RNA editing of the *AMD1* gene is important for ascus maturation and ascospore discharge in *Fusarium graminearum*

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Ascospores are the primary inoculum in the wheat scab fungus *Fusarium graminearum* that was recently shown to have sexual stage-specific A-to-I RNA editing. One of the genes with premature-stop-codons requiring A-to-I editing to encode full-length functional proteins is *AMD1* that encodes a protein with a major facilitator superfamily (MFS) domain. Here, we characterized the functions of *AMD1* and its UAG-to-UGG editing event. The *amd1* deletion mutant was normal in growth and conidiation but defective in ascospore discharge due to the premature breakdown of its ascus wall in older perithecia, which is consistent with the specific expression of *AMD1* at later stages of sexual development. Expression of the wild-type or edited allele of *AMD1* but not un-editable allele rescued the defects of *amd1* in ascospore discharge. Furthermore, Amd1-GFP localized to the ascus membrane and Amd1 orthologs are only present in ascocarp-forming fungi that physically discharge ascospores. Interestingly, deletion of *AMD1* results in the up-regulation of a number of genes related to transporter activity and membrane functions. Overall, these results indicated that Amd1 may play a critical role in maintaining ascus wall integrity during ascus maturation, and A-to-I editing of its transcripts is important for ascospore discharge in *F. graminearum*.

*Fusarium graminearum* is one of the causal agents of Fusarium Head Blight (FHB) or scab, a destructive disease of wheat and barley worldwide. Besides causing severe yield losses, the pathogen often contaminates infested grains with deoxynivalenol (DON), zearalenone, and other mycotoxins. *F. graminearum* overwinters on plant debris and discharges ascospores into the air in the spring to infect flowering wheat or barley heads. Unlike many other plant pathogenic fungi, sexual reproduction plays a critical role in the infection cycle of *F. graminearum* because ascospores are the primary inoculum of FHB. Under field conditions, conidia produced on diseased plant tissues are mainly for spreading infection to vegetative tissues of host plants because of the flowering time of wheat heads.

*F. graminearum* is a homothallic ascomycete and a tractable genetic system for studying sexual development because of its high homologous recombination frequency and fertility. In the past decade, numerous genes important for sexual reproduction have been identified, including a number of protein kinase, phosphatase, and transcription factor genes and other genes with diverse functions. Whereas many of these genes also are important for vegetative growth and asexual reproduction, some have specific functions during sexual reproduction in *F. graminearum*, such as *GAE1* and *PUK1* that have no other defects but ascospore release or morphology. Interestingly, for the two paralogs of CDK kinase Cdc2 and beta-tubulin, whereas they have overlapping function in vegetative growth, only Cdc2A and Tub1 are important for ascus and ascospore development, suggesting differences in cell cycle regulation and microtubule cytoskeleton between vegetative hyphae and ascogenous tissues in *F. graminearum*.

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Recently, A-to-I RNA editing was found to specifically occur during sexual reproduction in *F. graminearum*. In animals, A-to-I editing catalyzed by the adenosine deaminase acting on RNA (ADAR) enzymes is the most prevalent type of RNA editing. Although plants and fungi lack ADAR orthologs, more than 26,000 A-to-I editing sites were identified in *F. graminearum*, and majority of them occurred in the coding regions and caused amino acid changes. The *PUK1* protein kinase gene known to be important for ascospore development and release had two tandem premature stop codons UAG UAG in its open reading frame (ORF) that were edited to UGG UGG during sexual reproduction to encode full-length proteins. Additional 69 genes with premature stop codons in their ORFs that had *PUK1*-like editing events in perithecia were identified in *F. graminearum*, suggesting the importance of RNA editing during sexual reproduction.

