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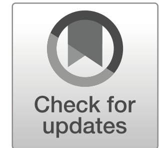

The transcription factor FgMed1 is involved in early conidiogenesis and DON biosynthesis in the plant pathogenic fungus *Fusarium graminearum*

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Abstract

Fusarium graminearum is a prominent fungal pathogen that causes economically important losses by infesting a wide variety of cereal crops. *F. graminearum* produces both asexual and sexual spores which disseminate and inoculate hosts. Therefore, to better understand the disease cycle and to develop strategies to improve disease management, it is important to further clarify molecular mechanisms of *F. graminearum* conidiogenesis. In this study, we functionally characterized the *FgMed1*, a gene encoding an ortholog of a conserved *MedA* transcription factor known to be a key conidiogenesis regulator in *Aspergillus nidulans*. The gene deletion mutants *ΔFgMed1* produced significantly less conidia, and these were generated from abnormal conidiophores devoid of phialides. Additionally, we observed defective sexual development along with reduced virulence and deoxynivalenol (DON) production in *ΔFgMed1*. The GFP-tagged FgMed1 protein localized to the nuclei of conidiophores and phialides during early conidiogenesis. Significantly, RNA-Seq analyses showed that a number of the conidiation- and toxin-related genes are differentially expressed in the *ΔFgMed1* mutant in early conidiogenesis. These data strongly suggest that FgMed1 involved in regulation of genes associated with early conidiogenesis, DON production, and virulence in *F. graminearum*.

Keywords Med1 · MedA · Conidiogenesis · *Fusarium graminearum*

Introduction

Fusarium head blight (FHB), also known as scab, caused by *Fusarium graminearum* (teleomorph *Gibberella zeae*) is a devastating disease affecting wheat and other small grains worldwide (McMullen et al. 1997). Under favorable conditions, this fungal disease can lead to severe yield losses and poor quality. Infested grains pose a serious safety threat to

Gaili Fan and Kai Zhang contributed equally to this work.

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human and animal health due to harmful mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), and zearalenone (Desjardins 2003; Pestka and Smolinski 2005). Despite the devastating issues caused by FHB, establishing effective disease management strategies has been very difficult. Fundamental biology understanding of pathogen-host association along with clearer characterization of the disease cycle is essential for spearheading new sustainable FHB management strategies. In many plant pathogenic fungi, conidia play a crucial role for disease dissemination as infectious propagules (Park and Yu 2012). The genetic and molecular mechanisms related to conidiation have been studied extensively in model fungi, such as *Neurospora crassa* and *Aspergillus nidulans*. In the past decades, a number of conidiation-related transcription factors (TFs) have been identified in plant pathogenic fungi, e.g., *Magnaporthe oryzae* and *Fusarium* species, associated with the regulation of cellular signal pathway genes (Odenbach et al. 2007; Ruiz-Roldan et al. 2015; Son et al. 2011; Zheng et al. 2012a, b).

The homeodomain transcription factors are evolutionarily conserved and regulate diverse developmental stages in fungi. Two homeobox genes in *Saccharomyces cerevisiae*, *MATa2* and *MATa1*, are known to be essential for mating (Ho et al. 2002). The first cloned homeobox gene *PAH1* in *Podospora anserina* was determined as a repressor of microconidiation (Arnaise et al. 2001). In *Fusarium* species, the conserved homeobox transcription factor Htf1 regulates phialide development and conidiogenesis via distinct signaling pathways (Zheng et al. 2012a, b). And in the rice blast fungus *M. oryzae*, homeodomain transcription factors are required for conidiation and appressorium development (Kim et al. 2009). Another family of transcription factors, the APSES TFs, is unique to fungi and regulates developmental processes in ascomycetes. StuA was the first APSES family TF to be characterized and was shown to be involved in conidiophore morphogenesis, notably in the formation of metulae and phialides in *A. nidulans* (Dutton et al. 1997). In *F. graminearum*, a *ΔFgStuA* mutant, showed significantly impaired spore production, lacked conidiophores and phialides, and did not develop perithecia and sexual ascospores (Lysoe et al. 2011). Lastly, Myb DNA-binding domain transcription factors are involved in various cellular processes in eukaryotes, including cell proliferation, apoptosis, differentiation, metabolism, and stress responses. In *F. graminearum*, 20 Myb-like proteins, e.g., Myt1, Myt2, and Myt3, were identified and most of them are required for normal sexual development (Kim et al. 2014; Lin et al. 2011, 2012; Son et al. 2011). In *Ashbya gossypii*, the deletion of *AgBAS1* gene that encodes Myb-like protein showed delayed spore germination (Mateos et al. 2006). The Myb-like transcription factor FlbD in *A. nidulans* controls asexual and sexual differentiation, as determined by the mutants exhibiting severe defects in conidiation and perithecia development (Arratia-Quijada et al. 2012).

Conidiation gene signaling in the model organism *A. nidulans* has been extensively studied, and the core regulatory pathway includes TFs BrlA-AbaA-WetA (Martinelli 1979). The TF MedA was first identified and characterized in *A. nidulans* as a temporal modifier upstream of these core regulatory pathway TFs (Chung et al. 2011). Deletion of *MedA* resulted in phialide production delay, reduced number of conidia as well as production of medusoid-like conidiophores (Clutterbuck 1969). In *A. fumigatus*, MedA controls adherence, host cell interactions, and virulence. However, conidiophore morphology is markedly different in *A. nidulans* and *A. fumigatus ΔmedA* mutants (Gravelat et al. 2010). The study of *MedA* in *Ustilago maydis* found that the gene was required for in vitro mating, pheromone response, and full virulence (Chacko and Gold 2012). In *M. oryzae*, the insertional mutants of MedA homolog Acr1 produced head-to-tail (acropetal) arrays of elongated conidia. Acr1 was regarded as a stage-specific negative regulator of conidiation that is required for establishing a sympodial pattern of spore formation (Nishimura et al. 2000). *F. oxysporum* Ren1, a Acr1 homolog, is required for proper differentiation of conidiogenesis for microconidia and macroconidia (Ohara et al. 2004).

With conidia production being a critical element in FHB disease cycle, there is a need to characterize the role of MedA homolog in conidia development and virulence in *F. graminearum*. Here, we identified the MedA ortholog FgMed1 and showed that null mutants are impaired in sexual and asexual development. The mutants also exhibited defects in DON production and virulence. Further intracellular localization study showed that FgMed1 protein localizes to the nuclei of conidiophores and terminal phialides. Transcriptome analyses further demonstrated that a number of the conidiation and toxin-related proteins are differentially expressed in the *ΔFgMed1* mutant, suggesting that FgMed1 is a transcriptional regulator that regulates the differentiation of sexual and asexual reproduction and virulence in *F. graminearum*.

Materials and methods

Strains and culture conditions

F. graminearum strain PH-1 originally from Fungal Genetics Stock Center in the USA (accession number NRRL 31084) was used as the wild-type strain in this study. *FgMed1* gene deletion strains (*ΔFgMed1-1, ΔFgMed1-2*) and the *FgMed1* complemented (FgMed1-Com) strain were generated in this study following our standard protocols (Zheng et al. 2015). All strains were routinely maintained on complete medium (CM) agar plate (Fan et al. 2017). TB3 medium (3 g yeast extract, 3 g acid hydrolysis casein, 20 g sucrose, and 15 g agar powder in 1 L water) was used for regeneration of protoplasts. Carboxymethyl cellulose medium (CMC) and carrot agar

(200 g carrots and 20 g agar in 1 L medium) were used for induction of asexual and sexual sporulation, respectively (Cappellini and Peterson 1965). All strains were stored as conidial suspensions in 20% glycerol at − 70 °C.

