

RESEARCH

Open Access



# Conserved Golgi nucleoside diphosphatases drive protein glycosylation and virulence in *Fusarium graminearum*

Soobin Shin<sup>1</sup>, Yejin Choi<sup>1</sup>, Thuy Thi Thanh Phan<sup>2,3</sup>, Seonwoo Song<sup>4</sup>, Nahyun Lee<sup>1</sup>, Jiyeun Park<sup>5</sup>, Sieun Kim<sup>6</sup>, Hosung Jeon<sup>1</sup>, Jung-Eun Kim<sup>7</sup>, Kyunghun Min<sup>8</sup>, Hun Kim<sup>9</sup>, Gyung Ja Choi<sup>9</sup>, Hyun Ah Kang<sup>4</sup>, Dae-Geun Song<sup>2,3\*</sup> and Hokyoung Son<sup>1,10,11,12\*</sup>

## Abstract

The Golgi apparatus orchestrates post-translational modification and vesicular trafficking, with guanosine diphosphatases (GDPases) facilitating nucleotide-sugar transport by hydrolyzing guanosine diphosphate (GDP) to guanosine monophosphate (GMP). Although GDPase functions are well known in yeast and human-pathogenic fungi, their roles in filamentous fungi, including plant pathogens, remain largely unexplored. Here, we investigated the biological functions of the Golgi GDPases Gda1 and Ynd1 in the wheat head blight pathogen *Fusarium graminearum*. While loss of *FgYND1* only resulted in minor growth problems, deletion of *FgGDA1* caused pleiotropic deficiencies in mycelial development, conidiation, sexual reproduction, and virulence. Notably, *FgYND1* overexpression partially rescued the defects of the *FgGDA1* deletion mutant in the developmental and invasive processes, indicating overlapping yet non-equivalent functions. Glycoproteomics analysis revealed global remodeling of glycoprotein profiles in the *FgGDA1* deletion mutant, with prominent changes among enzymes in carbon metabolism, consistent with disrupted Golgi-dependent glycosylation. According to our results, FgGda1 is the primary driver of protein glycosylation-dependent fungal development and virulence in *F. graminearum*, with FgYnd1 providing only partial redundancy.

**Keywords** Golgi, *GDA1*, *Fusarium graminearum*, Protein glycosylation

\*Correspondence:

Dae-Geun Song  
dsong82@kist.re.kr  
Hokyoung Son  
hogongi7@snu.ac.kr

<sup>1</sup> Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea

<sup>2</sup> Center for Natural Product Systems Biology, Korea Institute of Science and Technology (KIST), Gangneung 25451, Republic of Korea

<sup>3</sup> Natural Product Applied Science, KIST School, University of Science and Technology, Gangneung 25451, Republic of Korea

<sup>4</sup> Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

<sup>5</sup> Institute for Plant Sciences, University of Cologne, 50931 Cologne, Germany

<sup>6</sup> Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration, Wanju 55365, Republic of Korea

<sup>7</sup> Research Institute of Climate Change and Agriculture, Rural Development Administration, National Institute of Horticultural and Herbal Science, Jeju 63240, Republic of Korea

<sup>8</sup> Department of Microbiology, Pukyong National University, Pusan 48513, Republic of Korea

<sup>9</sup> Center for Eco-Friendly New Materials, Korea Research Institute of Chemical Technology, Daejeon 34114, Republic of Korea

<sup>10</sup> Plant Health Center, Seoul National University, Seoul 08826, Republic of Korea

<sup>11</sup> Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

<sup>12</sup> Plant Genomics and Breeding Institute, Seoul National University, Seoul 08826, Republic of Korea



© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

## Background

The Golgi complex is a central hub for protein glycosylation and trafficking. As proteins pass through the Golgi, they undergo extensive post-translational modifications (PTMs) such as glycosylation, sulfation, phosphorylation, and proteolysis (Zhang and Wang 2016; Schwabl and Teis 2022). Among these, protein glycosylation is particularly important, as it not only regulates protein folding and stability but also contributes to key biological processes including cellular interactions and signal transduction (He et al. 2024; Potel et al. 2025). The endoplasmic reticulum (ER)–Golgi–plasma membrane trafficking axis is then used to package and transport these altered proteins (Brandizzi and Barlowe 2013), ensuring efficient cellular functions and proper distribution to their final destinations (Day et al. 2013).