FGRRES_10094 (=FGSG_10094 of the previous annotation by the Broad Institute) was one of the five hypothetical genes with *PUK1*-like editing events that were selected for preliminary analysis for their roles in sexual reproduction. In this study, we further characterized the functions of FGRRES_10094 (named *AMD1* for ascus maturation and ascospore discharge) and its UAG to UGG editing event in ascospore development and release. The *amd1* deletion mutant was defective in ascospore discharge, likely due to the premature breakdown of its ascus wall. In addition to stage-specific editing, *AMD1* was specifically expressed at late stages of sexual development and its orthologs are only present in ascocarp-forming fungi. Expression of different mutant alleles of *AMD1* confirmed the importance of RNA editing. Furthermore, Amd1-GFP localized to the ascus membrane and deletion of *AMD1* results in the up-regulation of a number of genes related to transporter activity and membrane functions. Overall, our results indicated that *AMD1* may play a critical role in maintaining ascus wall integrity and A-to-I editing of its transcripts is important for ascospore discharge and auto-inhibition of ascospore germination in *F. graminearum*.

**Results**

**AMD1 encodes a protein unique to ascocarp-forming ascomycetes.** The ORF of FGRRES_10094 (named *AMD1* for ascus maturation and ascospore discharge) was predicted to contain one intron towards its 5'-end. However, our RNA-seq data showed that this intron was incorrectly predicted but the stop codon UAG (631–633) within it was changed to UGG by RNA editing in 97.6% of the transcripts in perithecia harvested at 8 days post-fertilization (dpf) (Fig. 1A). The actual *AMD1* ORF encodes a 1386 amino acid protein that contains a well-conserved major facilitator superfamily (MFS) domain and 11 transmembrane helixes (TM) (Fig. 1B). Interestingly, Amd1 appears to be a protein unique to ascocarp-forming ascomycetes because it lacks a distinct ortholog in the budding and fission yeasts and other Taphrinomycotina and Saccharomycotina species (Fig. 1C; Fig. S1). Amd1 orthologs are well conserved in Sordariomycetes, Dothideomycetes, and Leotiomycetes but not in Eurotiomycetes except Chaetothyriomycetidae species (Fig. 1C; Fig. S1). The distribution of Amd1 orthologs suggests that it may be functionally related to physical discharge of ascospores from asci and ascocarps (Fig. 1C).

**The expression of AMD1 is specific to late stages of sexual development.** Unlike in perithecia, *AMD1* transcripts were rare in RNA-seq data of hyphae and conidia, suggesting that *AMD1* was almost specifically expressed in perithecia. To verify this result, we assayed *AMD1* expression in PH-1 by qRT-PCR with RNA isolated from 12 h YEPD cultures and 8 dpf perithecia. Consistent with RNA-seq data, *AMD1* transcription was barely detectable in vegetative hyphae but its expression increased over 1,000 folds in perithecia (Fig. 2A). In RNA-seq generated with RNA isolated from mating cultures sampled at 1 and 2 dpf and perithecia collected 3–8 dpf (accession no. PRJNA384311), *AMD1* expression was barely detectable at early stages but began to increase at 5 dpf (Fig. 2B). The abundance of *AMD1* transcripts kept increasing from 6, 7, and 8 dpf (Fig. 2B). In comparison with 3 dpf young perithecia, the expression level of *AMD1* was up-regulated over 250 folds at 8 dpf. The timing of un-regulated expression of *AMD1* correlates with the ascus and ascospore development in perithecia.

**Ascospore discharge is blocked in the amd1 mutant.** The *AMD1* gene replacement construct was generated and transformed into the wild-type strain PH-1 in a previous study. The *amd1* mutant (Table 1) was normal in vegetative growth and conidiation. In comparison with PH-1, it had no obvious defects in virulence in infection assays with corn silks and wheat heads (Fig. S2). The *amd1* mutant also was normal in response to various stresses, including treatments with 0.75% SDS, 0.05% H2O2, and 0.7 M NaCl (Fig. S3). These results indicated that, consistent with stage-specific expression during sexual reproduction, *AMD1* is not important for hyphae growth, asexual reproduction, virulence, and stress response.

