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FpCzf14 is a putative C_2H_2 transcription factor regulating conidiation in *Fusarium pseudograminearum*

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Abstract

C_2H_2 zinc finger transcription factors such as FlbC and Msn2, have broad regulatory roles in fungal growth and conidiation. In the present study, we cloned and characterized a C_2H_2 zinc finger transcription factor gene, *FpCzf14*, in the wheat pathogen *Fusarium pseudograminearum*. FpCzf14 was localized to the nuclei. The expression of *FpCzf14* was significantly upregulated in conidia, suggesting that *FpCzf14* might contribute to conidiation. Further analysis of the *fpczf14*-deleted mutant (Δ*fpczf14*) demonstrated that it exhibited defect in conidiation, and this defect was restored in the complemented strain Δ*fpczf14*-C expressing *FpCzf14*, demonstrating that *FpCzf14* was essential for conidiation. Moreover, *FpCzf14* was required for mycelial growth and pathogenicity of *F. pseudograminearum*. Microscopic observation results showed that Δ*fpczf14* produced only very few penetration pegs and invasive hyphae inside host tissues compared with WT and Δ*fpczf14*-C. Additionally, results of reverse transcription quantitative PCR (RT-qPCR) showed that *FpCzf14* regulated expression of several conidiation-related genes in *F. pseudograminearum*. In conclusion, *FpCzf14*, as a core regulatory gene in conidiation, provides new insights into the mechanism of conidiation in *F. pseudograminearum*.

Keywords: *Fusarium pseudograminearum*, Transcription factors, FpCZF14, Conidiation, Pathogenicity

Background

Fusarium pseudograminearum, commonly found in soil and various decaying plant materials, is an important pathogen causing Fusarium crown rot (FCR) and Fusarium head blight (FHB) of wheat and barley (Kazan and Gardiner 2018; Zhou et al. 2019). Infection with *F. pseudograminearum* compromises grain yield and quality, and more importantly, *F. pseudograminearum* is a mycotoxin-producing species like some other *Fusarium* spp. that seriously threaten human health through the production of toxins (Tunali et al. 2012; Kazan and Gardiner 2018). In recent years, with the development and application of molecular biological techniques in plant pathogens, the functions of a few genes in *F. pseudograminearum* genome have been elucidated. For example, heat shock protein 70 (FpLhs1) is involved in protein secretion (Chen et al. 2019); Fdb3 and Tri5 function in mycotoxin production (Kettle et al. 2016; Powell et al. 2017); and *FpAda1* and *FpDep1* are necessary for growth and pathogenesis of *F. pseudograminearum* (Chen et al. 2020; Zhang et al. 2020). However, the functions of genes encoding C_2H_2 zinc finger (CZF) transcription factors (TFs) are unknown in *F. pseudograminearum*.

Zinc finger TFs are categorized into nine subfamilies on the basis of the number and position of the cysteine (Cys) and histidine (His) residues, including the Cys2/His2-type (C_2H_2), C_3H, C_3HC_4, C_2HC_5, C_4HC_3, C_2HC, C_4, C_6, and C_8 subfamilies (Klug 2010). Among them, C_2H_2, first identified as a DNA-binding motif in TFIIIA from *Xenopus laevis* in 1985, is considered the classical

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zinc finger (Brown et al. 1985). To date, many genes encoding for the CZF domain-containing proteins have been characterized from a wide variety of organisms, including animals, plants and fungi (Carrillo et al. 2017; Mackeh et al. 2018; Han et al. 2020). CZF TFs have been found to be involved in multiple cellular processes in plant pathogenic fungi. For example, among 47 CZF TFs that were deleted in *Magnaporthe oryzae* using gene knockout method, 44 were found to be required for fungal growth, development and pathogenicity (Cao et al. 2016). In *Fusarium graminearum*, 16 of 91 CZF TFs are involved in FHB (Son et al. 2011). *FolCZF1* that encodes a CZF TF in *F. oxysporum* f. sp. *lycopersici* is essential for conidiation, infection and fusaric acid production (Yun et al. 2019).

Over the whole life cycle, fungal pathogens of plants and animals often produce a diverse array of cell types ranging from hyphae to asexual and sexual reproductive structures in the external environment as well as inside host organisms. Asexual non-motile spores of higher fungi, called conidia, are the most common reproductive and infective cell types of many filamentous fungi. Asexual conidiation in the filamentous fungi *Aspergillus nidulans* and *Neurospora crassa* has been thoroughly investigated (Etxebeste et al. 2010; Park and Yu 2012; Ruger-Herreros and Corrochano 2020). The asexual reproductive cycle can be divided into two main phases: vegetative growth and development. When mycelia grow to a certain extent, the hyphae start to transform into aerial mycelium, thick-walled foot cells are formed, and multinuclear conidiophores develop at the tip or side of the aerial mycelia under external stimuli (Yu 2010). Over ten putative TFs have been identified to regulate conidiation in *A. nidulans* (Park and Yu 2012), such as CZF TF BrlA which acts in a core regulatory pathway of BrlA-AbaA-WetA (Boylan et al. 1987; Adams et al. 1988). Another putative CZF TF FlbC is an upstream activator of conidiation that directly binds to the cis-regulatory element of brlA to induce *brlA* expression (Kwon et al. 2010). Additionally, FlbC homologs in *M. oryzae* (FLB3) and *Fusarium verticillioides* (Ada1) also regulate asexual reproduction in fungi (Malapi-Wight et al. 2014; Matheis et al. 2017).

In this study, we identified and characterized a putative CZF TF-encoding gene, *FpCzf14*, which was significantly induced during conidiation of *F. pseudograminearum*. Furthermore, deletion of *FpCzf14* caused defect in growth, conidiation and pathogenicity of the pathogen, and led to low expression of many conidiation-related genes, suggesting that FpCzf14 is essential for conidiation of *F. pseudograminearum*.

