder JH, Lin F, Lu J (2016) Characterization of 47 Cys2-His2 zinc finger proteins required for the development and pathogenicity of the rice blast fungus *Magnaporthe oryzae*. New Phytol 211:1035-1051

Catlett NL, Lee B, Yoder OC, Turgeon BG (2003) Split-marker recombination for efficient targeted deletion of fungal genes. Fungal Genetics Reports 50:9-11

Chen H, Crabb JW, Kinsey JA (1998) The *Neurospora* aab-1 gene encodes a CCAAT binding protein homologous to yeast HAP5. Genetics148:123-130

Dong Y, Zhao Q, Liu X, Zhang X, Qi Z, Zhang H, Zheng X, Zhang Z (2015) MoMyb1 is required for asexual development and tissue-specific infection in the rice blast fungus *Magnaporthe oryzae*. BMC Microbiol 15: 37

Gomi K, Akeno T, Minetoki T, Ozeki K, Kunagai C, Okazaki N, Iimura Y (2000) Molecular cloning and characterization of a transcriptional cctivator gene, *amyR*, involved in the amylolytic gene expression in *Aspergillus oryzae*. Biosci Biotechnol Biochem 64:816-827

Hyon GS, Nga NTT, Chuma I, Inoue Y, Asano H, Murata N, Kusaba M, Tosa Y (2012) Characterization of interactions between barley and various host-specific subgroups of *Magnaporthe oryzae* and *M. grisea*. J Gen Plant Pathol 78:237-246

Jeon J, Lee GW, Kim KT, Park SY, Kim S, Kwon S, Huh A, Chung H, Lee DY, Kim CY, Lee YH (2020) Transcriptome profiling of the Rice blast fungus *Magnaporthe oryzae* and its host *Oryza sativa* during infection. Mol Plant Microbe Interact 33:141-144

Jones DAB, John E, Rybak K, Phan HTT, Singh KB, Lin SY, Solomon PS, Oliver RP, Tan KC (2019) A specific fungal transcription factor controls effector gene expression and

orchestrates the establishment of the necrotrophic pathogen lifestyle on wheat. Sci Rep 9**:**15884

Kato H, Yamamoto M, Yamaguchi-Ozaki T, Kadouchi H, Iwamoto Y, Nakayashiki H, Tosa Y, Mayama S, Mori N (2000) Pathogenicity, mating ability and DNA restriction fragment length polymorphisms of *Pyricularia* populations isolated from Gramineae, Bambusideae and Zingiberaceae plants. J Gen Plant Pathol 66:30-47

Kim HJ, Han JH, Kim KS, Lee YH (2014) Comparative functional analysis of the velvet gene family reveals unique roles in fungal development and pathogenicity in *Magnaporthe oryzae*. Fungal Genet Biol 66:33-43

Kwon NJ, Garzia A, Espeso EA, Ugalde U, Yu JH (2010) FlbC is a putative nuclear C2H2 transcription factor regulating development in *Aspergillus nidulans*. Mol Microbiol 77:1203-1219

Lu J, Cao H, Zhang L, Huang P, Lin F (2014) Systematic analysis of Zn2Cys6 transcription factors required for development and pathogenicity by high-throughput gene knockout in the rice blast fungus. PLoS Pathog 10:e1004432

Malapi-Wight M, Kim JE, Shim WB (2014) The N-terminus region of the putative C2H2 transcription factor Ada1 harbors a specie-specific activation motif that regulates asexual reproduction in *Fusarium verticillioides.* Fungal Genet Biol 62:25-33

Morita Y, Hyon G, Hosogi N, Miyata N, Nakayashiki H, Muranaka Y, Inada N, Park P, Ikeda K (2013) Appressorium-localized NADPH oxidase B is essential for aggressiveness and pathogenicity in the host-specific, toxin-producing fungus *Alternaria alternata* Japanese pear pathotype. Mol Plant Pathol 14:365-378

Nakayashiki H, Kiyotomi K, Tosa Y, Mayama S (1999) Transposition of the retrotransposon MAGGY in heterologous species of filamentous fungi. Genetics 153:693-703