Consistent with its high complexity, the Golgi apparatus serves as a home to numerous resident enzymes that sustain its functions. Among these, nucleoside diphosphatases (NDPases) are particularly important for Golgi metabolism, as they hydrolyze nucleoside diphosphates to monophosphates, thereby providing substrates for nucleotide sugar transport systems (Hirschberg et al. 1998). In the model organism *Saccharomyces cerevisiae*, two enzymes, Gda1p and Ynd1p, convert guanosine diphosphate (GDP) to guanosine monophosphate (GMP) (Abeijon et al. 1993; Gao et al. 1999). Because sugar residues are transported in the GDP-sugar form during glycoprotein biosynthesis in the Golgi, these enzymes play a critical role in GDP-sugar transport and in maintaining efficient protein glycosylation (Berninsone et al. 1994; D'Alessio et al. 2003). Therefore, *GDA1* and *YND1* contribute to key biological processes, as exemplified in human pathogenic fungi such as *Candida albicans* and *Cryptococcus neoformans*. In *C. albicans*, *GDA1* affects fungal development and protein glycosylation (Herrero Ana et al. 2002). Moreover, loss of *GDA1* in *C. neoformans* results in abnormal *O*-linked glycans assembled on cell wall mannoproteins (Jin et al. 2020). However, despite these findings, the roles of NDPases in filamentous fungi remain poorly understood, even though their multicellular development and host infection structures strongly suggest a critical requirement for precise glycosylation control.

*Fusarium graminearum* is an ascomycete fungus that causes Fusarium head blight (FHB) in wheat and barley, as well as ear rot and stalk rot in maize (Munkvold 2003; Goswami and Kistler 2004). Outbreaks of FHB result in not only severe yield losses but also grain contamination with various mycotoxins, particularly deoxynivalenol (DON) (Chen et al. 2019). FHB epidemics have repeatedly devastated wheat production, affecting about 20% of wheat-planted areas worldwide and causing yield losses

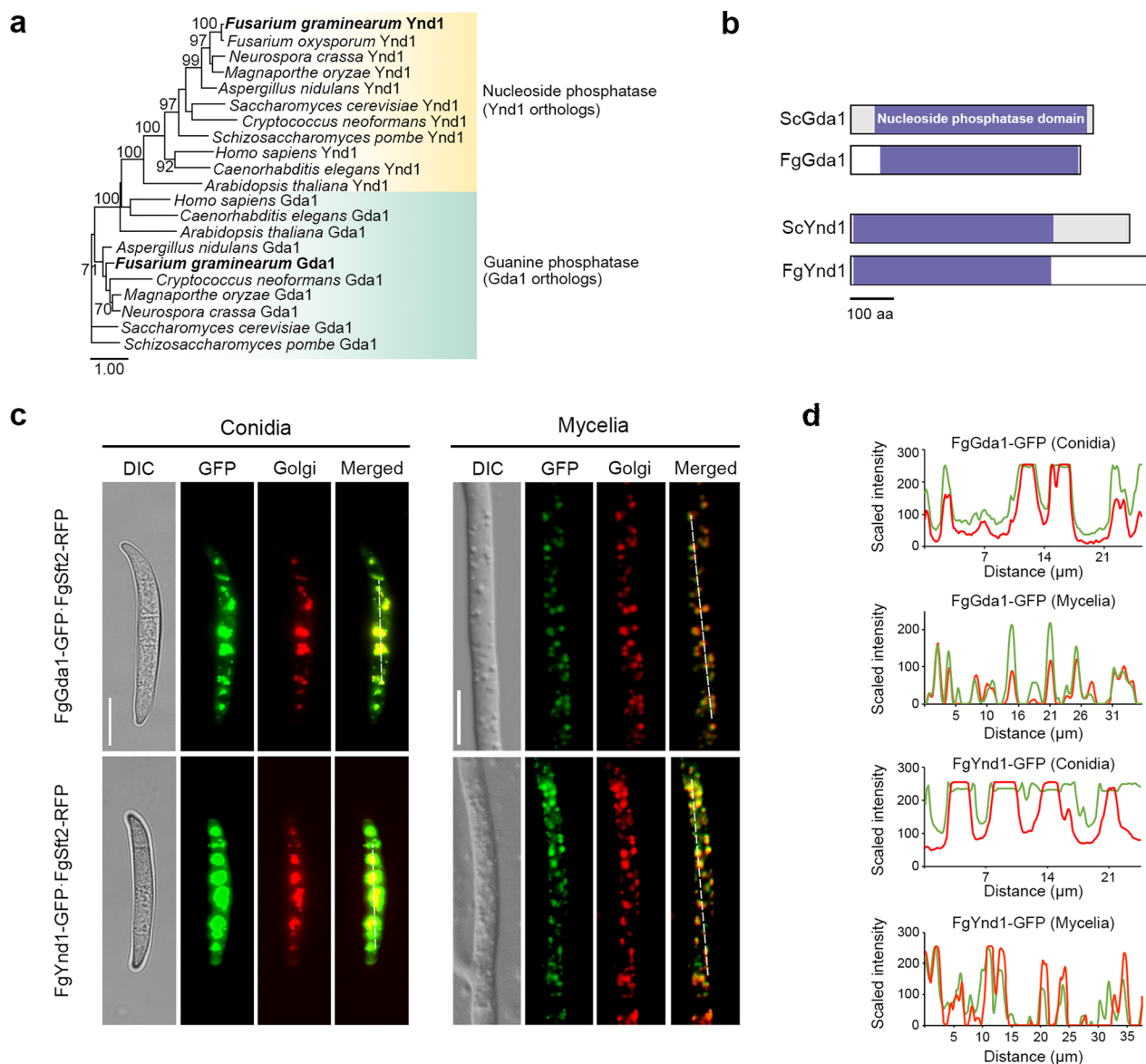
of over 3.4 million tons in China between 2000 and 2018 (Chen et al. 2019) and economic damages of 17 billion U.S. dollars in the United States between 1993 and 2014 (Wilson et al. 2017). Given the lack of complete resistance in wheat and maize, understanding the molecular mechanisms underlying fungal development and pathogenicity is essential for devising effective strategies to control FHB (Khan et al. 2020).