Results

Identification and expression of *FpCzf14*

FpCzf14 has a full-length sequence of 1842 bp without introns (FPSE_11518), and encodes a protein of 613 amino acids with a molecular weight of 64.72 kDa and an isoelectric point of 7.56. The FpCzf14 protein contains three CZF domains that are highly conserved among different CZF TFs (Fig. 1a). Protein database searches revealed FpCzf14 homologues in different filamentous fungi. Phylogenetic analysis showed that FpCzf14 shares high identities in amino acid sequence with nine homologs from filamentous fungi. FpCzf14 was closest to GzC2H014 from *F. graminearum*, a closely-related species of *F. pseudograminearum*, with which it shares 98.86% similarity (Fig. 1a). RNA-seq analysis revealed that *FpCzf14* was detectable at both vegetative growth and infection stages of *F. pseudograminearum* (Fig. 1b). The relative expression level of *FpCzf14* was further validated by reverse transcription-quantitative PCR (RT-qPCR), and the transcript level of *FpCzf14* increased to a maximum in conidia, suggesting a potential role of this gene in conidiation of *F. pseudograminearum* (Fig. 1c).

Targeted deletion of *FpCzf14* and subcellular localization of FpCzf14

The construct used for gene deletion was generated based on the sequence of *FpCzf14* (Fig. 2a). By screening, we obtained five transformants which gave the specific amplicon with the primers H2F + H2R, F3 + H1R, H1F + R3 and gave no amplicon with G1 + G2, confirming successful deletion of the *FpCzf14* gene in these five transformants (Fig. 2b). *Δfpczf14*-T1 was further confirmed by Southern blot analysis (Fig. 2c). For functional complementation of FpCzf14 in the *FpCzf14*-deleted mutant and also subcellular location assay, the *FpCzf14-GFP* fusion construct driven by the *FpCzf14* promoter was introduced into the mutant *Δfpczf14*-T1. The complemented strain was named *Δfpczf14*-C. Microscopic observation of conidia, conidial germination and mycelia of *Δfpczf14*-C indicated that FpCzf14 was diffuse in the cytoplasm and concentrated in the nuclei, as revealed by DAPI staining results (Fig. 2d).

FpCzf14 is required for growth and conidiation of *F. pseudograminearum*

To determine the role of FpCzf14 in hyphal growth, the wild-type (WT), *Δfpczf14* and *Δfpczf14*-C strains were inoculated on PDA plates and assayed for mycelial growth and colony morphology. In contrast with WT and the complemented strain *Δfpczf14*-C, the *FpCzf14*-deleted mutant *Δfpczf14* exhibited a significantly reduced colony growth rate (Fig. 3a, b). Microscopic observation showed that the *Δfpczf14* mutant produced fewer and