The *amd1* mutant also was normal in perithecia development and formed abundant melanized perithecia on carrot agar cultures at 7 dpf. However, ascospore cirrhi were rarely observed in mutant perithecia (Fig. 3A) even after prolonged incubation, suggesting its defects in ascospore release. To confirm this observation, we assayed ascospore discharge as previously described. Whereas abundant ascospores were forcibly discharged from wild-type perithecia after incubation for 16 h, under the same conditions, ascospore discharge was not observed in the *amd1* mutant (Fig. 3B). Therefore, *AMD1* is essential for forcible discharge of ascospores from perithecia in *F. graminearum*.

**AMD1 is required for ascus wall integrity.** Although the *amd1* mutant was defective in ascospore discharge, they formed abundant ascospores inside perithecia. However, when 8 dpf perithecia were examined, only scattered ascospores but not intact asci were observed in the *amd1* mutant (Fig. 3C), suggesting the breakdown of ascus wall. Under the same conditions, fascicles of asci were present in wild-type perithecia (Fig. 3C). To verify this observation, we examined perithecia with semi-thin sections of 8 dpf perithecia. In the wild type, asci with ascospores were observed (Fig. 3D). However, only scattered ascospores but not asci were observed in mutant...
perithecia (Fig. 3D). Because turgor pressure inside asci is important for the forcible discharge of ascospores, the premature breakdown of ascus wall in mutant perithecia may be directly responsible for its defects in ascospore release and formation of ascospore cirrhi.

To determine the timing of ascus wall disintegration, we examined ascospores and asci in perithecia sampled at 5, 6, 7, and 8 dpf. Both the wild type and \( \text{amd}1 \) mutant strains had fascicles of asci with 8-ascospores in 5 or 6 dpf perithecia. In 7 dpf perithecia, the ascus wall begun to disintegrate and the arrangement of ascospores in asci became loose in the \( \text{amd}1 \) mutant (Fig. 4). No asci were observed in mutant perithecia at 8 dpf (Fig. 4). These results indicate that the breakdown of ascus wall began at 7 dpf and completed by 8 dpf.

Germination of \( \text{amd}1 \) ascospores inside perithecia. Similar to the wild type, the \( \text{amd}1 \) mutant still produced four-celled ascospores. However, most of the mutant ascospores had germinated inside perithecia by 12 dpf (Fig. 4). Ascospore germination also was visible in semi-thin sections of mutant perithecia sampled at 10 dpf (Fig. 3D). Under the same conditions, ascospore germination was never observed inside perithecia formed by the wild type (Fig. 4). Extensive observations with mutant perithecia showed that ascospore germination only occurred after the breakdown of the ascus wall. Germination was not observed with ascospores that were still inside intact asci. These results showed that ascus wall integrity is important for preventing ascospore germination inside perithecia in \( F. \text{graminearum} \).

Interestingly, germ tubes were produced only from one end of mutant ascospores when they germinated inside perithecia (Fig. 5). When incubated in liquid complete medium (CM), both the wild-type and mutant ascospores first produced germ tubes from one end but germination from the other end also occurred rapidly. After incubation in CM for 6 h CM, approximately 25% of ascospores had germ tubes from both ends. The percentage of ascospores germinated from both ends increased to 85% in 10 h CM cultures (Fig. 5). These results indicated that mutant ascospores germinated in different manners under different conditions. Unlike germination in nutrient media, germination inside perithecia may involve different regulatory mechanisms.
The AMD1<sup>WT</sup> and AMD1<sup>TGG</sup> but not AMD1<sup>TAA</sup> alleles complement the amd1 mutant. For complementation assays, the wild-type AMD1 allele with the TA<sup>632</sup>G stop codon and its promoter region was amplified and fused with GFP to generate the AMD1<sup>WT</sup>-GFP construct, which was then transformed into the amd1 mutant. All the resulting amd1/AMD1<sup>WT</sup>-GFP transformants were normal in hyphal growth, conidiation, and sexual reproduction. Perithecia formed by the amd1/AMD1<sup>WT</sup>-GFP transformants formed ascospore cirrhi and had no ascospore germination inside perithecia (Fig. 3A), indicating the complementation of amd1.