In this study, we identified two GDPases, Gda1 and Ynd1, in *Fusarium graminearum*. Using biochemical and genetic approaches, we demonstrated that these enzymes are conserved as GDPases and are critical for both fungal development and virulence. Genetic epistasis analysis further revealed that Gda1 and Ynd1 possess shared but not completely overlapping functions. Moreover, Concanavalin A (ConA)-captured glycoproteomics analysis showed that the absence of *GDA1* altered the protein glycosylation profile, particularly in carbon metabolic enzymes. In contrast, cell wall glycan analysis indicated that *GDA1* had only a minor effect on *O*-glycan structure, suggesting that its role in *F. graminearum* differs from that in the human pathogenic fungus *C. neoformans*. Taken together, these findings led us to address two key questions: (i) are *FgGDA1* and *FgYND1* functionally conserved GDPases in *F. graminearum*? and (ii) how do they contribute to fungal development, glycosylation, and virulence? Overall, our study provides fundamental insights into Golgi-resident enzymes and their contributions to fungal development and virulence in phytopathogenic fungi.

## Results

### Identification of nucleoside diphosphatases FgGda1 and FgYnd1 in *F. graminearum*

Two GDPases, Gda1 and Ynd1, were identified and characterized in the model organism *Saccharomyces cerevisiae* (Abeijon et al. 1993; Gao et al. 1999). Two potential homologs were found in *F. graminearum* using their protein sequences as references (Fig. 1a). Phylogenetic analysis revealed a clear division of GDPases into two groups: one containing Gda1 and the other containing Ynd1. Therefore, we designated the *F. graminearum* homologs as *FgGDA1* (FGRAMPH1\_01G17201, formerly FGSG\_05143) and *FgYND1* (FGRAMPH1\_01G14889, formerly FGSG\_04270). FgGda1 and FgYnd1 exhibit 54% and 50% overall amino acid sequence identity with their respective counterparts in *S. cerevisiae*. InterPro analysis confirmed the presence of a conserved nucleoside phosphatase domain (IPR000407) in both proteins (Fig. 1b). Together, these data clearly indicate that FgGda1 and FgYnd1 are conserved GDPases in *F. graminearum* and cluster with orthologs from filamentous fungi.