Pham KTM, Inoue Y, Vu BV, Nguyen HH, Nakayashiki T, Ikeda K, Nakayashiki H (2015a) MoSET1 (histone H3K4 methyltransferase in *Magnaporthe oryzae*) regulates global gene expression during infection-related morphogenesis. PLoS Genet 11:e1005385

Pham KTM, Nguyen HH, Murai T, Chuma I, Tosa Y, Nakayashiki H (2015b) Histone H3K4 methyltransferase globally regulates substrate-dependent activation of cell-wall-degrading enzymes in *Magnaporthe oryzae*. J Gen Plant Pathol 81: 127-130

Shelest E (2008) Transcription factors in fungi. FEMS Microbiology Letters 286:145-151

338 Talbot NJ (2003) On the trail of a cereal killer: exploring the biology of *Magnaporthe*
339 *grisea*. Ann Rev in Microbiol 57:177-202

340 Urashima AS, Hashimoto Y, Don LD, Kusaba M, Tosa Y, Nakayashiki H, Mayama S (1999)
341 Molecular analysis of the wheat blast population in Brazil with a homolog of
342 retrotransposon MGR583. Ann Phytopathol Soc Jpn 65:429-436

343 Vu BV, Takino M, Murata T, Nakayashiki H (2011) Novel vectors for retrotransposon induced
344 gene silencing in *Magnaporthe oryzae* . J Gen Plant Pathol 77:147-151

345 Vu BV, Pham KT, Nakayashiki H (2013) Substrate-induced transcriptional activation of the
346 MoCel7C cellulase gene is associated with methylation of histone H3 at lysine 4 in the
347 rice blast fungus *Magnaporthe oryzae*. Appl Environ Microbiol 79:6823-6832

348

349

350 **Figure legends**

351

352 **Fig. 1** Quantitative RT-PCR analysis of five MoSET1-dependent transcription factors
353 at hyphal, conidial and infectious stages in *P. oryzae*. Actin was used to normalize
354 mRNA expression level. Data show fold change (relative to mRNA quantity in hyphae)
355 ± standard error (n = 3). Asterisks are given to indicate significant difference at $p < 0.05$
356 (*) and $p < 0.01$ (**) (two-tailed *t*-test).

357

358 **Fig. 2** Quantitative RT-PCR analysis of MGG_00472 and MGG_07386 mRNA in
359 candidates of their knock-down mutants. Actin was used to normalize mRNA
360 expression level. Data show fold change (relative to mRNA quantity in the wild-type
361 strain) ± standard error (n = 3). Different characters indicate significant differences by
362 Tukey's HSD ($p < 0.05$).

363

364 **Fig. 3** Phenotypic characterization of knock-out and -down mutants of MoSET1-
365 dependent TFs in *P. oryzae*. **a** Diameters of fungal colonies were measured at 9 days
366 after inoculation on rich agar medium. **b** Conidiation was evaluated by counting the
367 number of conidia under a light microscopy as described in details in Materials and
368 methods. **c-d** The rates of conidial germination (**c**) and appressorium formation (**d**) were
369 measured by observing conidial suspension on hydrophobic surface under a light
370 microscope after 5 h (conidial germination) and 24 h (appressorium formation)
371 incubation at 25℃.
372 Black bars indicate the wild-type strain Br48 (WT) and grey bars represent knock-out
373 and -down mutants of MoSET1-dependent TFs and their gene complemented strains
374 (cΔmgg_04699 and cΔmgg_06898). Data show fold change (relative to the wild-type
375 strain) ± standard error (n = 3). Different characters in the graphs indicate significant
376 differences by Tukey's HSD ($p < 0.05$) ND, not determined.

