New Phytologist Supporting Information

Methods S1

Article title: Plant defense compound triggers mycotoxin synthesis by regulating H2B ub1 and H3K4 me2/3 deposition

Authors: Tianling Ma, Lixin Zhang, Minhui Wang, Yiqing Li, Yunqing Jian, Liang Wu, H. Corby Kistler, Zhonghua Ma and Yanni Yin

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Determination of stress sensitivity, virulence and DON production

To determine sensitivity to various stresses, 5-mm mycelial plugs of each strain taken from a 3-day-old colony edge were inoculated on PDA supplemented without/with each stress agent, then incubated at 25°C for 3 days in the dark. The concentrations for each compound were indicated in figure legends. Each experiment was repeated three times independently.

For evaluating virulence on wheat spikelets, a 10-µl aliquot of conidial suspension (10^5 conidia/ml) was injected into a floret in the central section spikelet of single flowering wheat heads of susceptible wheat (Triticum aestivum) cultivar Jimai22. There were 20 replicates for each strain. At 15 d after inoculation, infected spikelets in each inoculated wheat head were recorded. For assessing virulence on wheat coleoptiles, the 2- to 3-mm top of the three-day-old coleoptiles was removed and the wounding top was directly inoculated with a 5-mm-diameter fresh mycelial plug. After inoculation, the seedlings were grown in a growth chamber at 25°C and 95% humidity for later lesion size measurements. Ten replicates were used for each strain. Each virulence experiment was repeated four times.

To determine DON production, vegetative hyphae of each strain were collected from a 24 h yeast extract peptone dextrose (YEPD) culture then inoculated to TBI liquid medium (Gardiner et al., 2009) using putrescine, spermidine, or glutamine (negative control) as nitrogen source at 28°C for 7 days in a shaker (150 rpm) in the dark. To detect carbon source effect on DON production, sucrose or glucose (negative control) were used as the carbon source in TBI with glutamine. Then the DON
production for each sample was extracted and quantified by using a DON Quantification Kit Wis008 (Wise Science, Zhenjiang, China). The dry weight of mycelium was used as an internal reference. To determine DON production during *F. graminearum* infection on wheat coleoptiles, infected wheat coleoptiles tissue was harvested 7 dpi, frozen in liquid nitrogen and stored at -80°C. The frozen samples were then ground to fine powder in a mortar using a pestle in the presence of liquid nitrogen. One gram of each sample was then re-suspended in 2 ml of water and then thoroughly mixed using a vortex shaker for 30 s. The mixture was then incubated for 30 min at 30°C in a water bath. All solids were removed by centrifugation and the supernatants were analyzed for DON content using a DON Quantification Kit Wis008 (Wise Science, Zhenjiang, China).

**Mutant generation and complementation, Flag- and GFP-fusion cassettes construction and in situ heterologous complementation of the BRE1**

Constructs for gene deletion and complementation of *F. graminearum* using a PEG-mediated protoplast transformation method were carried out as described previously (Yun *et al.*, 2013). The primers used to amplify the flanking sequences for each gene are listed in Table S1. Putative gene deletion mutants were identified by PCR assays, and the *FgRAD6, FgBRE1, FgDOT1* deletion mutants were further confirmed by a Southern blot assay. Constructions of Flag- and GFP- fusion cassettes using the double-joint PCR method were performed according to a previous study (Yun *et al.*, 2013). For in situ complementation of the heterologous *BRE1*, the
GFP-tag coding sequence, a gene replacement cassette carrying flanking sequences of *FgBRE1*, the geneticin (NEO) resistance gene and the coding sequence of *F. verticillioides FvBRE1*, or *Botrytis cinerea BcBRE1* or *Drosophila melanogaster DmBRE1* was constructed by double-joint PCR as described previously (Yun *et al.*, 2013). This cassette was used to transform protoplasts of ΔFgBre1 to generate heterologous Bre1 complementation strains ΔFgBre1-C<sup>Fv</sup>, ΔFgBre1-C<sup>Bc</sup> and ΔFgBre1-C<sup>Dm</sup>. Then western blot analyses were carried out to examine the expression of heterologous Bre1 proteins.

**Quantitative real-time polymerase chain reaction (qRT-PCR) analyses**

To determine the transcription of plant polyamine biosynthases, RNA was extracted from the samples collected as described in plant polyamine quantification assays, by using TaKaRa RNAiso Reagent (TaKaRa Biotechnology Co., Dalian, China). Each RNA sample was reversely transcribed with a HiScript II Q RT Kit (Vazyme, R223-01, Nanjing, China). RT-qPCR was carried out with primer sequences listed in Table S1 and *TaACTIN* gene was used as the internal control for normalization. The transcription of wheat polyamine biosynthases in non-infected heads at the corresponding time point was set to 1. Each experiment was repeated three times.

To detect the transcription of *F. graminearum* putrescine synthetase *FgODC*, RNA was extracted from hyphae cultured in YEPD, and hyphae during infection as described in plant polyamine quantification assays. *FgACTIN* gene expression was performed as a reference. The *FgODC* expression in vegetative hyphae was set to 1.
Each experiment was repeated three times.

To examine the transcription of DON biosynthesis genes ($FgTRIs$), RNA was extracted from the samples collected as described in DON production assays. $FgACTIN$ gene was used as the internal control for normalization and each experiment was repeated three times.

**Western blotting assays**

*F. graminearum* protein extraction was performed as described previously (Liu et al., 2015). The resulting proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to Immobilon-P transfer membrane (Millipore, Billerica, MA, USA). GFP- and Flag-tagged proteins were detected with monoclonal anti-GFP (ab32146, Abcam, Cambridge, UK) and anti-Flag (A9044, Sigma) antibodies, respectively. H2B ub1, H3K4me1, -me2 and -me3 levels were detected with the antibodies Cell Signalling 5546, ab8895, ab7766 and ab8580 (Abcam, Cambridge, MA, USA) respectively. H3 level was detected by an anti-H3 antibody (Huabio M1306-4) for the protein loading reference. Each experiment was repeated three times.