**Fig. 1** Characterization of the Golgi nucleoside diphosphatase FgGda1 and FgYnd1. **a** Phylogenetic tree of Gda1 and Ynd1 orthologs. Amino acid sequences were aligned using the MUSCLE algorithm, and a maximum likelihood phylogenetic tree was constructed using the GTR+gamma+I model with 1000 bootstrap replicates. Bootstrap values over 70 are presented at the nodes. The scale bar represents the number of amino acid substitutions per site. **b** Comparison of the domain architecture of Gda1 and Ynd1 in *S. cerevisiae* and *F. graminearum*. The nucleoside phosphatase domain (InterPro: IPR000407) was conserved in both FgGda1 and FgYnd1. **c** Subcellular localization of FgGda1 and FgYnd1. Each FgGda1 and FgYnd1 was fused with green fluorescent protein (GFP), and Golgi marker FgSft2 was fused with red fluorescent protein (RFP). The yellow color in the merged images indicates co-localization. Scale bar = 10  $\mu$ m. **d** The plots profiling the GFP and RFP intensities measured from the dot lines indicated in the photos

### FgGda1 and FgYnd1 localize to the Golgi

To assess the biological and pathological functions of *FgGDA1* and *FgYND1* in *F. graminearum*, we generated deletion mutants by replacing *FgGDA1* and *FgYND1* with a *GEN* in the wild-type strain, respectively. Southern blot hybridization verified that each gene had been

successfully deleted (Additional file 1: Figure S1a). Complementation strains (FgGDA1c and FgYND1c) were generated by introducing GFP-tagged ORF under their native promoters into the knockout mutants. In addition, overexpression transformants (FgGDA1oe and FgYND1oe) were constructed under the strong promoter ( $P_{RP27}$ ) and confirmed by quantitative reverse

transcription polymerase chain reaction (qRT-PCR) (Additional file 1: Figure S1b).

To determine subcellular localization, we co-expressed *FgGDA1-GFP* and *FgYND1-GFP* with the Golgi marker *FgSFT2-RFP* (Liu et al. 2005). Outcrossing produces strains such as  $\Delta Fggda1::FgGDA1-GFP-HYG$  *FgSFT2-RFP-GEN* and  $\Delta Fgynd1::FgYND1-GFP-HYG$  *FgSFT2::FgSFT2-RFP-GEN*. As expected, both fusion proteins showed distinct co-localization with FgSft2-RFP throughout observed developmental stages, including conidia and hyphae (Fig. 1c, d). These findings revealed that FgGda1 and FgYnd1 are Golgi-localized GDPases, consistent with their conserved roles in other fungi.

### ***FgGDA1* is essential for vegetative growth, sexual development, and conidiation in *F. graminearum***

Previous studies have demonstrated that Golgi-localized Gda1 and Ynd1 contribute to fungal growth and development in the model organism *S. cerevisiae* as well as in the human pathogenic fungi *C. albicans* and *C. neoformans* (Abeijon et al. 1993; Gao et al. 1999; Herrero Ana et al. 2002; Jin et al. 2020). Therefore, we investigated whether FgGda1 and FgYnd1 play comparable roles in plant pathogenic fungi. We comprehensively analyzed phenotypic alterations in vegetative growth, asexual/sexual reproduction, mycotoxin production, and virulence in the wild-type and deletion mutants.

Deletion of *GDA1* resulted in severe defects in vegetative growth on both complete medium (CM) and minimal medium (MM), whereas deletion of *FgYND1* caused only mild growth reduction (Fig. 2a). Overexpression of either *FgGDA1* or *FgYND1* also altered colony morphology and slightly impaired vegetative growth compared with the wild type. Regarding sexual reproduction, the  $\Delta Fggda1$  mutant completely failed to produce perithecia (Fig. 2b). Conidiation was likewise dramatically reduced in  $\Delta Fggda1$ , while both *FgGDA1oe* and *FgYND1oe* strains produced approximately 65% fewer conidia than the wild type (Fig. 2c). Moreover,  $\Delta Fggda1$  produced morphologically abnormal conidia with significantly reduced length (Fig. 2d, e). However, these mutants did not exhibit significant differences in deoxynivalenol (DON) production compared with the wild type (Additional file 1: Figure S2).