377

378 **Fig. 4** Inoculation test of knock-out and -down mutants of MoSET1-dependent TFs in
379 *P. oryzae*. **a** Infection assay was performed on the wheat cultivar Norin 4 at 23℃. Four
380 to five days after inoculation, symptoms on the inoculated plants were evaluated. Letters

under pictures of infected leaves indicate disease index values by a grading method (Hyon et al., 2012). This experiment was repeated at least three times, and representative samples are presented. **b** The rates of infection hyphae formation in infected leaves. The black bar indicates the wild-type strain Br48 (WT) and grey bars represent knock-out and –down mutants of MoSET1-dependent TFs and a gene complemented strain (cΔmgg_04699). Error bars represent standard errors of the mean (n = 10). Different characters in the graph indicate significant differences by Tukey's HSD ($p < 0.05$).

Fig. 5 Schematic diagram of putative MoSET1 regulatory network during infection-related morphogenesis in *P. oryza*e.

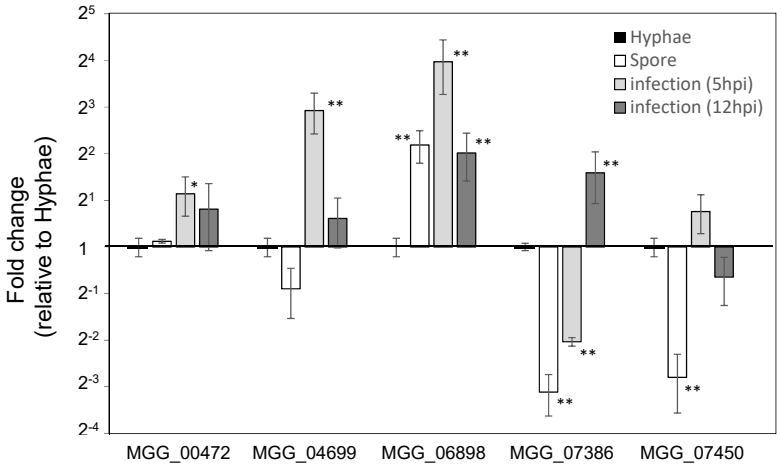

Fig 1. Quantitative RT-PCR analysis of five MoSET1-dependent transcription factors at hyphal, conidial and infectious stages in *P. oryzae*. Actin was used to normalize mRNA expression level. Data show fold change (relative to mRNA quantity in hyphae) ± standard error (n = 3). Asterisks are given to indicate significant difference at $p < 0.05$ (*) and $p < 0.01$ (**) (two-tailed t-test).

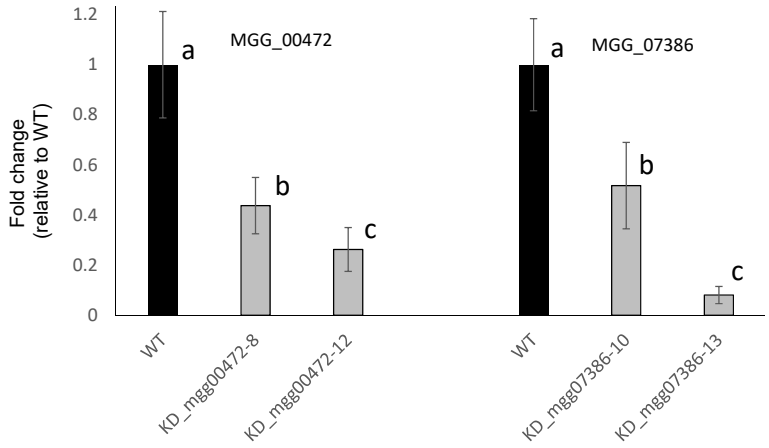

Fig 2. Quantitative RT-PCR analysis of MGG_00472 and MGG_07386 mRNA in candidates of their knock-down mutants. Actin was used to normalize mRNA expression level. Data show fold change (relative to mRNA quantity in the wild-type strain) ± standard error (n = 3). a-c, Different characters indicate significant differences by Tukey's HSD ($P < 0.05$).