To determine the function of A<sup>632</sup>-to-I editing, we also generated the AMD1<sup>TGG</sup>-GFP (edited) and AMD1<sup>TAA</sup> (uneditable) constructs by introducing the A<sup>632</sup>G and G<sup>633</sup>A mutations, respectively, and transformed them into the amd1 mutant. The resulting transformants were screened by PCR and examined for defects in sexual
All the amd1/AMD1TAA transformants had similar defects with the original amd1 mutant in ascospore release and ascus wall integrity (Fig. 3A), indicating the essential role for RNA editing in AMD1 function. However, expression of the AMD1TGG allele fully complemented the ascospore release defects of amd1 (Fig. 3A). The amd1/AMD1TGG-GFP transformants were normal in ascospore discharge and formed ascospore cirri as frequently as the wild type. Therefore, expression of AMD1TGG, similar to the wild-type allele, fully complemented the amd1 mutant, suggesting that the unedited transcripts (2.4%) of AMD1 had no functions during sexual reproduction.

Amd1-GFP localizes to the ascus membrane. None of the amd1/AMD1WT-GFP transformants had detectable GFP signals in vegetative hyphae and conidia (Fig. S4), which was consistent with the specific expression of AMD1 in perithecia. When perithecia of different development stages were examined, no GFP signals were observed in asci of 5 dpf perithecia or ascospores outside asci in older perithecia (Fig. 6). Amd1-GFP mainly localized to the ascus membrane in 8 dpf perithecia (Fig. 6). To our knowledge, this is the first report on proteins reproduction. All the amd1/AMD1TAA transformants had similar defects with the original amd1 mutant in ascospore release and ascus wall integrity (Fig. 3A), indicating the essential role for RNA editing in AMD1 function. However, expression of the AMD1TGG allele fully complemented the ascospore release defects of amd1 (Fig. 3A). The amd1/AMD1TGG-GFP transformants were normal in ascospore discharge and formed ascospore cirri as frequently as the wild type. Therefore, expression of AMD1TGG, similar to the wild-type allele, fully complemented the amd1 mutant, suggesting that the unedited transcripts (2.4%) of AMD1 had no functions during sexual reproduction.

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Figure 3. The amd1 mutant was defective in ascospore release and ascus wall integrity. (A) Mating cultures of the wild-type PH-1 (WT), amd1 mutant, and transformants of amd1 expressing the AMD1WT-, AMD1TGG-, or AMD1TAA-GFP construct were examined 8 days post-fertilization (dpf). Arrows point to cirri. (B) Ascospore discharge was assayed with 7 dpf perithecia of the same set of strains. Ascospores discharged from perithecia were accumulated as whitish masses when examined after incubation for 16 h. (C) The same set of strains were examined for asci and ascospores in 8 dpf perithecia. No intact asci were observed in the amd1/AMD1TAA transformant. Bar = 20 μm. (D) Semi-thin sections of representative perithecia of PH-1 (WT) and the amd1 mutant that were fixed and stained with 0.5% (wt/vol) toluidine blue. Arrows mark the germinated ascospores. Bar = 20 μm.
localizes to the ascus membrane in filamentous ascomycetes. The subcellular localization pattern of Amd1 is consistent with its TM helixes and functions in maintaining ascus wall integrity and ascospore discharge in *F. graminearum*.

**Constitutive expression of the AMD1TGG allele has no effects on hyphal growth and conidiation.** Although *AMD1* transcripts were rare in hyphae, it is possible that the existence of the UA632G stop codon is to
avoid accidental expression of Amd1 proteins, which may be detrimental to vegetative growth in *F. graminearum*. To test this hypothesis, we generated the PRP27-AMD1TGG-GFP construct and transformed it into the amd1 mutant. The resulting transformants had no obvious defects in vegetative growth and conidiation (Table S1). In 8 h germlings, localization of Amd1-GFP to the cytoplasm membrane was not observed but GFP signals were observed in peri-nuclear regions that may be related to the endoplasmic reticulum due to overexpression (Fig. S4). These results indicate that expression of AMD1 by the strong, constitutive RP27 promoter 17, 18 had no effects on vegetative growth and asexual reproduction, and the localization of Amd1 to the ascus membrane may depend on its interacting proteins that are specifically expressed during sexual reproduction.