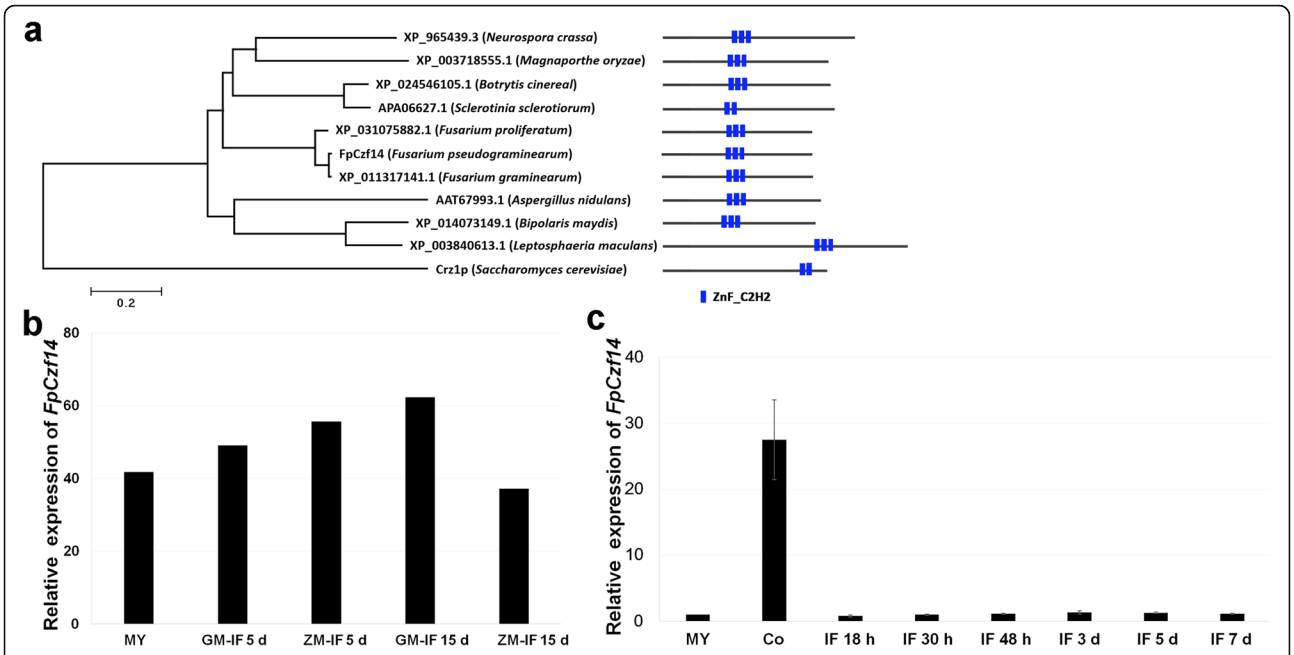

Fig. 1 Identification and characterization of FpCzf14 in *F. pseudograminearum*. **a** Phylogenetic analysis and CZF domain identification of FpCzf14 homologues in several organisms. **b** Transcriptional levels of *FpCzf14* in mycelia and at the infection stage of *F. pseudograminearum*. Raw transcriptome data were previously submitted to the NCBI database (Submission ID: SUB5545839) (Chen et al. 2019). My, mycelia; GM-IF 5 d and GM-IF 15 d indicate samples from susceptible wheat cultivar Guomai301 at 5 and 15 days post-inoculation (dpi) with *F. pseudograminearum*, respectively; ZM-IF 5 d and ZM-IF 15 d indicate samples from resistant wheat cultivar Zhoumai24 at 5 and 15 dpi with *F. pseudograminearum*, respectively. dpi. **c** Transcription profile of *FpCzf14* in mycelia, conidia, and infected wheat coleoptiles from 18 h post-inoculation (hpi) to 7 dpi analyzed by RT-qPCR. My, mycelia; Co, conidia; IF 18 h, 30 h, 48 h, 3 d, 5 d, and 7 d indicate samples from susceptible wheat cultivar Aikang58 at 18 hpi, 30 hpi, 48 hpi, 3 dpi, 5 dpi, and 7 dpi, respectively

curved hyphal branches (Fig. 3c), which indicated that FpCzf14 is involved in hyphal growth.

To estimate the effect of *FpCzf14* on conidiation of *F. pseudograminearum*, the conidial yields of the WT, *Δfpczf14* and *Δfpczf14*-C strains were individually quantified from 4-day-old cultures in CMC, CL and MB liquid at 25 °C, 150 rpm. The results showed that conidial production in the WT strain began at 20 h, and peaked at 4 d. However, *Δfpczf14* cultured in these media could not produce conidia until 10 d. The complemented strain *Δfpczf14*-C did not show any defects in conidiation (Fig. 4 and Additional file 1: Figure S1a). To exclude the possibility that the defect in conidiation in *Δfpczf14* was a consequence of a reduced growth rate, we inoculated WT, *Δfpczf14* and *Δfpczf14*-C on SNA, CLA and MA plates for conidia production. After 10 d, WT and *Δfpczf14*-C produced abundant normal conidia on all above media. After 17 days, when the colony size of *Δfpczf14* was the same as that of WT, we observed that *Δfpczf14* failed to produce any conidia on CLA and MA, and only very few small conidia (< 20 conidia in one plate) were observed on SNA (Fig. 4 and Additional file 1: Figure S1b). Cumulatively, this suggested that *FpCzf14* was essential in regulating conidia production in *F. pseudograminearum*.

FpCzf14 deletion reduces the pathogenicity of *F. pseudograminearum*

To investigate the involvement of FpCzf14 in virulence of *F. pseudograminearum*, mycelia agar plugs of the WT, *Δfpczf14* and *Δfpczf14*-C strains were inoculated onto wheat coleoptiles and barley leaves. After incubation for 24 h at 25 °C in the dark, mycelia agar plugs were removed from plants. After that, the plant samples were placed under the same conditions for 3 d to allow lesion expansion. The *Δfpczf14* strain caused very small disease lesions, whereas the lesions caused by the WT and complemented strains were obviously larger than those of *Δfpczf14* (Fig. 5a-c). Disease symptoms were further observed in a pot inoculation experiment. Most wheat seedlings inoculated by WT and *Δfpczf14*-C were obviously slow-growing at 10 dpi; in contrast, wheat seedlings inoculated with *Δfpczf14* mutant only showed mild symptoms (Fig. 5d). Upon infection with WT and *Δfpczf14*-C, the disease severity indexes (DSI) of wheat seedlings were 21.03 ± 1.2% and 21.74 ± 5.2%, respectively. In contrast, the DSI of wheat seedlings infected with *Δfpczf14* was only 2.62 ± 1.5% (Fig. 5e). We further examined infection structures of these *F. pseudograminearum* strains on wheat coleoptile