Nucleic acid manipulations, construction of *FgMed1* deletion mutants and Southern blot

The procedure for isolation of genomic DNA was described earlier (Fan et al. 2017). Total RNA samples were isolated from frozen mycelia with Eastep™ Total RNA extraction Kit (Promega (Beijing) Biotech Co., Ltd, China, LS1030) according to the manufacturer's instruction. PCR primers (Supplementary Table S1) used in this study were synthesized at Beijing Liuhe Huada Genomics Technology Co., LTD, Beijing, China. The gene replacement of *FgMed1* in *F. graminearum* strain PH-1 was generated according to the split-marker recombination approach (Catlett et al. 2003; Son et al. 2014a, b). *F. graminearum* protoplast preparation and fungal transformation were performed following the protocol previously described (Yang et al. 2015). The transformants were selected on hygromycin-resistant CM culture plates, verified by PCR with genes specific primers, and further characterized by RT-PCR. In total, 5 colonies were selected, and 2 of these were selected for subsequent phenotypic analysis. Southern blot was performed as described (Matar et al. 2017). The genomic DNA of *F. graminearum* was digested by *Xho*I and separated by agarose gel. The probe for hybridization was amplified from genomic DNA of PH-1 by PCR using primers Med1AF and Med1AR (Supplementary Table S1). Then, the probe was labeled with the DIG-High Prime DNA Labeling and Detection Starter Kit (Roche, Mannheim, Germany).

Vegetative growth and osmotic sensitivity test

For vegetative growth assay, mycelial pellets with equal colony diameter (5 mm) were collected with a cork borer, placed at the center of SYM medium, and were incubated at 28 °C in inverted manner. For the osmotic sensitivity test, mycelial pellets with equal colony diameter were collected with a cork borer, placed on SYM plates amended with 1 M sorbitol, 0.5 M NaCl, 0.05 mM H_2O_2, 0.2 mg/ml CFW, 0.05% SDS, or 0.2 mg/ml Congo red, and incubated in the dark at 28 °C to monitor fungal growth. Colony diameter was measured and photographed after 4 days of incubation. All tests were performed in triplicates.

Asexual and sexual production

For asexual production assay, three agar blocks carrying fresh mycelium were inoculated into a 30 ml CMC broth at 28 °C on a rotary shaker for 2–4 days. Quantification of conidia was performed with a hemocytometer and also photographed at different time stages with the Olympus BX51 Research Microscope (Olympus Co. Tokyo, Japan). Nuclei in conidia and phialides were visualized with 4′,6-diamidino-2-phenylindole (DAPI) (10 mg/ml, Sigma-Aldrich Co., St. Louis, USA) staining. For spore germination test, freshly harvested macroconidia were suspended in CM Petri dish with gentle agitation, and observed at 4 h, 12 h, 24 h, and 48 h time points. All experiments were replicated three times. For ascospore production, mycelia agar blocks were cultivated on carrot agar plates for 1 week and pressed down with a spreader after applying 2.5% sterilized Tween 60 per 20 ml Petri dish to induce sexual reproduction (Pasquali and Kistler 2006). Perithecium formation and ascospore production were examined under a dissecting microscope after incubation for 2 weeks at 28 °C under UV light.

Virulence and DON production assays

Virulence assays on flowering wheat heads were performed as previously described (Liu et al. 2013; Zheng et al. 2015). We inoculated agar blocks (5 mm in diameter) with mycelia on the flowering wheat heads, incubated in 100% humidity, and observed symptoms 14 days after inoculation. The disease was calculated by the average disease index (diseased spikelets per head). Disease index was measured by the number of symptomatic spikelet 15 days after inoculation. Mean and standard error were calculated with results from three independent experiments. At least three wheat heads were examined in each repeat. For the DON production test, autoclaved rice grains were inoculated with mycelia of the wild-type and mutant strains, cultured at 28 °C for 3 weeks. DON analysis was performed as previously described (Bluhm et al. 2007).

Construction of FgMed1-GFP fusion vector and cellular localization of FgMed1

The FgMed1 protein was tagged with eGFP at the N-terminus by fusing the respective DNA sequences under control of its native promoter. The *FgMed1* gene plus 1722-bp promoter region was amplified using genomic DNA extracted from wild-type PH-1 as a template using appropriate primers (Supplementary Table S1) (Zheng et al. 2015). Then, a SOE-PCR were applied to obtain the native promoter-FgMed1-GFP, cloned into a T vector (pMDTM 18/19-T Vector Cloning Kit, Takara), which was verified by sequencing. The constructed vector was co-transformed into protoplasts of *ΔFgMed1* mutant along with pKNTG vector (Khang et al. 2005) harboring the neomycin-resistance marker. Transformants were screened by PCR with primer pairs of pGFPF/FgMed1CR and by phenotypic restoration to *ΔFgMed1*. GFP fluorescence was followed using a Leica TCS SP5 inverted confocal laser scanning microscope (Leica, Germany).

RNA-sequencing and bioinformatics analysis

Three biological replicates of the wild-type PH-1 and *ΔFgMed1* mutants were grown in CMC for 24 h on a rotary shaker (150 rpm). Mycelia and conidia were harvested with Miracloth and were washed with distilled water. Total RNA was extracted with Eastep^{TM} Total RNA extraction Kit (Promega (Beijing) Biotech Co., Ltd, China, LS1030) according to the manufacturer's instruction. RNA-sequencing libraries were created using Illumina Truseq^{TM} RNA sample prep Kit (Illumina, Inc., San Diego, USA). Sequencing was performed on an Illumina HiSeq4000 instrument (Illumina, Inc., San Diego, USA) using the reagents provided in the Illumina Hiseq4000 Truseq SBS Kit (Illumina, Inc., San Diego, USA). The gene differential expression was analyzed by Cuffdiff (http://cole-trapnell-lab.github.io/cufflinks/cuffdiff/index.html). The gene enrichment was carried out with Gene Ontology (http://www.geneontology.org/). The relative transcript abundance was measured in FPKM (expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced). The $\log_2$ ratios of the FPKM values were used to identify differentially expressed genes. All raw data files are available from the NCBI database (accession number PRJNA516068).

Quantitative real-time PCR (qRT-PCR) assays

For the qRT-PCR assays, fresh mycelia of *ΔFgMed1* and wild-type strain PH-1 samples were collected at the time points of 12 h, 24 h, 36 h, and 48 h under CMC medium at 28 °C on a rotary shaker. For the expression level of core conidiation regulators, the samples of wild-type PH-1 and *ΔFgMed1* mutant were collected at 24 h after grown in CMC on a rotary shaker at 28 °C. RNA was extracted using RNAiso Reagent (TaKaRa Biotechnology Co., Dalian, China) according to the manufacturer's instructions. qRT-PCR was carried out using primer pairs listed in Supplementary Table S1. The β-tublin gene FGSG_09530 was used as an endogenous control. Relative abundance of transcripts was calculated by the $2^{-\Delta\Delta Ct}$ method. Quantitative RT-PCR was conducted at least twice with three independent biological replicates.

Results

Sequence analysis of the MedA ortholog in *F. graminearum*

FgMed1 (FGSG_02471.3), a homolog of *A. nidulans* MedA, was originally identified by a BLASTp searching for *A. nidulans* MedA. We identified a single homolog of FgMed1 in the *F. graminearum* genome, which is a 728-amino-acid hypothetical protein (41% identity) (Supplementary Fig. S1A). In addition, the FgMed1 transcript contains a ~ 1273 bp 5′untranslated region (5′UTR), and its open reading fragment contain two introns. A previous study in *A. fumigatus* confirmed that nuclear localization of MedA was mediated via three nuclear localization signal (NLS) sites NLS2, NLS3 within the conserved C-terminal domain. In our study, we performed sequence alignment analysis of FgMed1 orthologues against other fungi (Al Abdallah et al. 2012), and learned that NLS sites are highly conserved (Supplementary Fig. S1B).

FgMed1 deletion and complementation

To analyze the function of FgMed1, we constructed a null mutant (*ΔFgMed11*) from wild-type strain PH-1 by homologous recombination strategy as described previously (Fan et al. 2017). Transformants were selected on hygromycin-amended medium, and five individual targeted deletion mutants were created. Two mutants, designated *ΔFgMed1-1 and ΔFgMed1-2*, were chosen for further confirmed by RT-PCR and Southern blot analysis (Supplementary Fig. S2A). In order to confirm that phenotypic defects in mutants were caused by *FgMed1* gene deletion, we complemented the mutant with a wild-type *FgMed1* gene with its native promoter, and the *FgMed1* was fused to a *GFP* for gene localization observation. Subsequently, we obtained the complemented strain Fg*Med1*-Com and confirmed the recovery of *FgMed1* transcription (Supplementary Fig. S2B).