**The expression of AMD1 is reduced in the Fgkin1 mutant.** In *F. graminearum*, FgKin1, a microtubule affinity-regulating protein kinase (MARK), is also required for ascospore discharge and prevention of ascospore germination inside perithecia 19. The Fgkin1 and amd1 mutants has similar defects in ascospore discharge and disintegration of the ascus wall (Fig. 7A). Similar to amd1, germination of ascospores from one end also was observed inside Fgkin1 perithecia 19 (Fig. 7A). When assayed by qRT-PCR with RNA isolated from 7 dpf perithecia, the AMD1 expression level was reduced approximately 5 folds in the Fgkin1 mutant in comparison with that of the wild type (Fig. 7B). It is possible that the FgKin1 kinase controls ascus wall integrity by somehow regulating the expression of AMD1 in *F. graminearum*.

**Deletion of AMD1 affects more than 300 genes expression.** To identify genes affected by AMD1 deletion, we conducted RNA-seq analysis with RNA isolated from perithecia sampled at 7 dpf. In comparison with the wild type, 53 and 263 genes were up- and down-regulated over two folds, respectively, in the amd1 mutant.
(Table S2). Among the up-regulated genes, Gene Ontology (GO) enrichment analysis showed that 19 genes each related to transporter activity and membrane were significantly enriched (Fig. S5), suggesting that deletion of AMD1 may affect cross-membrane transportation and membrane functions. Among the down-regulated genes, approximately half of them encode hypothetical proteins or proteins of unknown functions (Table S2) and no significant enrichment of any GO terms was observed. However, several genes that may be related to cell wall synthesis, modifications, or integrity were down-regulated in the mutant, including FGRRES_12586, FGRRES_07238, FGRRES_17404, FGRRES_02262, FGRRES_10920, FGRRES_13169, and FGRRES_03674. Reduced expression of these genes may be related to the defects of amd1 in ascus wall integrity.

Discussion

The AMD1 gene requires A-to-I RNA editing during sexual reproduction to encode a full-length protein. Interestingly, its orthologs are only present in ascomycetes that form asci inside ascocarps and eject ascospores from asci. Most of the Eurotiomycetes that form cleistothecia such as Aspergillus nidulans lack AMD1 orthologs. It is tempting to speculate that AMD1 is functionally related to the physical ejection of ascospores from asci and its orthologs may evolve only in ascomycetes that are more advanced than those forming cleistothecia20. Ascospores are not ejected from naked asci formed by Taphrinomycotina and Saccharomycotina species or cleistothecia.

The forcible ejection of ascospores is functionally related to the generation of turgor pressure in ascites22,23. In F. graminearum, individual mature asci extend through the ostiole prior to ascospore discharge22. It is likely that the ascus wall was degraded in the amd1 mutant before asci were mature and ready for ascospore ejection. Although its exact function is not clear, AMD1 may be involved in strengthening or the modification of ascus wall at later stages. It is also possible that mannitol accumulation and ion fluxes important for ascus turgor generation25 were affected in the amd1 mutant, which in turn may affect ascus turgor and ascus wall modifications. The specific expression of AMD1 at late sexual stages and its localization to the ascus membrane supported the likely functions of Amd1 proteins in maintaining ascus wall integrity. Furthermore, RNA-seq analysis showed that several genes related to cell wall modification or integrity were down-regulated in mutant perithecia. Interestingly, 19 genes each encoding proteins that are functionally related to transporter activity and membrane functions were upregulated in the amd1 mutant, which accounted for over two thirds of the 53 up-regulated genes. Most of these genes with up-regulated expression in amd1 had no or little expression in 8 dpf perithecia in the wild type22, suggesting that their up-regulation may be related to the breakdown of ascus wall and membrane in the mutant.