To evaluate the role of *FgGDA1* and *FgYND1* in fungal virulence, we performed point inoculation of flowering wheat heads.  $\Delta Fggda1$  mutants colonized the inoculated spikelet but failed to spread to adjacent spikelets, while the wild-type,  $\Delta Fgynd1$ , complementation, and overexpression strains developed clear head blight symptoms 21 days post-inoculation (dpi) (Fig. 3a, b). To further monitor hyphal spread,  $\Delta Fggda1$  mutants expressing cytosolic GFP were inoculated onto wheat heads.

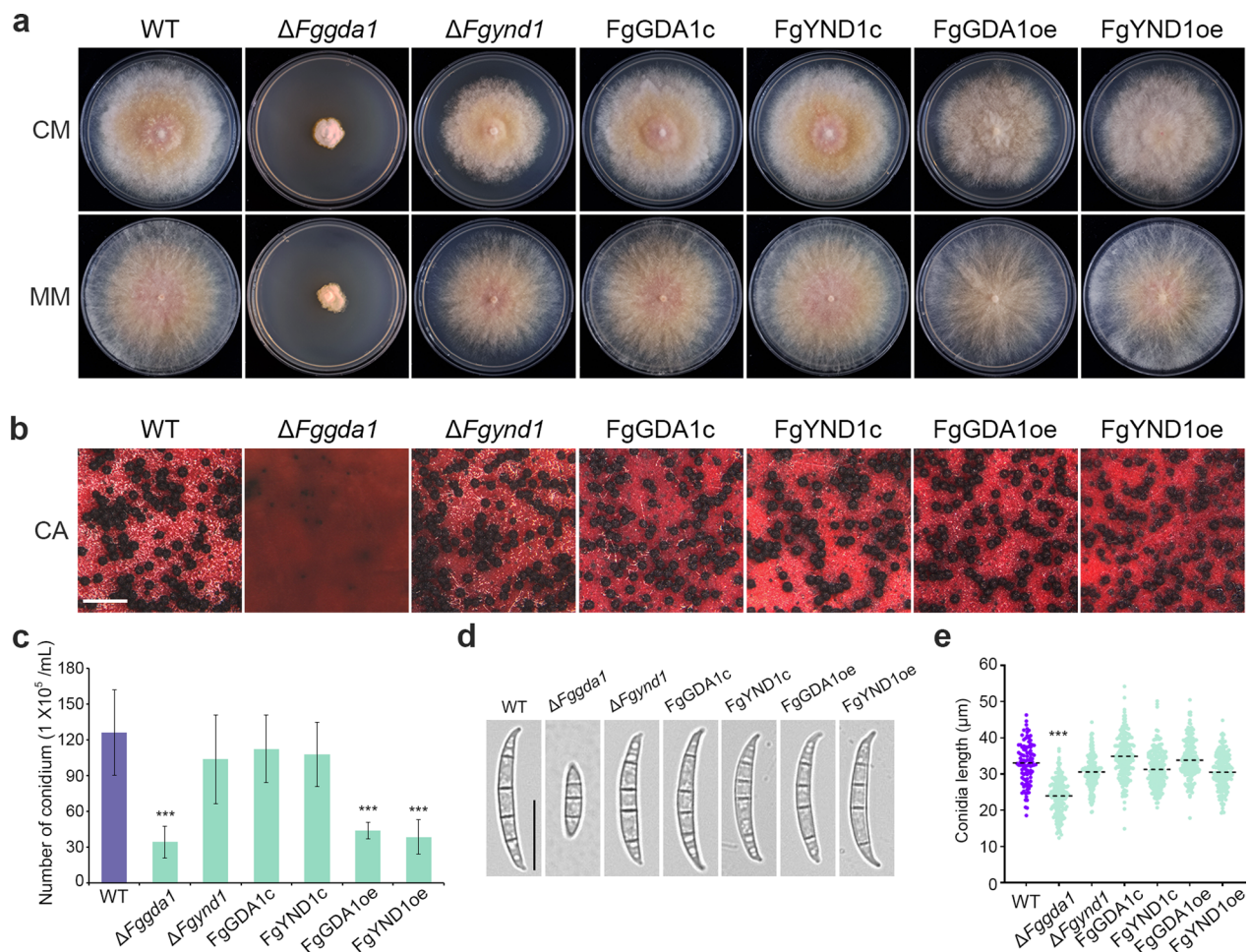
At 6 dpi, hyphae of the strain carrying the wild-type *FgGDA1* allele spread to neighboring spikelets through rachis nodes, but  $\Delta Fggda1$  failed to spread beyond the inoculated spikelet (Fig. 3c). These results indicate that *FgGDA1* is essential for fungal development and virulence in *F. graminearum*, whereas *FgYND1* contributes only marginally.