FgMed1 is involved in vegetative growth

F. graminearum vegetative growth was evaluated by measuring colony diameter on CM agar after incubating for 4 days at 28 °C. The results showed *ΔFgMed1* mutants grew approximately 17% slower than the wild-type and FgMed1-Com strains but with normal mycelial morphology. The two mutants showed a diameter growth of 6.46 ± 0.07 and 6.38 ± 0.10 cm on CM after 4 days at room 28 °C whereas wild-type and FgMed1-Com measured 7.78 ± 0.10 and 7.67 ± 0.11 respectively (Fig. 1a, Table 1). These results suggest that *FgMed1* plays a role in vegetative growth.

To determine whether the slower growth was caused by the stress responses or cell wall integrity, *F. graminearum* strains were exposed to several stress-induced reagents as 1 M sorbitol, 0.5 M NaCl, 0.05 mM H_2O_2, and several cell membrane damage reagents, e.g., 0.2 mg/ml CFW, 0.05% SDS, and 0.2 mg/ml Congo red. However, there were no significant differences in mycelial growth among all the tested strains under the exposure of the stress-induced reagents and cell membrane damage reagents (data not shown). These results suggested that *FgMed1* is involved in vegetative growth but does not play a role in maintaining cell membrane integrity of *F. graminearum.*

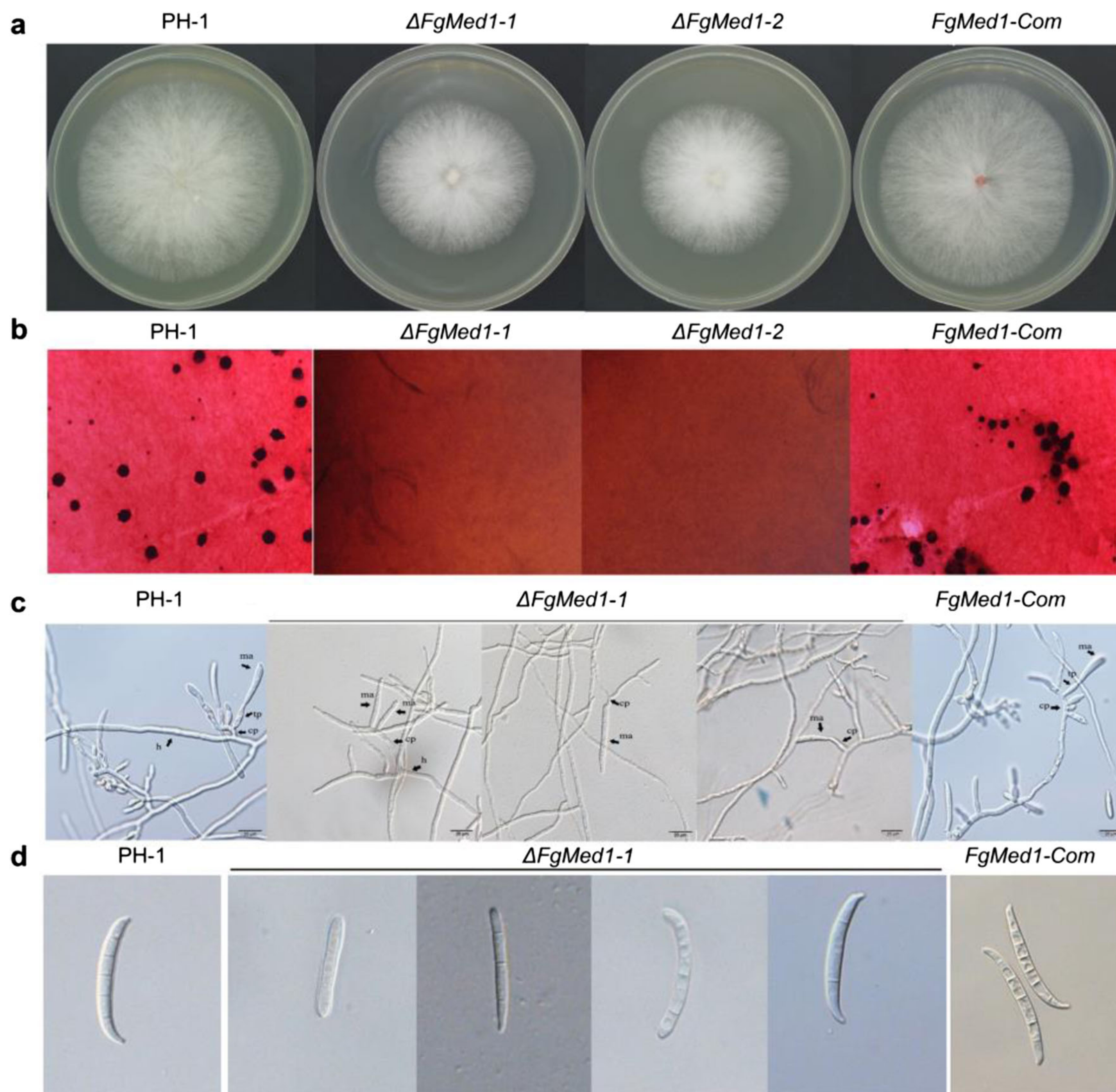

Fig. 1 Mycelial growth and macroconidial defect in *ΔFgMed1* mutant. **a** Mycelial growth of *F. graminearum* strains on complete media (CM). **b** Self-fertility of *F. graminearum* strains. Six-day-old carrot agar culture was mock-fertilized to induce sexual reproduction and incubated for an additional 2 weeks. **c** *FgMed1* regulates the development of macroconidia production. *ΔFgMed1* produced the macroconidia without terminal phialides. **d** Morphology of macroconidia produced by PH-1 and *ΔFgMed1* mutants. *ΔFgMed1* produced both normal and abnormal macroconidia

Table 1 Characterization of *ΔFgMed1* and complementation transformants

Strain	Growth (cm)[a]	Conidiation (10^4/ml)[b]	Germination (%)				DON (ppm)[c]
			4 h	12 h	24 h	48 h	
PH-1	7.78 ± 0.10	144 ± 11.79	56.00 ± 9.57	100	100	100	1101.1 ± 196.8
ΔFgMed-1	6.46 ± 0.07*	0.03 ± 0.02*	6.87 ± 2.58*	25.61 ± 6.35*	66.03 ± 6.30*	88.34 ± 7.09	2.1 ± 1.0*
ΔFgMed-2	6.38 ± 0.10*	0.04 ± 0.01*	7.27 ± 1.53*	30.48 ± 5.16*	68.78 ± 4.80*	82.63 ± 4.55	3.5 ± 1.6*
Med1-Com	7.76 ± 0.11	139 ± 7.37	63.67 ± 8.02	100	100	100	1293.3 ± 250.8

Mean and standard deviation were calculated from three independent replicates

[a] Growth rate was assayed with 4-day-old CM agar cultures

[b] Conidiation was measured with 6-day-old CMC cultures

[c] Deoxynivalenol was determined with 3-week-old rice grain cultures, and a blank control for DON quantification was 0

*Data were performed three replicates with the protected Fisher's Least Significant Difference test. Indicated significant difference ($P = 0.05$)

FgMed1 is essential for sexual and asexual reproduction

The MedA was known to regulate conidial development in several other fungal species (Al Abdallah et al. 2012; Gravelat et al. 2010; Ohara et al. 2004), and we performed asexual and sexual reproduction tests in *F. graminearum* to study the function of FgMed1. For the sexual reproduction test, mutants failed to generate perithecia and ascospores after a cross (Fig. 1b), which indicated that FgMed1 is essential for sexual reproduction in *F. graminearum*. Asexual conidia production was also dramatically affected by the gene mutation. Cultures of the *ΔFgMed1* mutants produced few macroconidia on CMC medium when compared with wild-type and *FgMed1-Com* strains. After 6 days of incubation, only 300 ± 200 and 400 ± 100 macroconidia/ml were obtained from *ΔFgMed-1* and *ΔFgMed1-2* mutants, in contrast to 144 ± 11.79 × 10^6 and 139 ± 7.37 × 10^6 macroconidia/ml in PH-1 and FgMed1-Com strains, respectively (Table 1).