Another defect of the amd1 mutant was the germination of ascospores from one end inside perithecia after the breakdown of ascus wall. This defect is similar to that of the Fgkin1 mutant19. Kin1 is a MARK kinase that is involved in microtubule based transportation via phosphorylation of microtubule-associated proteins25. In this study, we showed that the expression level of AMD1 was decreased approximately 5 folds in the Fgkin1 mutant, which may be directly related to the effects of Fgkin1 in ascospore discharge and germination. Because Amd1 localized to the ascus membrane but Fgkin1 localizes to the septal pore19, they may not directly interact with each other and AMD1 expression may be indirectly regulated by FgKIN1. Nevertheless, unlike AMD1, FgKIN1 is constitutively expressed, and the Fgkin1 mutant had a reduced growth rate19. Therefore, the Fgkin1 kinase must have other downstream targets and more diverse functions than Amd1 in F. graminearum. The gea1 mutant is another mutant in F. graminearum that had similar defects with amd1 in ascospore discharge and germination11. However, different from Amd1, Gea1 protein localizes to the cytoplasm membrane of ascospores and some gea1 ascospores had morphological defects. Nevertheless, it will be important to determine the relationships among Amd1, Fgkin1, and Gea1 during ascus maturation and ascospore ejection.

Like the Fgkin1 mutant19, ascospores of the amd1 mutant germinated from one end inside perithecia but germinated from both ends when cultured in CM. These observations suggest that the two ends of mature ascospores are not equal and the presence of nutrients may promote the production of germ tubes from both ends of ascospores in F. graminearum. However, it is puzzling how the fungus distinguishes one end from the other in four-celled ascospores derived from two rounds of mitosis and cytokinesis. It is also not clear what molecular mechanisms are responsible for the auto-inhibition of ascospore germination inside perithecia. If F. graminearum produces certain metabolites or ascospore surface compounds that function as auto-inhibitory factors to prevent ascospores from germination inside perithecia, the amd1 and Fgkin1 mutants may be defective in the production or accumulation of these compounds. It will be important to assay for defects of the amd1 and Fgkin1 mutants in the accumulation of mannitol and ions enriched inside ascI and Gea1 if they are responsible for auto-inhibition of ascospore germination in F. graminearum.

AMD1 is one of the 60 genes with premature stop codons in the coding regions that require A-to-I editing to encode full-length proteins in F. graminearum15. Because RNA editing is incomplete, even though the editing level was 97.6% at A632, the unedited transcripts were still present and might encode a small peptide. Nevertheless, the amd1/AMD11078-GFP transformants were similar to the wild type and complemented transformants in ascospore discharge, indicating that the unedited transcripts had no detectable functions if they indeed encoded a small peptide in F. graminearum. However, there are 9 other nonsynonymous editing events identified in the AMD1 transcripts, including two in the MFS domain (Fig. 1A). Five of editing sites have editing levels higher than 90% and another 7 had editing levels ranging from 30–90%. Therefore, RNA editing not only enables AMD1 to encode a full-length functional protein but also introduces amino acid sequence variations in F. graminearum. It will be interesting to determine the functions of these nonsynonymous editing sites in AMD1.