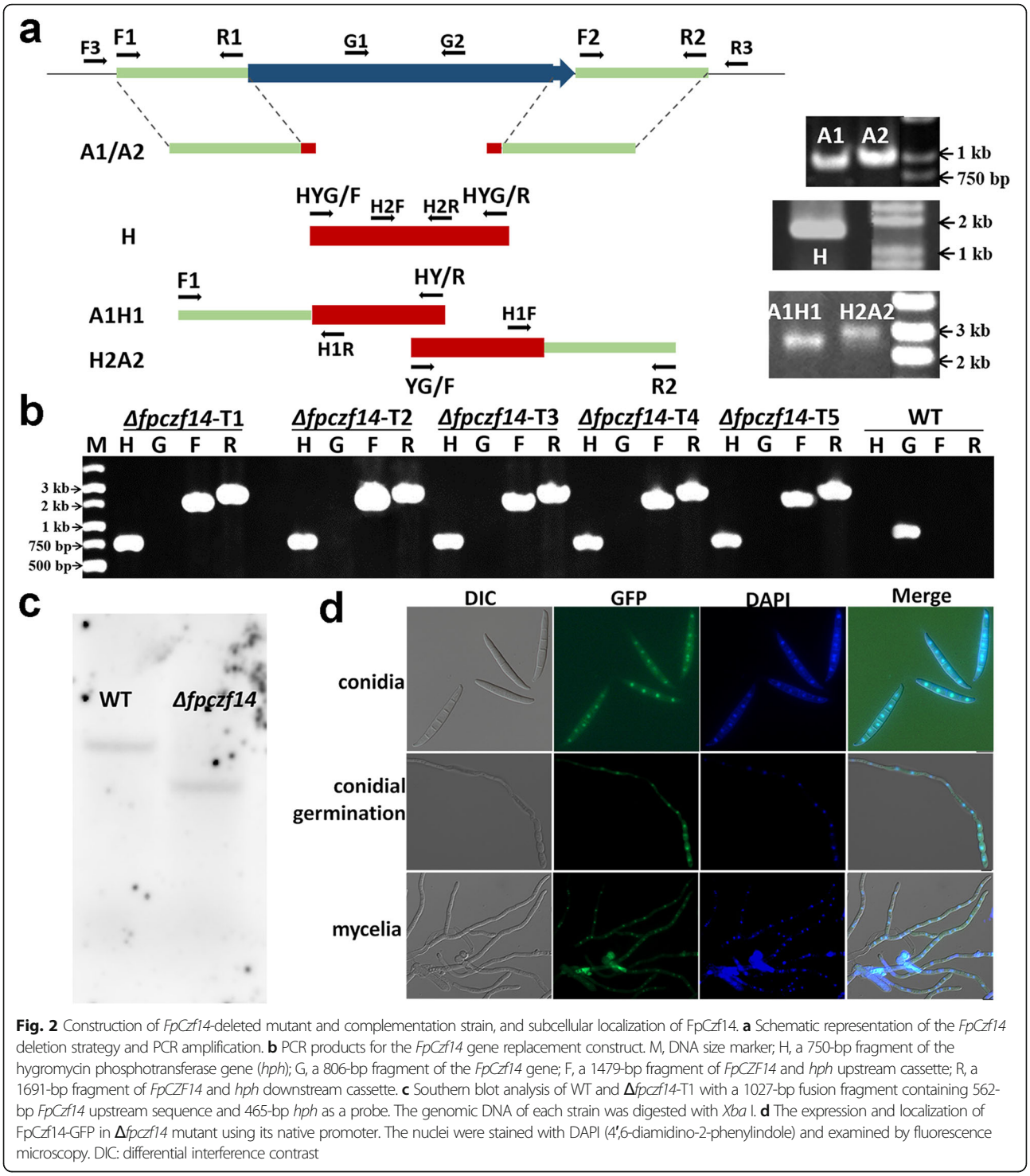

Fig. 2 Construction of *FpCzf14*-deleted mutant and complementation strain, and subcellular localization of FpCzf14. **a** Schematic representation of the *FpCzf14* deletion strategy and PCR amplification. **b** PCR products for the *FpCzf14* gene replacement construct. M, DNA size marker; H, a 750-bp fragment of the hygromycin phosphotransferase gene (*hph*); G, a 806-bp fragment of the *FpCzf14* gene; F, a 1479-bp fragment of *FpCZF14* and *hph* upstream cassette; R, a 1691-bp fragment of *FpCZF14* and *hph* downstream cassette. **c** Southern blot analysis of WT and Δ*fpczf14*-T1 with a 1027-bp fusion fragment containing 562-bp *FpCzf14* upstream sequence and 465-bp *hph* as a probe. The genomic DNA of each strain was digested with *Xba* I. **d** The expression and localization of FpCzf14-GFP in Δ*fpczf14* mutant using its native promoter. The nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole) and examined by fluorescence microscopy. DIC: differential interference contrast

epidermis. The *Δfpczf14* mutant produced very few penetration pegs and invasive hyphae inside wheat epidermis. By contrast, WT and *Δfpczf14*-C produced very high numbers of infectious hyphae (Fig. 5f). The results indicated that FpCzf14 was essential for the full virulence of *F. pseudograminearum*.

FpCzf14 regulates the expression of conidiation-related genes

Very few genes related to conidiation have been reported in *F. pseudograminearum*. In the present study, eight homologs of conidia-related genes of *A. nidulans* and *N. crassa* (Oiartzabal-Arano et al. 2016; Ruger-Herreros