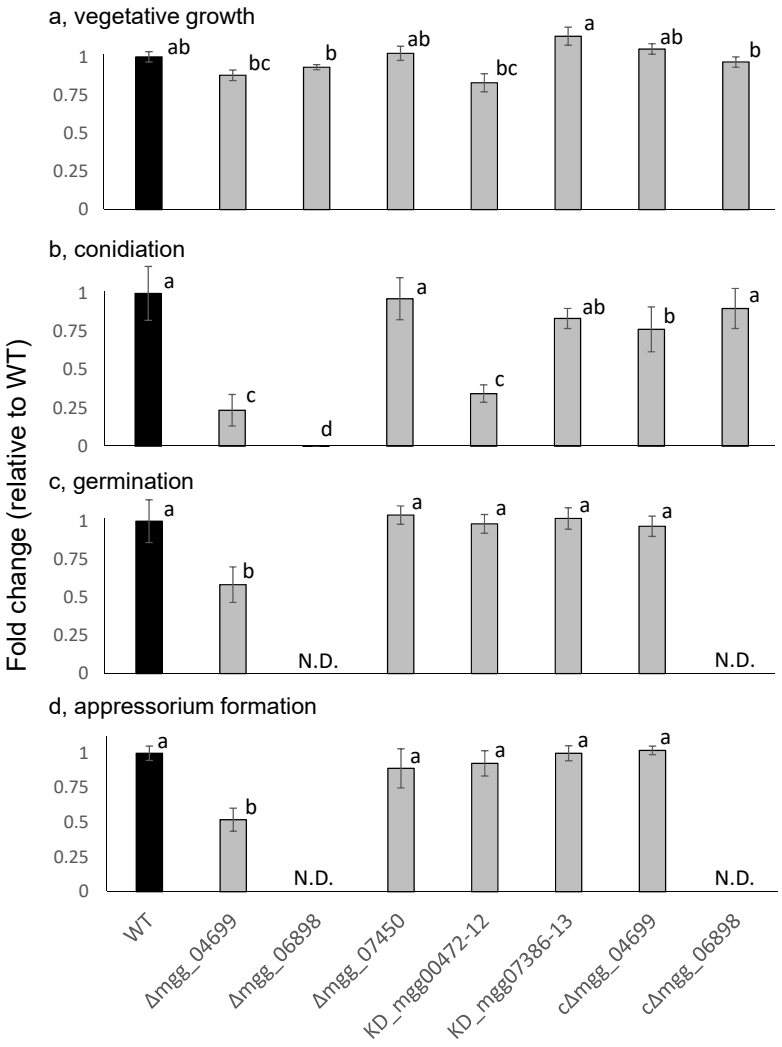

Fig 3. Phenotypic characterization of knock-out and -down mutants of MoSET1-dependent TFs in *P. oryzae*. a, Diameters of fungal colonies were measured at 9 days after inoculation on rich agar medium. **b,** Conidiation was evaluated by counting the number of conidia under a light microscopy as described in details in Materials and method. **c-d,** The rates of conidial germination (c) and appressorium formation (d) were measured by observing conidial suspension on hydrophobic surface under a light microscope after 5 h (conidial germination) and 24 h (appressorium formation) incubation at 25℃.

Black bars indicate the wild-type strain Br48 (WT) and grey bars represent knock-out and –down mutants of MoSET1-dependent TFs and their gene complemented strains (cΔmgg_04699 and cΔmgg_06898). Data show fold change (relative to the wild-type strain) ± standard error (n = 3). a-d, Different characters in the graphs indicate significant differences by Tukey's HSD ($P < 0.05$). ND, not determined.