Methods

Strains and culture conditions. The F. graminearum wild-type strain PH-128 and all the transformants generated in this study were routinely maintained on potato dextrose agar (PDA) plates at 25°C. Conidiation in...
Generation of the **AMD1**\(^{WT}\), **AMD1**\(^{TG}\), **AMD1**\(^{TA}\), and P\(_{RP27-AMD1}\)^{TG} transformatants. For complementation assays, the entire **AMD1** gene including its promoter region was amplified with primers 094-NF and 094-R (Table S3) and co-transformed with Xhol-digested pFL2 (carrying the geneticin resistance marker) into yeast strain XK1–25 by the gap repair approach.\(^{15, 16}\) The **AMD1**\(^{WT}\)•GFP fusion construct was rescued from Trp\(^{+}\) yeast transformants and confirmed by sequencing analysis. The same yeast gap repair approach was used to generate the **AMD1**\(^{TG}\)•GFP, P\(_{RP27-AMD1}\)^{TG}•GFP, and **AMD1**\(^{TA}\)•GFP constructs. To introduce the A632G and G633A mutations, **AMD1** was amplified with primer pairs 094E-F/094E-R and 094S-F/094S-R (Table S3), respectively. All the resulting mutant alleles of **AMD1** were verified by sequencing and transformed into the **amd1** mutant. Transfectants of **amd1** expressing the **AMD1**\(^{WT}\), **AMD1**\(^{TG}\), P\(_{RP27-AMD1}\)^{TG}, and **AMD1**\(^{TA}\)•GFP constructs were identified by PCR and examined for GFP signals by epifluorescence microscopy.

**Specimen preparation for semi-thin sections.** Perithecia collected from mating cultures at 8 or 10 dpf were fixed with 4% (vol/vol) glutaraldehyde in 0.1 M phosphate buffer (pH 6.8) overnight at 4 °C. Samples were then dehydrated in a series of acetone consisting of 30, 50, 70, 80, 90, and 100% (vol/vol). The dehydrated samples were embedded in Spurr resin as described.\(^{33}\) Semi-thin sections (1 μm in thickness) were stained with 0.5% (wt/vol) toluidine blue before being examined with an Olympus BX-53 microscope.

**qRT-PCR analysis.** For qRT-PCR assays, RNA samples were isolated with the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) from perithecia collected at 7 dpf. The Fermentas First cDNA synthesis kit (Hanover, MD, USA) was used for cDNA synthesis. The **TUB2** beta-tubulin gene was used as the internal control and the relative expression of each gene was calculated with the 2^−ΔΔCt\(^{32}\) method. Data from three biological replicates were used to calculate the mean and standard deviation of the expression levels.\(^{32}\)

**Plant infection assays.** For infection assays with flowering wheat heads of cultivar Xiaoyan 22, conidia were harvested from 5-day-old CMC cultures and re-suspended to 2.0 × 10^8 conidia/ml in sterile distilled water. For each head, the fifth spikelet from the base of the inflorescence was inoculated with 10 μl of conidium suspensions as described.\(^{36, 37}\) FHB symptoms were examined at 14 day post-infection to estimate the disease index.\(^{38, 39}\) Corn silks were infected with culture blocks and examined as described.\(^{40}\)

**RNA-seq analysis.** Perithecia of PH-1 and **amd1** mutant were harvested from carrot agar cultures at 7 dpf and used for RNA extraction with TRIZol (Invitrogen, USA). For each strain, RNA was isolated from two biological replicates. RNA-seq libraries were prepared with the NEBNext Ultra\(^{®}\) Directional RNA Library Prep Kit (NEB, USA) following the instruction provided by the manufacturer and sequenced with Illumina HiSeq 2500 with the paired-end 2 × 150 bp model at the Novogene Bioinformatics Institute (Beijing, China). For each replicate, at least 24 Mb paired-end reads were obtained. The resulting RNA-seq reads were mapped onto the reference genome of **F. graminearum** strain PH-1\(^{15, 16, 41}\) by HISAT2\(^{42}\). The number of reads (count) mapped to each gene were calculated by featureCounts.\(^{43}\) Differential expression analysis of genes was performed using the edgeRun package\(^{44}\) with the exactTest function. Genes with a FDR (false discovery rate) of below 0.05 and |log2(FC)| (log fold change) of above 1 were regarded as differentially expressed genes.

**Data availability.** RNA-seq data were deposited at NCBI SRA database under accession number SRP100650.

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Author Contributions
J.R.X. and H.L. conceived and designed the experiments, S.C., Y.H., C.H., Y.X., C.W., H.Z., and H.L. performed the experiments and data analyses. S.C., H.L., and J.R.X. wrote the manuscript.

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