Normally, the wild-type produces macroconidia on solitary terminal phialides or on multiple terminal phialides borne on conidiophores. However, the mutant only produced macroconidia directly from the abnormal conidiophores without terminal phialides (Fig. 1c, d), which may be directly responsible for the drastic reduction of conidia in the *ΔFgMed1* mutant. Moreover, *ΔFgMed1* produced both normal and abnormal macroconidia. The tips of the abnormal conidia in the *ΔFgMed1* mutant appeared bulbous without a foot cell and an apical cell (Fig. 1c). These results indicated that *FgMed1* plays an important role in the development of both sexual reproduction as well as conidiation.

To further study the function of *FgMed1* in the development of conidiation, we monitored conidia germination in *ΔFgMed1* mutants. The spores collected from PH-1 and *ΔFgMed1* were incubated in liquid CM with gentle agitation. The aberrant macroconidia in the mutants was able to germinate but we saw a significant lag time when compared to the wild-type macroconidia. After 4 h, over 56% macroconidia of PH-1 showed at least one germ tube. However, only about 6~7% mutant macroconidia germinated. After 12 h, approximately all PH-1 macroconidia germinated but the germination rate of *ΔFgMed1* mutants only reached about 25~30%. After 48 h, *ΔFgMed1* macroconidia did reach a germination rate of 82~88% (Table 1).

FgMed1 affected the pathogenicity and DON production

Subsequently, we questioned whether FgMed1 plays a role on pathogenicity, since fungal reproduction and virulence have shown association in other plant pathogenic fungi. We inoculated wheat heads with mycelial plugs of fungal strains (Zheng et al. 2015), and the assays showed that the *ΔFgMed1-1* and *ΔFgMed1-2* mutants produced moderate scab symptom with a disease index of 6.28 ± 0.86 and 6.76 ± 0.91, respectively (Supplementary Fig. S3). The average disease index of PH-1 and *FgMed1-Com* was 11.35 ± 0.81 and 12.46 ± 1.00, respectively. We then asked whether this outcome is correlated with mycotoxin deoxynivalenol (DON) production, which is reported as a virulence factors in *F. graminearum* (Proctor et al. 1995). DON production, assayed in 3-week-old rice grain cultures, showed that *ΔFgMed1* mutants have a very low level of DON than the wild-type, suggesting that FgMed1 plays an important role in DON biosynthesis (Table 1).

Intracellular localization of the GFP-tagged FgMed1

The sequence alignment analysis of FgMed1 ortholog against those of other fungi found that nuclear localization signal (NLS) sites are highly conserved in the conserved C-terminal domain of MedA homologs (Supplementary Fig. 1B). We examined the cellular localization of FgMed1 fused to enhanced green fluorescent protein (eGFP) to understand the temporal and spatial pattern of FgMed1. We examined the eGFP expression at different time points (12 h, 24 h, 36 h, and 48 h) after inoculating the mycelia of FgMed1-eGFP strain into CMC medium. The eGFP signals were not detectable until 12 h post inoculation (hpi). The signals were extremely strong at 24 hpi (Fig. 2), but no signal could be detected beyond 36 hpi. Monitoring of the eGFP fusion protein and 4,6-diamidino-2-phenylindole (DAPI)-stained nuclei by fluorescent microscopy confirmed the nuclear localization of FgMed1-eGFP in conidiophores and terminal phialides. These results demonstrated that *FgMed1* is important for *F. graminearum* conidiogenesis.

Moreover, we tested the expression patterns of FgMed1 in the wild-type strain PH-1 at 12-h, 24-h, 36-h, and 48-h time points. The real-time PCR results showed that the FgMed1 expression level increased from 12 to 24 hpi and then decreased from 24 to 48 hpi (Fig. 3). The highest FgMed1 expression was detected at 24 hpi during the sporulation stage. This result corresponded with our eGFP localization study. Therefore, we concluded that the transcription factor FgMed1 is activated and localized to the nucleus, and is predicted to participate in regulating conidiogenesis.

RNA-sequencing based transcriptome analysis

To further gain insight into the FgMed1 function on downstream genes, we conducted a RNA-seq study of wild-type PH-1 and *ΔFgMed1* deletion mutant strains at 24 h after the induction of conidiogenesis. Differentially expressed genes (DEGs) were identified by 1.2-fold (log2_fold change) in FKPM values (FPKM values of zero were converted to 1 for the calculation of fold change). A total of 1158 up-regulated (>

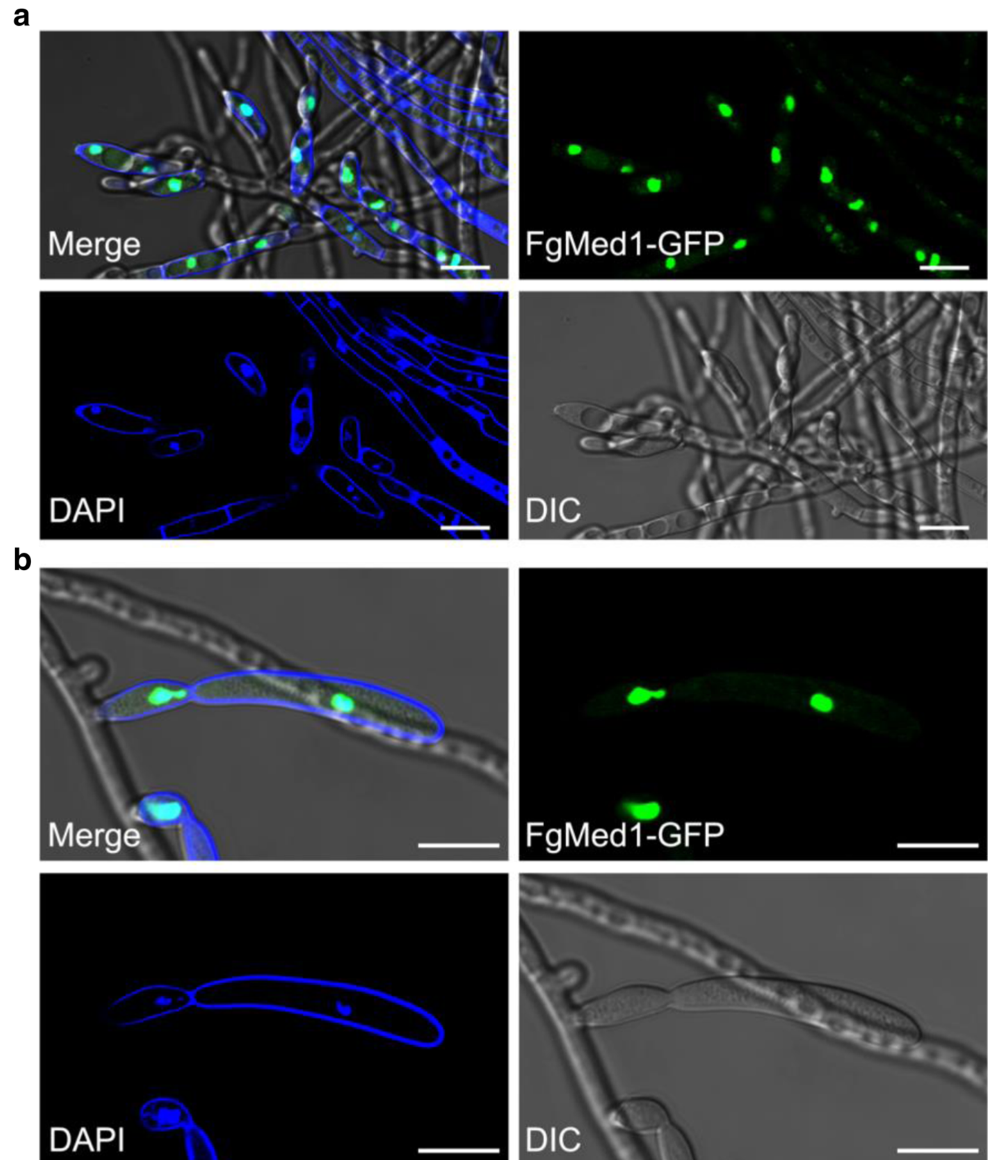

Fig. 2 Subcellular distribution of FgMed1-GPF fusion protein. Colocalization analyses with FgMed1-GPF and DAPI double labeling in *F. graminearum*. **a**, **b** The signals were localized in the nuclei of conidiophores and terminal phialides. Scale bar = 10 μm