### ***FgYND1* partially compensates for the loss of *FgGDA1***

Next, we asked whether the two Golgi GDPases could compensate for each other's loss. To investigate their genetic relationship, we measured the expression of *FgYND1* or *FgGDA1* in the  $\Delta Fggda1$  and  $\Delta Fgynd1$  mutant strains. Interestingly, when one GDPase-encoding gene was knocked out, the expression of the other gene was increased, suggesting that *FgGDA1* and *FgYND1* influence each other's expression (Additional file 1: Figure S3). Furthermore, we generated the following strains through genetic crosses of single-gene mutants:  $\Delta Fggda1$ -*FgYND1oe*, *FgGDA1oe*- $\Delta Fgynd1$ , and  $\Delta Fggda1$ - $\Delta Fgynd1$ . Overexpression of *FgYND1* partially alleviated the vegetative growth defect of  $\Delta Fggda1$  (Fig. 4a), whereas simultaneous deletion of both genes was nearly lethal, consistent with findings in *C. neoformans* (Jin et al. 2020). In sexual development assays,  $\Delta Fggda1$ -*FgYND1oe* partially restored perithecia production to some extent (Fig. 4b), but rosettes remained abnormal, often immature, and empty perithecia were frequently observed (Fig. 4c). Conidial morphology was largely restored and conidiation modestly recovered (Fig. 4d, e). In virulence assays, increased *FgYND1* expression partially rescued the defects of  $\Delta Fggda1$  on coleoptiles (Fig. 4f, g) and enhanced disease progression on wheat heads, though full head blight symptoms were not achieved (Fig. 4h, i). Collectively, these genetic and morphological data indicate that although *FgGDA1* and *FgYND1* function in a shared Golgi pathway, *FgYND1* provides only limited compensation, and *FgGDA1* is indispensable for full development and virulence.