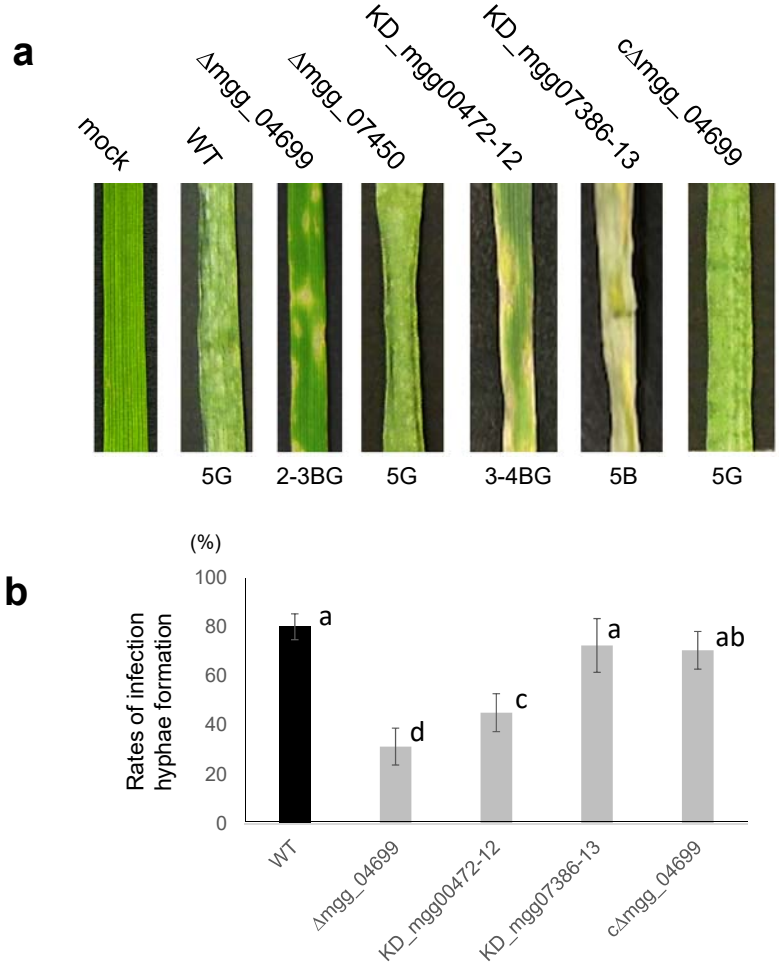

Fig. 4. Inoculation test of knock-out and -down mutants of MoSET1-dependent TFs in *P. oryzae*. **a,** Infection assay was performed on the wheat cultivar Norin 4 at 23℃. Four to five days after inoculation, symptoms on the inoculated plants were evaluated. Letters under pictures of infected leaves indicate disease index values by a grading method (Hyon et al., 2012). This experiment was repeated at least three times, and representative samples are presented. **b,** The rates of infection hyphae formation in infected leaves. The black bar indicates the wild-type strain Br48 (WT) and grey bars represent knock-out and –down mutants of MoSET1-dependent TFs and a gene complemented strain (cΔmgg_04699). Error bars represent standard errors of the mean (n = 10). a-d, Different characters in the graph indicate significant differences by Tukey's HSD ($P < 0.05$).

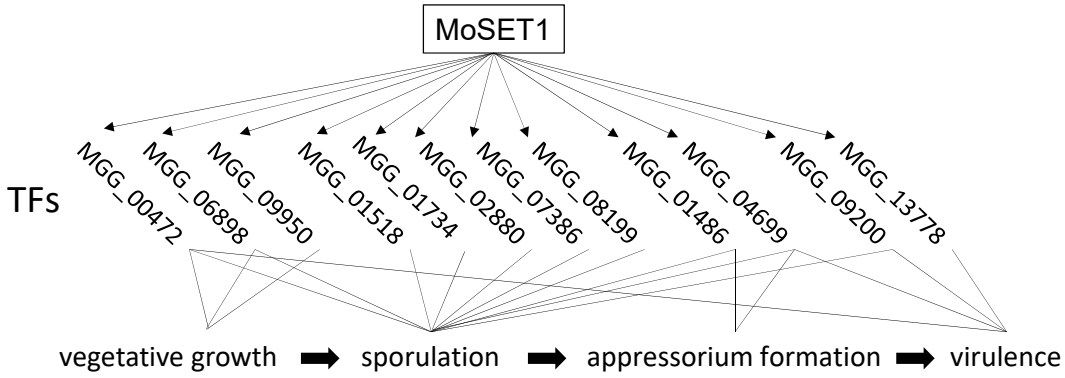

Fig. 5. Schematic diagram of putative MoSET1 regulatory network during infection-related morphogenesis in *P. oryzae*.