1.2-fold) genes and 581 down-regulated (< 1.2-fold) genes were identified in *ΔFgMed1* when compared to those DEGs in PH-1 (Supplementary Fig. S4). When the DEGs were mapped to the chromosomes, they were distributed evenly except in chromosome 4 (Supplementary Fig. S5). Using the WEGO (Web Gene Ontology Annotation Plot) to plot GO annotation (Ye et al. 2018), the 1158 up-regulated genes and 581 down-regulated were grouped into three functional categories, 552 and 289 to molecular function, 391 and 200 to biological process, and 152 and 110 to cellular component, respectively (Fig. 4a, b) (Supplementary Table S2). For the down-regulated genes, most genes were associated with the organic substance metabolic process, primary metabolic process, hydrolase activity, organic cyclic compound binding, and heterocyclic compound binding. For the up-regulated genes, most genes were classified into oxidoreductase activity, organic substance metabolic process, and heterocyclic compound binding.

Functional categorization of the targeted DEGs was further conducted manually by the gene annotation into XI groups through the NCBI blast and the published literature (Table 2 and Table 3). Not surprisingly, twenty genes were identified to spore-related protein group (including asexual or sexual development) according to the reported literature (Table 2). Among these were *GzBrlA FgAbaA* and *FgwetA*, the orthologs of *A. nidulans brlA*, *abaA*, and *wetA*, respectively, which make up the well-characterized conidiation regulatory pathway. Furthermore, the velvet protein FgVeA was also identified, which is known to control asexual and sexual development as well as secondary metabolisms in several fungal species (Jiang et al. 2011). Additional genes, such as *FgFGP1*, *MAT1-2-1*, *FgPBS2*, and *GzRFX1*, that are involved in

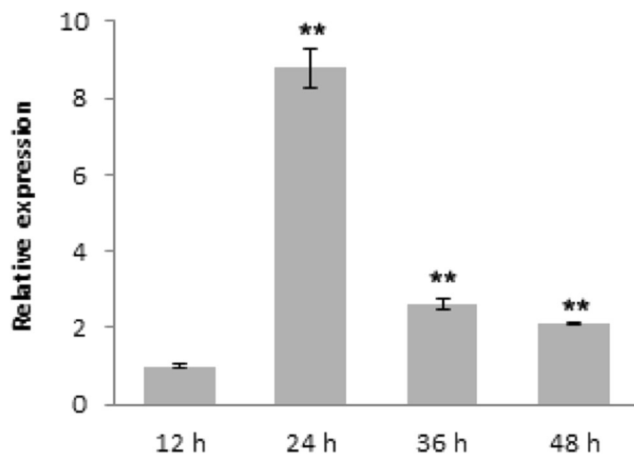

Fig. 3 Expression levels of *FgMed1* at different time points (12 h, 24 h, 36 h, and 48 h) after inoculation in CMC medium. qRT-PCR was used to quantify transcript level of *FgMed1* relative to that of the reference gene β-tubulin using the $2^{-\Delta\Delta Ct}$ method. Error bars represent the standard deviation. Double asterisks indicated significant difference ($P = 0.01$)

asexual or sexual development were also identified. A number of genes involved in mycotoxin and aurofusarin biosynthesis were identified, including a transcription factor gene *DAL81* (FGSG_01134), *ZEB2* (FGSG_02398) and aurofusarin biosynthesis genes. Some DEGs were classified to other groups, including integral membrane protein, vegetative incompatibility protein HET-E-1, chitin synthase related protein, multiple antibiotic resistance protein, F-box Protein, and osmotic related protein (Table 3). These results indicated that FgMed1 is a transcription factor with a broad regulatory role in diverse cellular processes, including control of asexual, sexual development, virulence, and toxin production as well as secondary metabolisms in *F. graminearum*.

The genetic relationship of Med1 and the conidiation regulators genes *brlA*, *abaA*, and *wetA*

RNA-seq results showed that the expression of the central conidiation regulator genes *brlA*, *abaA*, and *wetA* was all down-regulated in the *ΔFgMed1* mutant. To confirm the reduction in expression of *brlA*, *abaA*, and *wetA* in the *ΔFgMed1* mutant, qRT-PCR were conducted using the RNA extracted at 24 h after the induction of conidiogenesis. The results showed a relative down-regulation in expression of *brlA*, *abaA*, and *wetA* genes in the *ΔFgMed1* mutant when compared to the wild-type strain (Fig. 5), suggesting that *FgMed1* is an upstream activator of the central conidiation regulators *brlA*, *abaA*, and *wetA*.

Discussion

In this study, we characterized FgMed1 in *F. graminearum*, the homolog of *A. nidulans* MedA, by generating deletion mutants and analyzing the phenotypic defects. We found that *FgMed1* was specifically involved in conidiogenesis and conidium formation in *F. graminearum*. The *FgMed1* deletion mutants failed to produce perithecia and ascospores after a cross, which indicated that *FgMed1* is essential for sexual reproduction in *F. graminearum*. Moreover, the *FgMed1* deletion mutants produce macroconidia directly from the abnormal conidiophores in the absence of terminal phialides. Subsequently, we found that *FgMed1* affected the pathogenicity and DON production. The intracellular localization of the GFP-tagged *FgMed1* confirmed the nuclear localization of FgMed1-GFP in the cell of conidiophores and terminal phialides in *F. graminearum*. Furthermore, the RNA-seq analysis showed an overall gene expression profile and identified some potential targeted genes that FgMed1 regulated in *F. graminearum*.

Thus, we conclude that the transcription factor FgMed1 is involved in regulation of diverse cellular processes, including control of asexual sporulation, sexual development, and virulence as well as toxin production in *F. graminearum.*

The TF MedA has been characterized as a key developmental regulator and has been studied extensively in the model organism *A. nidulans*. Orthologs of MedA in other filamentous fungi have also been studied, including in *A. fumigatus*, *F. oxysporum*, *M. oryzae, N. crassa*, and *U. maydis*. The studies showed that MedA orthologs play a conserved role in conidiation, albeit some different phenotypic consequences in each species. The deletion of MedA led to medusoid-like conidiophores in *A. nidulans*, but was normal in *A. fumigatus* (Chung et al. 2011). In *U. maydis*, MedA is needed for successful sexual mating (Chacko and Gold 2012). In *M. oryzae and F. oxysporum*, the MedA mutation showed deficiencies in conidiophore architecture (Lau and Hamer 1998; Ohara et al. 2004), which are very similar to the FgMed1 mutant producing macroconidia directly from the abnormal conidiophores without terminal phialides. Moreover, *N. crassa acon-3*, an ortholog of MedA, is required for early conidiophore development and female fertility, and can complement the conidiation defects in *A. nidulans ΔmedA* (Chung et al. 2011). Our results, along with published reports, showed that MedA orthologs play a conserved role in conidiation in fungi.