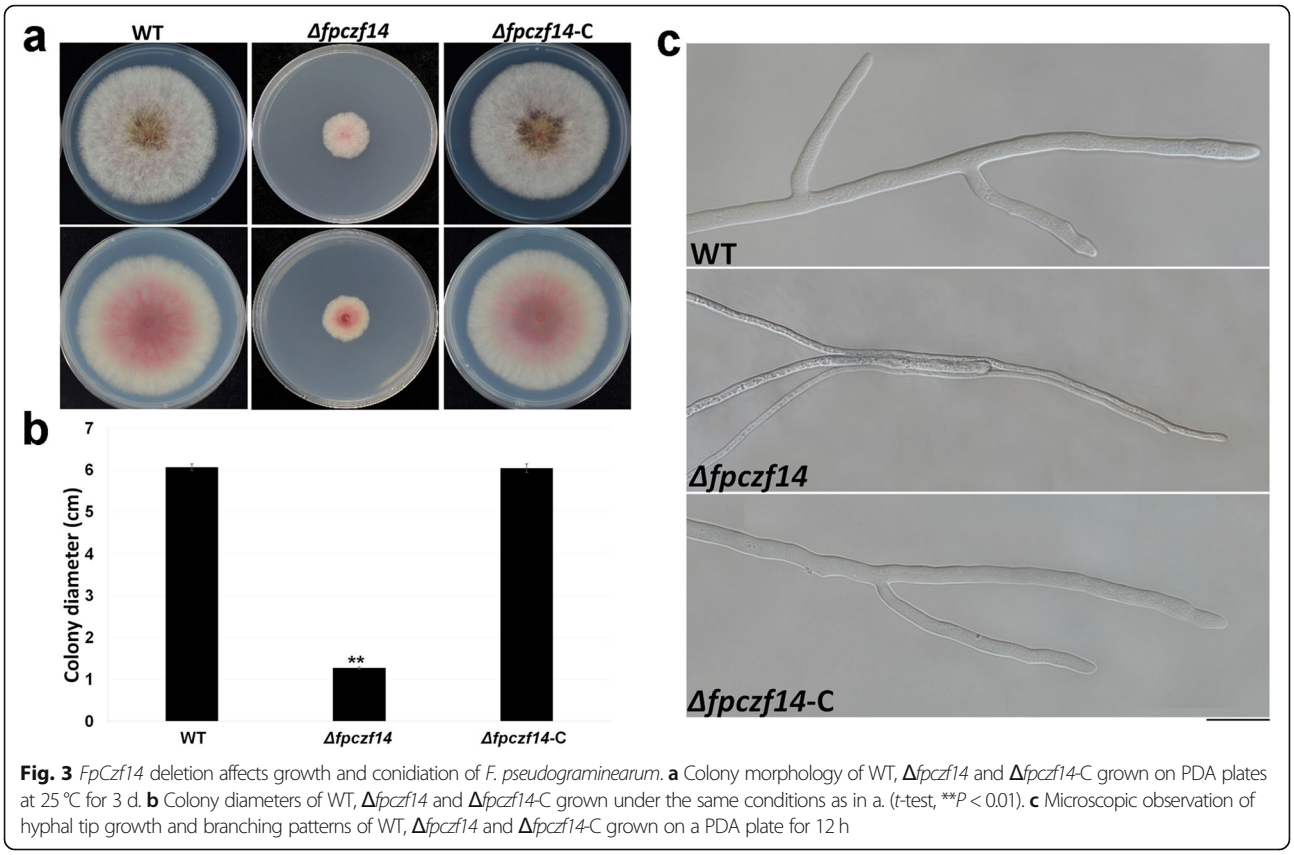

Fig. 3 *FpCzf14* deletion affects growth and conidiation of *F. pseudograminearum*. **a** Colony morphology of WT, Δ*fpczf14* and Δ*fpczf14*-C grown on PDA plates at 25 °C for 3 d. **b** Colony diameters of WT, Δ*fpczf14* and Δ*fpczf14*-C grown under the same conditions as in a. (*t*-test, $^{**}P < 0.01$). **c** Microscopic observation of hyphal tip growth and branching patterns of WT, Δ*fpczf14* and Δ*fpczf14*-C grown on a PDA plate for 12 h

and Corrochano 2020) were identified in *F. pseudograminearum* (Table 1). To determine if FpCzf14 regulates conidiation by controlling these conidia-related genes, we analyzed the expression levels of these genes in WT, *Δfpczf14* and *Δfpczf14*-C. The expression levels of six genes – *FPSE_11664* (*abaA*), *FPSE_02736* (*flbC*), *FPSE_01067* (*SteA*), *FPSE_04527* (*fluG*), *FPSE_11746* (*pdeB*) and *FPSE_01933* (*medA*) – were down-regulated in *Δfpczf14* compared with those in WT and *Δfpczf14*-C (Fig. 6). In contrast, the genes *FPSE_02660* (*wetA*) and *FPSE_02622* (*stuA*) showed similar expression profiles among WT, *Δfpczf14* and *Δfpczf14-C* (Fig. 6). The results indicated that FpCzf14 might play roles in conidiation by regulating the expression of these conidia-related genes.

Discussion

Wheat FCR caused by *F. pseudograminearum* has been spreading rapidly and has resulted in huge economic losses in the Huanghuai wheat-growing area of China since 2012 (Li et al. 2012; Zhou et al. 2019). However, only a few studies have evaluated the pathogenic mechanism of *F. pseudograminearum*. CZF TFs are conserved and have been demonstrated to play important roles in eukaryotes. In this study, we identified a CZF TF gene, *FpCzf14*, in *F. pseudograminearum* and explored its biological roles. FpCzf14 has three highly conserved CZF motifs, and its homologues are widely present in the fungal kingdom, implying that FpCzf14 may be essential for many fungal species to maintain their normal growth and development, as well as other functional activities. Transcriptome data combined with RT-qPCR results indicate that FpCzf14 is expressed stably in mycelia and during infection stages of *F. pseudograminearum*, but is significantly upregulated at the conidiation stage.

Of FpCzf14 homologues, MoNSDC from *M. oryzae* and GzC2H014 from *F. graminearum* have been functionally characterized (Son et al. 2011; Cao et al. 2016). A *MoNSDC*-deleted *M. oryzae* strain showed defects in growth and conidiation (Cao et al. 2016). GzC2H014 functioned in mycelial growth, sexual development, deoxynivalenol production and virulence, but was not involved in conidiation of *F. graminearum* (Son et al. 2011). Conidia production was normal in *GzC2H014*-deleted mutant of *F. graminearum*. FpCzf14 shares over 98% amino acid identity with GzC2H014 from *F. graminearum*. Deletion of *FpCzf14* not only inhibited growth and virulence of *F. pseudograminearum*, but also resulted in the developmental failure of conidia. This suggested that FpCzf14 might have distinct strategies in regulating fungal conidiation.