**Affinity capture-mass spectrometry analysis**

$FgAReA$ was tagged with GFP at its N-terminus and transferred into the $FgAREA$ deletion mutant, and the resulting transformant was used for protein extraction. After protein extraction, the supernatant was transferred into a sterilized tube. An aliquot of
25 μl of GFP-trap agarose beads (ChromoTek, Martinsried, Germany) was added to capture GFP-FgAreA interacting proteins, following the manufacturer’s instructions. After incubation at 4°C overnight, the agarose beads were washed three times with 1000 μl of TBS (20 mM Tris-HCl, 500 mM NaCl, pH7.5). Proteins binding to the beads were then boiled with 50 μl TBS supplemented with 10 μl 10% SDS. After centrifugation at 5000 g for 5 min at 4°C, the supernatant was digested with trypsin and tryptic peptides were analyzed by Shanghai Applied protein technology Co., Ltd. using mass spectrometry (Ding et al., 2010; Yin et al., 2018). False discovery rate (FDR) was used to determine relative protein confidence. The peptide-to-spectrum matches (PSMs) were quantified to determine relative protein abundance (Kruger et al., 2019). Candidate interacting proteins were filtered based upon high confidence (≤1% FDR) and reproducible presence across samples (PSM>5).

**Electrophoretic mobility shift assay (EMSA)**

The cDNA encoding full-length FgBre1 or FgBre1 lacking the bZIP domain were amplified and cloned into pGEX-4T-3 vector to generate GST-tagged protein. The resulting construct was transformed into the *Escherichia coli* strain BL21 (DE3) after verifying the cDNA sequence. The recombinant GST-FgBre1 and GST-Bre1^ΔbZIP^ were purified using GST agarose (Thermo Fisher Scientific, Waltham, USA). The GST protein was used as negative control. The promoter sequence of *FgTRI1* and *FgTRI5* was amplified using the relevant primers listed in Table S1. EMSA was performed as described previously (Wang et al., 2019). Briefly, reaction mixtures containing
purified proteins, promoter DNAs and 10×Binding buffer (100 mM Tris-HCl (PH 7.5), 0.5M NaCl, 10 mM DTT, 10 mM EDTA, 50% glycerol) were incubated for 20 min at 25°C. The reactions were electrophoresed on 1.2% agarose gel in 0.5×TAE for 45 min in 80 V under low temperature. Signals were detected by a J3-3000 imaging system after dying DNA with ethidium bromide (EB) for 15 min. The experiment was conducted independently three times.

**Yeast two-hybrid (Y2H) assays**

To construct plasmids for Y2H analysis, the coding sequence of each gene was amplified from cDNA of the wild type with corresponding primer pairs (Table S1). The cDNA fragments were cloned into the yeast GAL4-binding domain vector pGBKT7 and GAL4-activation domain vector pGADT7 (Clontech, Mountain View, CA, USA), respectively. Pairs of Y2H plasmids were co-transformed into *S. cerevisiae* strain Y2H Gold following the lithium acetate/single-stranded DNA/polyethylene glycol transformation protocol. The plasmid pair pGBKT7-53 and pGADT7-T was used as a positive control. The plasmid pair pGBKT7-Lam and pGADT7-T was used as a negative control. Transformants were grown at 30°C for 3 days on synthetic medium (SD) lacking Leu and Trp, and then serial dilutions of yeast cells (cells/ml) were transferred to SD without His, Leu and Trp to assess protein-protein interaction. Three independent experiments were performed to confirm each Y2H result.

**References**

Ding SL, Liu W, Iliuk A, Ribot C, Vallet J, Tao A, Wang Y, Lebrun MH, Xu JR. 2010. The tig1 histone deacetylase complex regulates infectious growth in the rice blast fungus *Magnaporthe oryzae*. *The Plant Cell* **22**: 2495-2508.
Gardiner DM, Kazan K, Manners JM. 2009. Nutrient profiling reveals potent inducers of trichothecene biosynthesis in *Fusarium graminearum*. *Fungal genetics and biology* 46: 604-613.

Kruger AN, Brogley MA, Huizinga JL, Kidd JM, de Rooij DG, Hu Y, Mueller JL. 2019. A neo-functionalized X-Linked ampliconic gene family is essential for male fertility and equal sex ratio in mice. *Current Biology* 29: 3699.

Liu Y, Liu N, Yin Y, Chen Y, Jiang J, Ma Z. 2015. Histone H3K4 methylation regulates hyphal growth, secondary metabolism and multiple stress responses in *Fusarium graminearum*. *Environmental Microbiology* 17: 4615-4630.

Wang Z, Ma T, Huang Y, Wang J, Chen Y, Kistler HC, Ma Z, Yin Y. 2019. A fungal ABC transporter FgAtm1 regulates iron homeostasis via the transcription factor cascade FgAreA-HapX. *PLoS Pathogens* 15: e10077919.

Yun Y, Liu Z, Zhang J, Shim WB, Ma Z. 2013. The MAPKK FgMkk1 of *Fusarium graminearum* regulates vegetative differentiation, multiple stress response, and virulence via the cell wall integrity and high-osmolarity glycerol signaling pathways. *Environmental Microbiology* 16: 2023-2037.

Yin Y, Wang Z, Cheng D, Chen X, Chen Y, Ma Z. 2018. The ATP-binding protein FgArb1 is essential for penetration, infectious and normal growth of *Fusarium graminearum*. *New Phytologist* 219: 1447-1466.