One of our key aims in this study is to investigate the molecular mechanisms underlying conidiogenesis in *F. graminearum*. Therefore, the RNA-seq experiments were conducted by comparing wild-type and *ΔFgMed1* strains during conidiation. 1158 up-regulated and 581 down-regulated genes in *ΔFgMed1* were identified when compared to PH-1. Significantly, the key genetic components of *brlA*, *abaA*, and *wetA* involved in conidiation were identified in the model organism *A. nidulans*, these key cores regulatory proteins were studied extensively, and MedA was identified as a temporal modifier of expression of these genes. However, in *A. fumigatus*, it was determined that the expression of the core conidiation pathway genes was independent of *MedA* (Gravelat et al. 2010). In our RNA-seq study, we

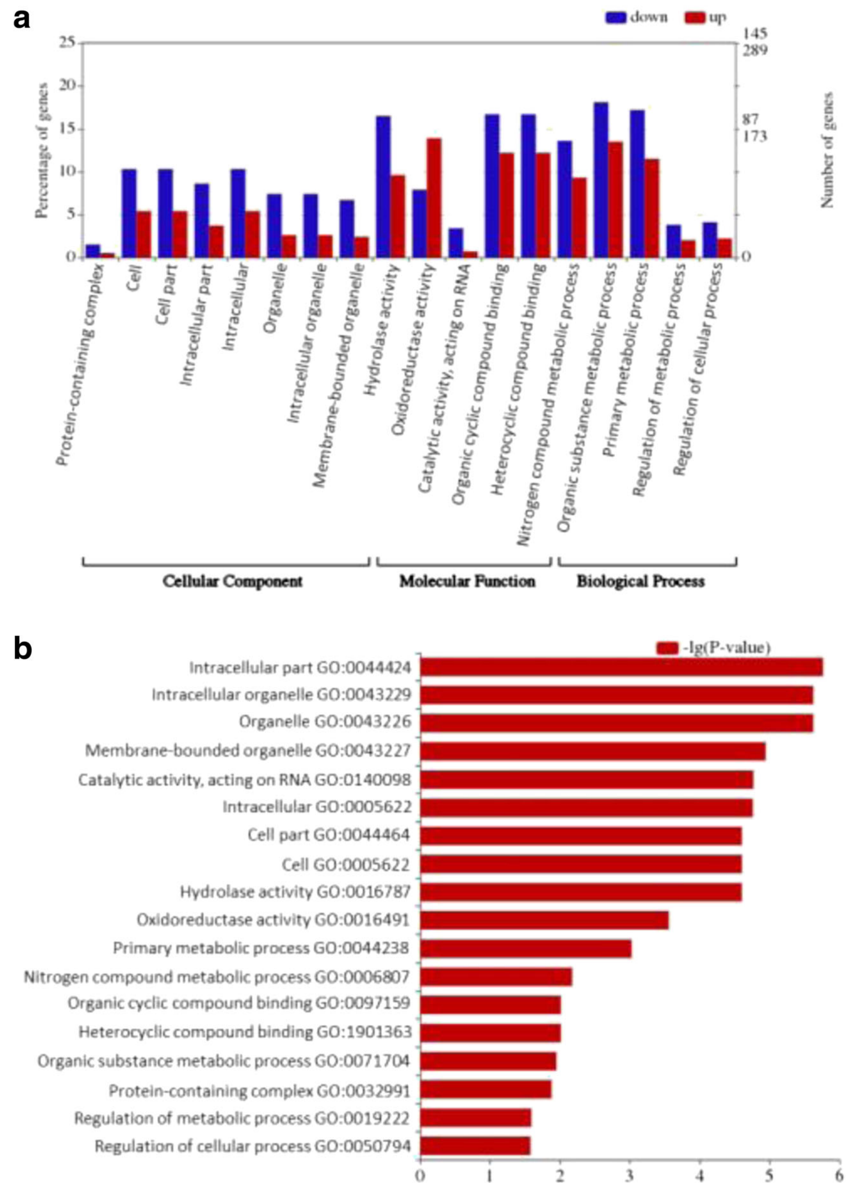

Fig. 4 Gene ontology (GO) of FgMed1 targeted DEGs by WEGO 2.0 (Ye et al. 2018). **a** The WEGO histogram of FgMed1 targeted DEGs. The *x*-axis displays the GO terms. The right *y*-axis shows the gene numbers, while the left *y*-axis shows the percentages (the *y*-axis was log scaled). **b** Log of *P* values of GO terms indicated the significant differences between the down and up-regulated genes

found the expression of *FgAbaA*, *FgWetA*, and *FgBrlA* was all significantly reduced in the *ΔFgMed1*, which was also confirmed by qPCR. These indicate that the conidiation core regulatory pathway *FgBrlA-Aba-WetA* regulated by FgMed1 is conserved in *F. graminearum*. The other conidiation-related genes, such as *FgFGP1*, *FgVEA*, *GzRFX1*, and *FgFlbB*, were also identified in the RNA-seq data. VeA is a key light-dependent developmental regulator that functions as a repressor of conidiation and activator of sexual development (Jiang et al. 2011; Kim et al. 2015; Park and Yu 2012). In our study, *FgVEA* was significantly down-regulated in *ΔFgMed1* when compared to PH-1, suggesting that *FgMed* is required for the activation *FgVEA* gene expression.

In *F. graminearum*, sexual reproduction is mainly controlled by two closely linked *MAT* loci which are important regulators. The phenotypic changes caused by *MAT* deletions and gene expression patterns in *F. graminearum* strongly suggested that *MAT* genes are involved in both the early and late stages of sexual development (Kim et al. 2015). From the RNA-seq data, we found that mating-type control genes *MAT1-1-1*, *MAT1-2-1*, and *MAT1-2-3* were all up-regulated in *ΔFgMed1*. Previous genome-wide microarray analysis showed that a putative chitin binding protein FGSG_05847 was regulated by the *MAT* loci and predicted to be involved in the early stage of perithecium formation during sexual development (Kim et al. 2015). In our study, FGSG_05847 was highly up-regulated in the *ΔFgMed1* mutant. Since FGSG_05847 was a target gene of *MAT* loci, thus, we assume FgMed1 controls the expression of FGSG_05847 through *MAT* loci. All these results seem that FgMed1 is a negative regulator of the sexual reproduction, but the *ΔFgMed1* mutant failed to generate perithecia and ascospores in our test.

Table 2 Functional classification of DEGs in *F. graminearum* according to reported literature