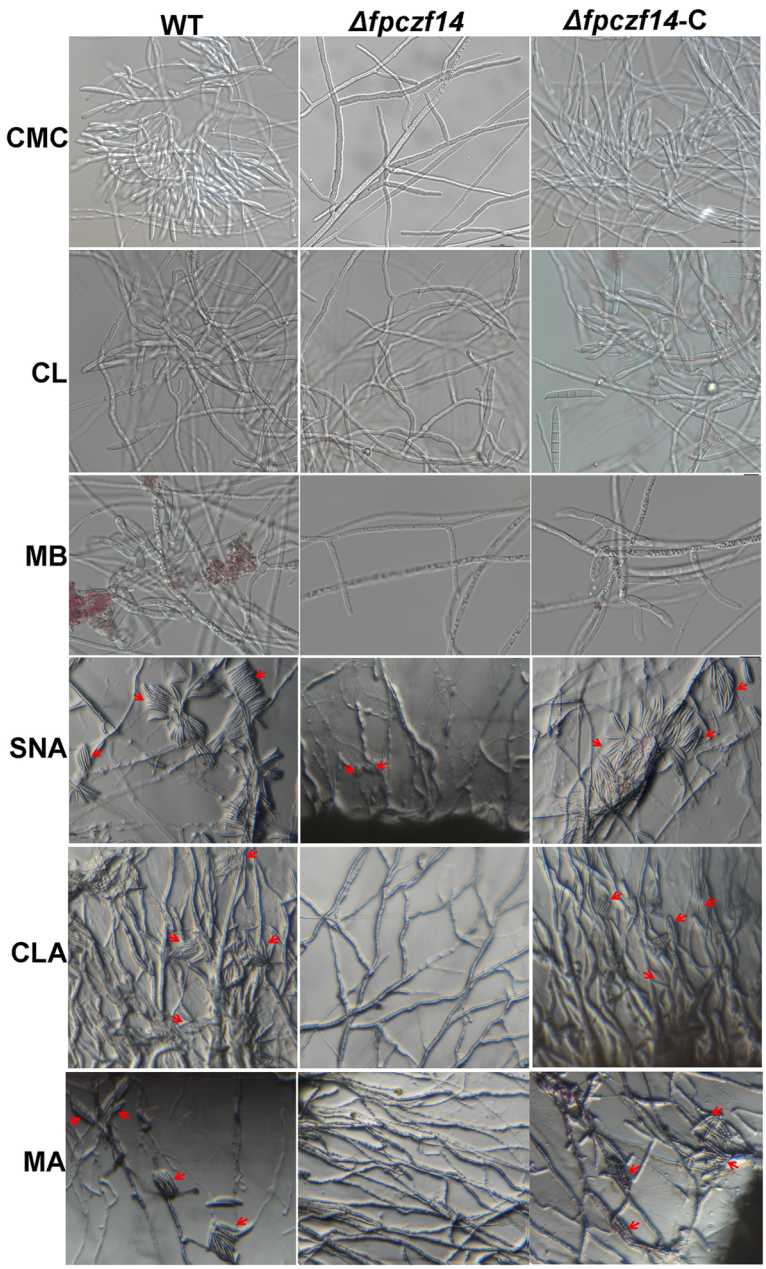

Fig. 4 Microscopic observation of conidia formation by WT, Δ*fpczf14* and Δ*fpczf14*-C in CMC, CL and MB liquid media (top three rows). Microscopic observation of conidia formation by WT, Δ*fpczf14* and Δ*fpczf14*-C on SNA, CLA and MA plates (bottom three rows). Red arrows point to conidia

Some CZF TFs from *Fusarium* species have been reported to be involved in conidiation. For instance, pcs1 positively regulates conidiation of *F. graminearum*. Deletion of *pcs1* resulted in a significant reduction in conidial production, and overexpression of *pcs1* increased conidial production (Jung et al. 2014); Ada1, a putative homologue of *A. nidulans* FlbC, regulated asexual reproduction in *F. verticillioides* (Malapi-Wight et al. 2014). Conidiation is well known to be strictly controlled by a central regulatory pathway of three TFs BrlA, AbaA, and WetA which act in concert with other genes, such as FLBs, as an integral part of the fungal life cycle (Park and Yu 2012). According to the RT-qPCR results, the expression levels of *FPSE_11664* (*abaA*), *FPSE_02736* (*flbC*), *FPSE_01067* (*SteA*), *FPSE_04527* (*fluG*), *FPSE_11746* (*pdeB*) and *FPSE_01933* (*medA*) were down-regulated in *FpCzf14*-deleted mutant of *F. pseudograminearum*, indicating that *FpCzf14* might affect conidiation by regulating these genes. Overall, FpCzf14 is an important putative transcription regulator, which is required for growth, conidial development and