Protein name	Gene ID	log2 (mutant/WT)	Reference	Phenotype by gene deletion
Group I: spore-related protein				
FgAbaA	FGSG_11850	− 4.88175	Son et al. 2013	No C
FgFlbB	FGSG_03597	− 3.30734	Park et al. 2012	No defects
FgwetA	FGSG_10166	− 3.25882	Son et al. 2014a, b	Fewer C, abnormal C
FgFGP1	FGSG_12164	− 3.05366	Jonkers et al. 2012	Reduced V, loss trichothecene toxin, fewer AS, fewer C
FgVEA	FGSG_11955	− 1.68832	Son et al. 2014a, b	Increase C, reduced VG, V, T
GzRFX1	FGSG_07420	− 1.57302	Min et al. 2014	Reduced C, T, abnormal C, no V, fewer P
GzBrlA	FGSG_07310	− 1.36292	Son et al. 2011	Reduced P, no AS/AP
	FGSG_09000	3.01534	Son et al. 2011	Delay PM
MAT1-1-1	FGSG_08892	2.58236	Kim et al. 2015	Smaller P, no AS/AP
Early growth response protein	FGSG_10470	2.64434	Son et al. 2011	Reduced VG, no P, no AS/AP
Fibronectin-attachment protein	FGSG_03916	2.49105	Kim et al. 2015	No P
3-dehydroquinate synthetase	FGSG_07578	2.32675	Kim et al. 2015	Fewer P, no AS
Homocysteine transferase	FGSG_10825	1.95316	Kim et al. 2015	No P, no aerial mycelia, reduced V, T
MAT1-2-1	FGSG_08893	1.74494	Kim et al. 2015	Smaller P, no AS/AP
MAT1-2-3	FGSG_08894	1.83666	Kim et al. 2012	No defects
C2H2 finger protein	FGSG_07052	1.56345	Son et al. 2011	Reduced P, C, no V
GzC2H044	FGSG_06701	1.44392	Son et al. 2011	Delay PM
Cyclin b3	FGSG_07132	− 1.51016		NDM
Probable chitin binding protein	FGSG_05847	5.02958	Kim et al. 2015	NDM
FgPBS2	FGSG_08691	1.54299	Zheng et al. 2012a, b	No P, reduced C, VG, V
Group II: metabolism-associated genes				
ArginineN-methyltransferase 1	FGSG_01134	− 1.69907	Wang et al. 2012	Reduced V, sensitivity to oxidative and membrane stresses
Secreted lipase protein	FGSG_05906	− 4.33655	Kim et al. 2015	Reduced V, reduced extracellular lipolytic activity
Linoleic acid isomerase protein	FGSG_02668	6.87132	Zhang et al. 2017	Reduced VG, V, sensitive to linoleic acid
Group III: tox-related protein				
DAL81-TF	FGSG_02068	− 3.53383	Son et al. 2011	Reduced DON, ZEA
Tri15-TF	FGSG_03881	3.22055	Son et al. 2011	No defects
Tox1	FGSG_09570	3.40111		NDM
Toxin	FGSG_10566	2.22988		NDM
ZEB2	FGSG_02398	3.68937	Son et al. 2011	No ZEA
PKS11	FGSG_01790	4.62333	Gaffoor et al. 2005	Increased VG
PKS15/PLSP1	FGSG_04488	2.47607	Gaffoor et al. 2005	No defects
PKS9	FGSG_12121	1.64139	Gaffoor et al. 2005	Increased VG
Group IV: aurofusarin biosynthetic gene cluster				
GIP2/AurR1	FGSG_02320	4.94804	Lysoe et al. 2006	
GIP3/AurO	FGSG_02321	5.60605	Lysoe et al. 2006	
GIP4/AurT	FGSG_02322	2.78154	Lysoe et al. 2006	
GIP5/AurR2	FGSG_02323	1.99058	Lysoe et al. 2006	
GIP6	FGSG_02325	7.81311	Lysoe et al. 2006	
GIP7/AurJ	FGSG_02326	7.41204	Lysoe et al. 2006	
GIP8/AurF	FGSG_02327	8.23295	Lysoe et al. 2006	
GIP10/AurL2	FGSG_02330	− 4.12908	Lysoe et al. 2006	
GzORF1	FGSG_02319	4.45981	Kim et al. 2006	
GzMCT	FGSG_02331	2.45889	Kim et al. 2006	
PKS12	FGSG_02324	6.82031	Kim et al. 2005	
GIP1	FGSG_02328	7.34478	Kim et al. 2005	

AP ascospore, *AS* asci, *C* conidiation, *H* hyphal growth, *P* perithecia, *PM* perithecia maturation, *SD* sexual development, *T* mycotoxin production, *V* virulence, *VG* vegetative growth, *ZEA* zearalenone, *DON* deoxynivalenol, *No defects* similar with wild-type, *NDM* no deletion mutant were reported

Therefore, the sexual development pathway regulated by FgMed1 still needed further study.

MedA orthologs are also known to govern virulence in some fungal species. For example, the *MedA* deletion showed an attenuated virulence in *A. fumigatus*, an invertebrate and a mammalian pathogen model (Gravelat et al. 2010). In *M. oryzae*, the deletion of *ACR1* resulted in reduced ability in host surface attachment and a reduction in appressoria formation, which ultimately led to the reduction in pathogenicity (Lau and Hamer 1998). In

Table 3 Functional classification of DEGs according with NCBI blast

NCBI blast	Gene_ID	log2 (mutant/WT)
Group V: cell wall related protein		
Cell wall glycoprotein [*Ustilaginoidea virens*]	FGSG_12439	− 8.88969
Cell wall glycoprotein [*Fusarium langsethiae*]	FGSG_02961	− 7.73147
Cell wall glucanase [*Fusarium langsethiae*]	FGSG_07944	− 3.07125
Cell wall proline rich [*Fusarium langsethiae*]	FGSG_07364	1.41584
Cell wall protein PRY3 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_02744	1.45159
Related to cell wall protein PhiA [*Fusarium proliferatum*]	FGSG_08122	2.00277
Cell wall mannoprotein [*Fusarium fujikuroi*]	FGSG_05232	3.111
Cell wall protein PhiA [*Fusarium fujikuroi*]	FGSG_04074	3.94381
Cell wall protein PhiA [*Fusarium fujikuroi*]	FGSG_03662	3.68157
Related to cell wall protein cwl1 [*Fusarium fujikuroi* IMI 58289]	FGSG_09796	1.59899
Antigenic cell wall [*Fusarium langsethiae*]	FGSG_03123	2.75272
Group VI: integral membrane protein		
Integral membrane protein pth11 [*Fusarium fujikuroi*]	FGSG_02818	− 7.43186
Related to integral membrane protein PTH11	FGSG_10593	− 7.3185
Related to integral membrane protein	FGSG_04825	− 7.09829
Related to integral membrane protein [*Fusarium proliferatum* ET1]	FGSG_07595	− 6.23723
Related to integral membrane protein PTH11 [*Fusarium mangiferae*]	FGSG_03897	− 5.84077
Related to integral membrane protein PTH11 [*Fusarium mangiferae*]	FGSG_03588	− 5.76483
Integral membrane protein [*Fusarium langsethiae]*	FGSG_03277	− 4.98464
Integral membrane protein pth11 [*Fusarium fujikuroi*]	FGSG_03688	− 4.85213
Related to integral membrane protein [*Fusarium mangiferae*]	FGSG_03561	− 4.71855
Integral membrane protein [*Verticillium alfalfae* VaMs.102]	FGSG_05692	− 4.3665
Related to integral membrane protein pth11 [*Fusarium proliferatum*]	FGSG_13461	− 3.81059
Integral membrane protein PTH11 [*Fusarium fujikuroi*]	FGSG_10983	− 3.28223
Integral membrane protein [*Metarhizium rileyi* RCEF 4871]	FGSG_03128	1.43866
Integral membrane protein [*Fusarium langsethiae*]	FGSG_12474	2.97103
Integral membrane protein [*Verticillium alfalfae* VaMs.102]	FGSG_08342	− 1.91426
Integral membrane protein PTH11 [*Fusarium fujikuroi*]	FGSG_03962	1.64575
Integral membrane [*Fusarium langsethiae*]	FGSG_06118	4.01832
Integral membrane protein [*Fusarium langsethiae*]	FGSG_12475	4.02565
Integral membrane protein [*Fusarium avenaceum*]	FGSG_07792	5.94998
Group VII: vegetative incompatibility protein HET-E-1		
Vegetative incompatibility protein HET-E-1 [*Fusarium oxysporum* f. sp. *cubense* race 1]	FGSG_13472	1.59129
Vegetative incompatibility protein HET-E-1 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_03995	1.9999
Vegetative incompatibility protein HET-E-1 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_13978	2.07979
Vegetative incompatibility protein HET-E-1 [*Fusarium langsethiae*]	FGSG_04859	2.22494
Vegetative incompatibility protein HET-E-1 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_08144	2.35764
Vegetatible incompatibility protein HET-E-1 [*Fusarium fujikuroi*]	FGSG_02035	2.39764
Vegetative incompatibility protein HET-E-1 [*Colletotrichum higginsianum* IMI 349063]	FGSG_11377	2.46431
Vegetative incompatibility protein HET-E-1 [*Tolypocladium ophioglossoides* CBS 100239]	FGSG_04764	2.57365
Vegetative incompatibility protein HET-E-1 [*Fusarium oxysporum* f. sp. *cubense* race 1]	FGSG_10569	2.86247
Vegetatible incompatibility protein HET-E-1 [*Fusarium fujikuroi*]	FGSG_11067	3.42848
Vegetatible incompatibility protein HET-E-1 [*Fusarium fujikuroi*]	FGSG_01249	3.64696
Vegetative incompatibility protein HET-E-1 [*Fusarium langsethiae*]	FGSG_07622	3.70422
Vegetative incompatibility protein HET-E-1 [*Fusarium langsethiae*]	FGSG_11017	4.10775
Vegetative incompatibility protein HET-E-1 [*Madurella mycetomatis*]	FGSG_04587	4.1102
Related to vegetatible incompatibility protein HET-E-1 [*Fusarium mangiferae*]	FGSG_10601	4.17975
Vegetative incompatibility protein HET-E-1 [*Madurella mycetomatis*]	FGSG_04805	4.25273