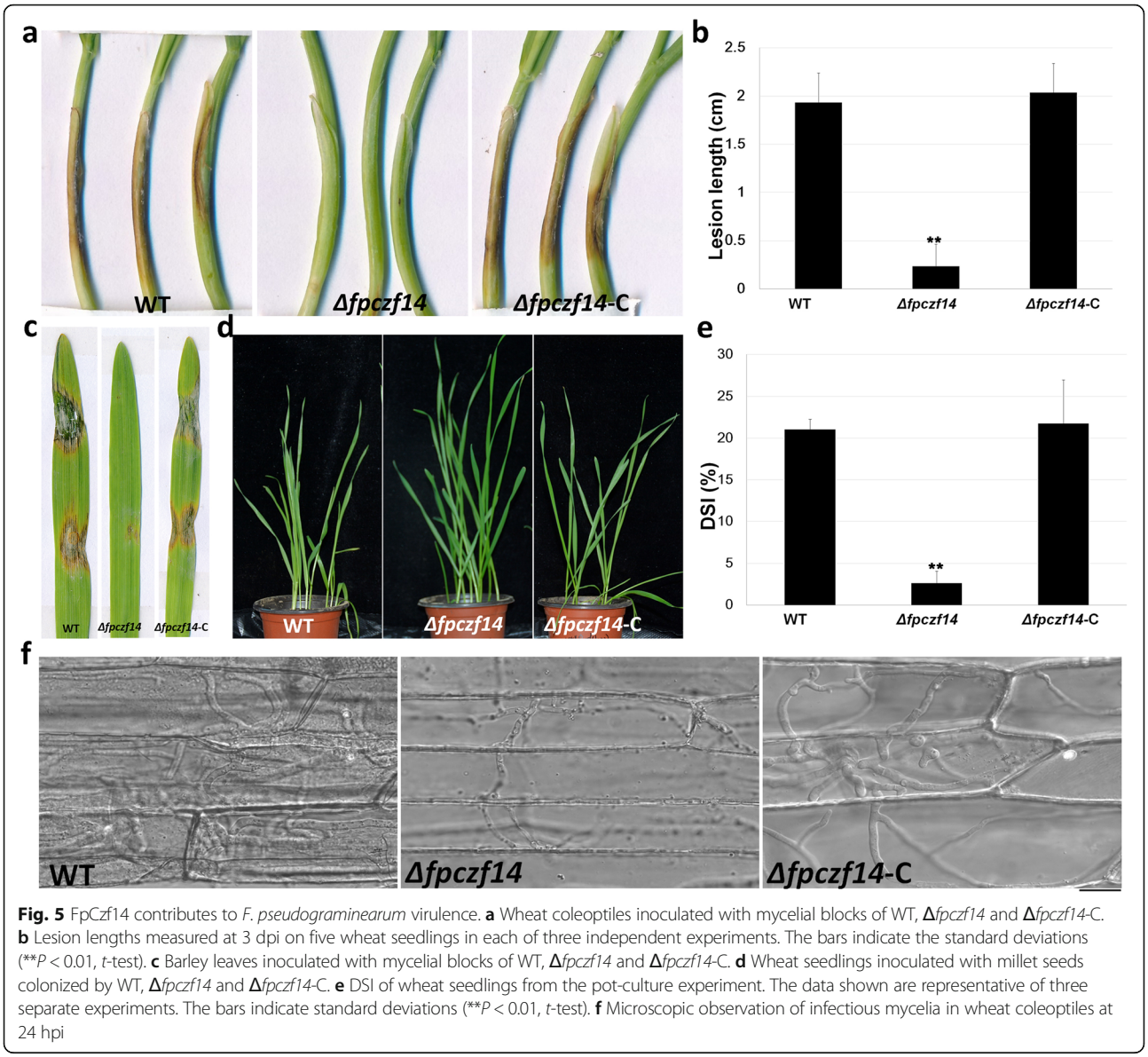

Fig. 5 FpCzf14 contributes to *F. pseudograminearum* virulence. **a** Wheat coleoptiles inoculated with mycelial blocks of WT, Δ*fpczf14* and Δ*fpczf14*-C. **b** Lesion lengths measured at 3 dpi on five wheat seedlings in each of three independent experiments. The bars indicate the standard deviations (**$P < 0.01$, *t*-test). **c** Barley leaves inoculated with mycelial blocks of WT, Δ*fpczf14* and Δ*fpczf14*-C. **d** Wheat seedlings inoculated with millet seeds colonized by WT, Δ*fpczf14* and Δ*fpczf14*-C. **e** DSI of wheat seedlings from the pot-culture experiment. The data shown are representative of three separate experiments. The bars indicate standard deviations (**$P < 0.01$, *t*-test). **f** Microscopic observation of infectious mycelia in wheat coleoptiles at 24 hpi

Table 1 Homologous genes related to conidiation among *Aspergillus nidulans*, *Neurospora crassa* and *Fusarium pseudograminearum*

Fusarium pseudograminearum	*Aspergillus nidulans*	*Neurospora crassa*
FPSE_02660	*wetA*	*NCU01033*
FPSE_11664	*abaA*	–
FPSE_02736	*flbC*	*flb-3*
FPSE_01067	*SteA*	*SteA*
FPSE_04527	*fluG*	*NCU04264*
FPSE_02622	*stuA*	*Asm-1*
FPSE_11746	*pdeB*	*acon-2*
FPSE_01933	*medA*	*acon-3*

'-' indicates no homologous gene

virulence in *F. pseudograminearum*. Further studies will reveal its regulatory network, illuminating the related regulatory mechanism.

Conclusions

We identified a CZF TF FpCzf14 from *F. pseudograminearum* in this study. The expression of *FpCzf14* was induced in conidia, and the *FpCzf14*-deleted *Δfpczf14* strain exhibited a significant decrease in growth, conidiation and pathogenicity. RT-qPCR analysis further revealed that FpCzf14 had specific effects on other known conidiation regulatory genes. Taken together, the results indicate that FpCzf14 regulates conidiation in *F. pseudograminearum*. This study provides new insights into the mechanisms underlying *F. pseudograminearum* development and virulence.

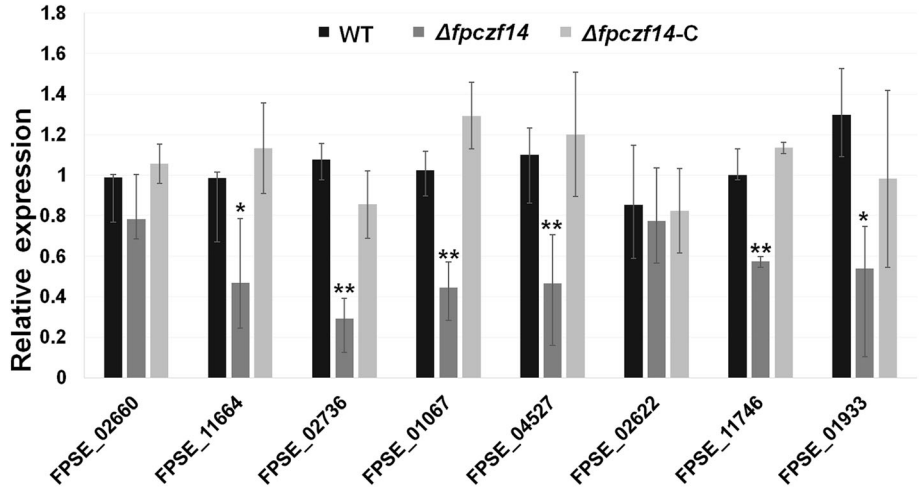

Fig. 6 RT-qPCR analyses of eight conidiation-related genes in WT, Δ*fpczf14* and Δ*fpczf14*-C (*t*-test, *$P < 0.05$, *$P < 0.01$)

Methods

Strains and culture conditions

The WT strain of *F. pseudograminearum* used for transformation and phenotypic analyses was Wz2-8A, which was isolated in Wuzhi, Henan Province of China (Zhou et al. 2019). Both WT and genetically engineered strains in this study were cultured on potato dextrose agar (PDA) medium, containing 200 g/L potato, 20 g/L glucose and 20 g/L agar. For long-term storage, mycelial or conidial cultures were stored as 30% glycerol stocks at − 80 °C.

Bioinformatics

FpCzf14 was first identified from the *F. pseudograminearum* CS3096 database (Gardiner et al. 2018) and then cloned from *F. pseudograminearum* strain Wz2-8A for further analysis. *FpCzf14* homologs in other fungi were gathered by protein BLAST algorithm at the database of the National Centre for Biotechnology (NCBI). The C_2H_2 domains were predicted using the SMART program. The phylogenetic tree of FpCzf14 with nine homologs was generated using MEGA 5 by the neighbor-joining method with 1000 replicates for bootstrap analysis (Tamura et al. 2011). Crz1p, a C_2H_2 zinc finger protein from the unicellular fungus *Saccharomyces cerevisiae*, was used as an out-group.