Table 3 (continued)

NCBI blast	Gene_ID	log2 (mutant/WT)
Group VIII: chitin synthase related protein		
Endochitinase 1 [*Fusarium langsethiae*]	FGSG_11903	− 5.75513
Endochitinase 1 [*Fusarium langsethiae*]	FGSG_11904	− 5.48938
Chitin binding protein [*Fusarium fujikuroi*]	FGSG_05663	− 2.17117
Cutinase transcription factor 1 beta [*Madurella mycetomatis*]	FGSG_02088	− 2.02903
Chitinase [*Fusarium langsethiae*]	FGSG_05969	1.68025
Chitin synthase regulatory factor 3 [*Fusarium langsethiae*]	FGSG_08673	1.73431
Chitin synthase [*Colletotrichum tofieldiae*]	FGSG_10013	1.94457
Chitinase 1 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_04143	2.44803
Chitinase 2 [*Madurella mycetomatis*]	FGSG_02170	2.78651
Chitinase A1 [*Fusarium oxysporum* f. sp. *cubense* race 1]	FGSG_03212	3.84356
Chitin synthase 6 [*Fusarium oxysporum* f. sp. *cubense* race 4]	FGSG_06550	1.36639
Cutinase precursor [*Fusarium graminearum* PH-1]	FGSG_03457	− 18.4062
Chitinase 1 [*Fusarium oxysporum* Fo47]	FGSG_03591	− 2.94982
Group IX: multiple antibiotic resistance protein		
ABC2 [*Fusarium pseudograminearum* CS3096], multidrug resistance protein [*Fusarium fujikuroi*]	FGSG_08308	− 4.64129
Related to multidrug resistance-associated protein [*Fusarium proliferatum*]	FGSG_06141	− 2.90595
ABC multidrug [*Fusarium langsethiae*]	FGSG_11240	− 2.38325
Drug resistance protein [*Beauveria bassiana* D1-5]	FGSG_07632	2.45993
ABC multidrug transporter [*Aspergillus fumigatus* Af293]	FGSG_11988	3.09518
Multidrug resistance protein [*Fusarium langsethiae*]	FGSG_02316	3.40314
AcrB/AcrD/AcrF family protein [*Brevibacterium mcbrellneri*]	FGSG_07997	3.49781
Multidrug resistant protein [*Fusarium fujikuroi*]	FGSG_07564	3.59197
Mfs-multidrug-resistance transporter [*Fusarium langsethiae*]	FGSG_02966	4.18428
Related to multidrug resistant protein [*Fusarium fujikuroi* IMI 58289]	FGSG_02869	6.11049
GroupX: F-box protein		
F-box protein Fbl2 [*Fusarium fujikuroi*]	FGSG_06969	− 2.76214
F-box domain, Skp2-like protein [*Ophiocordyceps sinensis* CO18]	FGSG_01401	1.47392
F-box domain-containing protein [*Beauveria bassiana* ARSEF 2860]	FGSG_11995	1.69746
F-box containing protein [*Fusarium langsethiae*]	FGSG_12026	1.73044
F-box domain protein [*Metarhizium anisopliae*]	FGSG_01700	2.09088
F-box domain-containing protein [*Arthroderma otae* CBS 113480]	FGSG_08770	2.91803
Group XI: osmotic related protein		
Osmotic growth protein 1 [*Fusarium oxysporum* f. sp. *cubense* race 1]	FGSG_09373	2.79515
Nik-1 protein (os-1p protein) [*Fusarium langsethiae*]	FGSG_02449	− 1.34133
Nik-1 protein (Os-1p protein) [*Fusarium fujikuroi*]	FGSG_08031	− 1.64029
MAP kinase kinase PBS2 [*Fusarium graminearum* PH-1]	FGSG_08691	1.54299
Related to plant PR-1 class of pathogen related proteins [*Fusarium fujikuroi* IMI 58289]	FGSG_03109	− 7.23949

U. maydis, the *MedA* mutants exhibited significantly reduced virulence as well. In our study, we also observed reduced virulence in *ΔFgMed1* mutant when compared to the wild-type. There are multiple prognoses for the impairment in virulence of *ΔFgMed1*. First, the deletion *FgMed1* led to a significant reduction in conidia production, a delay in conidial germination, and a slight inhibition in mycelium growth. Secondly, the production of DON in *ΔFgMed1* was dramatically reduced.

Interesting, our RNA-seq data showed that though the expression of DON and ZEA toxin-related gene FGSG_02068 (DAL81-TF) was down-regulated in *ΔFgMed1*, other toxin-related genes, such as FGSG_03881, FGSG_09570 (tox1), FGSG_10566 (toxin), FGSG_02398 (ZEB2), FGSG_01790, FGSG_04488, and FGSG_12121, were up-regulated. The aurofusarin biosynthetic gene cluster was identified in our RNA-seq data; 12 aurofusarin biosynthesis genes, *GIP1*, *GIP2*, *GIP3*, *GIP4*, *GIP5*, *PKS12*, *GIP6*, *GIP7*, *GIP8*, *GIP10*,

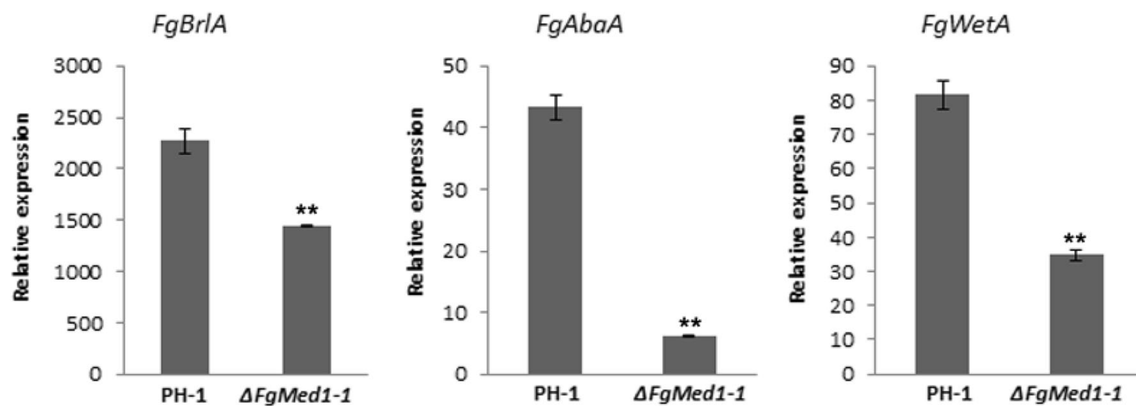

Fig. 5 Real-time PCR analyses of the central conidiation regulators: *brlA*, *abaA*, and *wetA*. The relative quantity of the transcripts of each gene was compared between the *ΔFgMed1* mutant and wild-type strain. The experiment was repeated three times with similar results and the representative data from one experiment was presented. The stable expressed β-tublin gene (FGSG_09530) was used to normalize all candidate genes. Error bars represent the standard deviation. Double asterisks indicated significant difference ($P = 0.01$)

GzORF1, and *GzMCT*, were found. Our RNA-seq results indicate that all these genes are up-regulated in *ΔFgMed1* except for *GIP10*, strongly suggesting that FgMed1 is a negative regulator of the aurofusarin biosynthesis gene cluster.

In this study, we mapped the DEGs in the chromosomes to have a better understanding of the overall gene expression profile. However, the results showed that the genes are distributed randomly except in chromosome 4, where we did not observe gene distribution at one terminal (Supplementary Fig S5). This is because the region of terminus of chromosome 4 represents the rDNA clusters. In conclusion, we demonstrated that the MedA ortholog FgMed1 in *F. graminearum* is involved in the sexual and asexual development, DON production, and virulence. Significantly, we learned that FgMed1 regulates the expression of a wide range of genes during early conidiogenesis. This functional characterization of FgMed1 expands our understanding of how conidiogenesis and toxin production are closely associated with virulence in *F. graminearum*.

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Compliance with ethical standards

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

Conflict of interest The authors declare that they have no conflict of interest.

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