RNA isolation and RT-qPCR

Total RNA was extracted from lyophilized mycelia, conidia and infected plants using EASYspin plant RNA Kit (Aidlab, China), following the manufacturer's instructions. Digestion of contaminating DNA and reverse transcription of total RNA were performed using a PrimeScript™RT reagent Kit with gDNA Eraser catalog (TaKaRa, Dalian, China). Real-time quantitative PCR (qPCR) was performed with the Applied Biosystems 7000 Real-Time PCR system using SYBR green dye for fluorescence detection. The primer pairs used for qPCR are listed in Additional file 2: Table S1. The expression level for each gene was normalized to that of the *F. pseudograminearum TEF1* gene and the relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method.

Generation of deletion mutants

The *FpCzf14* gene replacement constructs were generated by the split-marker approach (Catlett et al. 2003). Primers were listed in Additional file 2: Table S1 and a schematic diagram of the primers used for generating deletion mutant and PCR amplification was shown in Fig. 2a. *FpCzf14* upstream and downstream flanking fragments (~ 1 kb) were amplified by PCR and ligated to the hygromycin phosphotransferase (*hph*) cassette by overlapping PCR and then transformed into protoplasts of Wz2-8A by polyethylene glycol (PEG)-mediated protoplast fungal transformation (Liu and Friesen 2012). Correct transformants were verified by PCR and Southern blotting. Southern blot analysis was performed using the DIG High Prime DNA Labeling and Detection Starter kit I according to the manufacturer's instructions (Roche Diagnostics, Mannheim, Germany). Hybridization was performed with a DIG-labelled probe specific for the upstream sequences of the *FpCzf14* and *hph* gene amplified using the probe primer pairs (Additional file 2: Table S1).

FpCzf14 complementation and subcellular localization

To further confirm that differences in phenotype between the mutant (*Δfpczf14*) and the WT strain (Wz2-8A) were due to the loss of *FpCzf14*, the genomic sequence containing the full-length *FpCzf14* gene without stop codon together with its 1531-bp promoter region,

was amplified from the WT genome via PCR, and ligated into the pKNTG vector, and introduced into *Δfpczf14* by PEG-mediated transformation. The complementation strains were verified by PCR and green fluorescence assays. Conidia, germinated conidia and mycelia were collected and stained by DAPI for observation of the subcellular localization of FpCzf14 using a Nikon Ti-s instrument.

Growth, conidiation and pathogenicity assays

Mycelial growth and colony morphology of WT, *Δfpczf14* and complemented transformant (*Δfpczf14*-C) were measured when these strains were incubated on PDA plates at 25 °C for 24 h and 3 d. Conidiation was measured by counting the number of conidia produced respectively in carboxymethyl cellulose (CMC) liquid, carnation leaf (CL) liquid and mung bean (MB) liquid media at 25 °C for 4 d with shaking at 150 rpm (Chen et al. 2016; Chen et al. 2019). Conidia were imaged and counted using a Nikon Ti-s instrument. Furthermore, the formation of conidia was also evaluated on spezieller nährstoffarmer agar (SNA), carnation leaf agar (CLA), and maltose agar (MA) plates. The amount of conidia in WT and *Δfpczf14*-C strains was measured after a 10-day incubation at 25 °C in darkness, and it was measured in *Δfpczf14* after a 17-day incubation under the same condition (Inoue et al. 2002; Droce et al. 2017; Lu et al. 2019). Conidia were imaged using a Nikon SMZ25 stereomicroscopy.

The pathogenicity experiments were performed on 3-day-old wheat coleoptiles and 10-day-old barley leaves. The lengths or diameters of disease lesions were recorded at 3 dpi, and epidermal cells were viewed under a Nikon Ti-s instrument. Infection assay by pot-culture experiment was conducted with 0.5% inoculation millet in sterile soil using pre-geminated wheat seed under high relative humidity conditions at 25 °C with a photoperiod of 16 h/8 h (light/dark cycle). Wheat growth was photographed at 10 d. The disease severity index (DSI) was assessed on each plant within each pot using a 0 to 7 rating scale (Smiley et al. 2005). DSI (%) = [Σ (class value × the number of plants in each class value)/(total number of plants × the highest-class value)] × 100.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s42483-020-00074-7.

Additional file 1: Figure S1. Conidiation quantification and colonies morphology. **a** Number of conidia produced by WT, Δ*fpczf14* and Δ*fpczf14*-C in CMC, CL and MB. Data shown are representative of three separate experiments. The bars indicate standard error (**$P < 0.01$, *t*-test). **b** Colonies of WT, Δ*fpczf14* and Δ*fpczf14*-C on SNA, CLA and MA plates.

Additional file 2: Table S1. Primers used in the study.

Abbreviations

CL: Carnation leaf; CLA: Carnation leaf agar; CMC: Carboxymethyl cellulose; CZF: C_2H_2 zinc finger; DAPI: 4′,6-diamidino-2-phenylindole; dpi: Days post-inoculation; DSI: Disease severity index; GFP: Green fluorescent protein; hpi: Hours post-inoculation; MA: Maltose agar; MB: Mung bean; RT-qPCR: Reverse transcription quantitative PCR; PCR: Polymerase chain reaction; PDA: Potato dextrose agar; PEG: Polyethylene glycol; SNA: Spezieller nährstoffarmer agar; TFs: Transcription factors

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Authors' contributions

LC and HL conceived and designed the research. LC, JZ, HX, YM, YL and MP performed the experiments and analyzed the data. XX provided assistance for fungal infection. BS contributed constructions. LC and HL wrote the paper. YS revised the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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