### **Glycoproteome data reveal a strong link between *FgGDA1* and protein glycosylation**

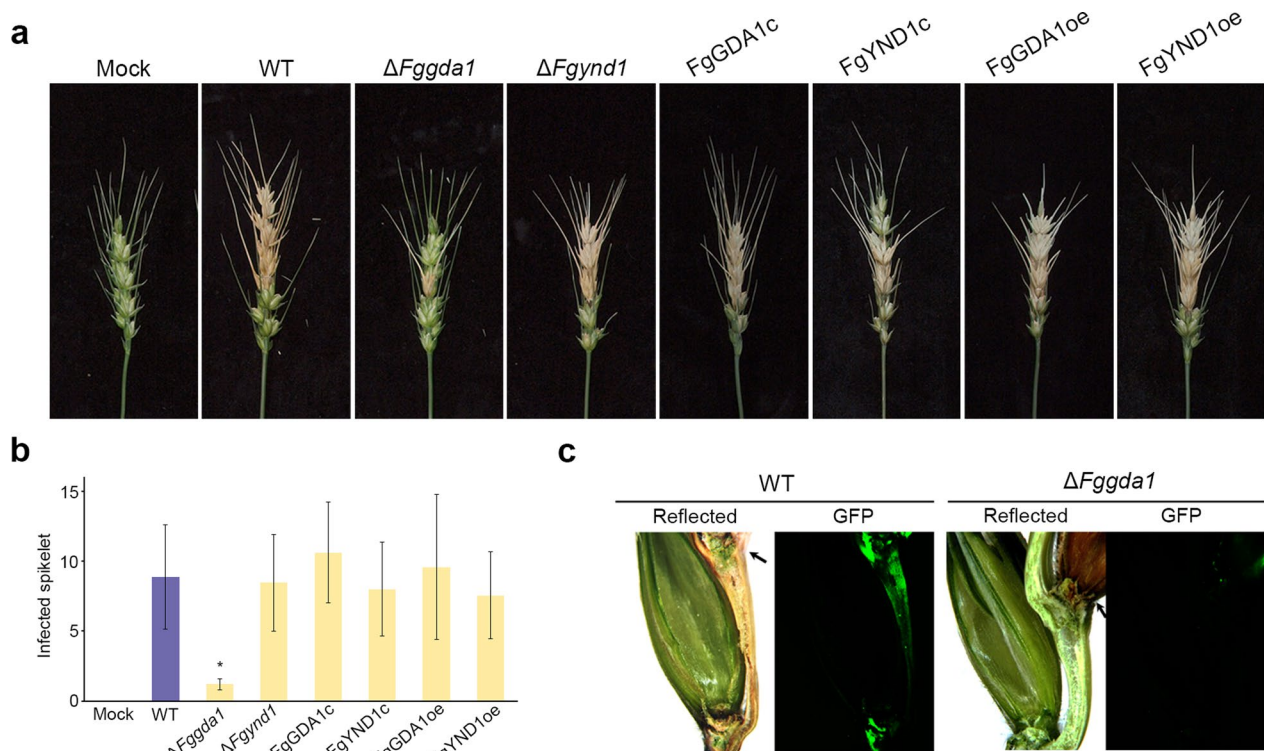
Previous studies showed that Gda1 modulates protein glycosylation and cell wall glycan structures in several yeast species (Abeijon et al. 1993; Herrero Ana et al. 2002; Jin et al. 2020). We therefore hypothesized that *FgGDA1* regulates protein glycosylation in *F. graminearum*, thereby contributing to fungal virulence. To test this, we performed Concanavalin A (ConA)-based glycoproteome profiling of the wild-type and  $\Delta Fggda1$  strains. A total of 125 glycoproteins were identified, of which 55 were upregulated and 21 were downregulated in the  $\Delta Fggda1$  mutant.



**Fig. 2** Phenotypic characterization of the *FgGDA1/FgYND1* deletion mutant strains. **a** Mycelial growth of *F. graminearum* strains on complete medium (CM) and minimal medium (MM). Pictures were taken 4 days after inoculation. **b** Perithecia formation. The perithecia were imaged 7 days after sexual induction on carrot agar. Scale bar = 1000  $\mu$ m. **c** Asexual reproduction. The number of conidia produced by each strain was counted after 5 days of cultivation in carboxymethyl cellulose (CMC) medium. Error bars represent standard deviations of biological replicates ( $n=3$  for wild-type,  $n=6$  for  $\Delta Fggda1$ ,  $\Delta Fgynd1$ , FgGDA1c, FgYND1c, FgGDA1oe, and FgYND1oe). **d** Macroconidia morphology. Macroconidia were induced in CMC medium for 5 days and subsequently observed by bright field (BF) microscopy. Scale bar: 20  $\mu$ m. **e** Dot plot showing the conidial length of each strain. The dashed line represents the median value. The length of 100 conidia induced on CMC medium was measured for each strain. Significant difference ( $***P < 0.001$ ), compared to the  $\Delta FgGda1$  mutant strain was indicated by asterisks

Principal component analysis (PCA) revealed a clear separation between the wild type and  $\Delta Fggda1$ , suggesting a strong impact of *FgGDA1* on global glycoprotein composition (Fig. 5a). Consistently, gene ontology (GO) enrichment of upregulated glycoproteins highlighted carbohydrate derivative metabolic processes (GO:1901135) (Fig. 5b), and functional enrichment network analysis further showed carbon metabolism as a central node among affected glycoproteins (Fig. 5c). These data suggest that *FgGDA1* strongly influences the glycosylation profiles of proteins, particularly those associated with carbon metabolism.

We also measured the expression of known *N*- and *O*-mannosylation-related genes (*OST1*, *OST5*, *PMT1*, and *PMT2*) in the plant pathogenic fungi (Xu et al. 2020; Cui et al. 2023; Moon et al. 2025). Compared with the wild-type strain, the  $\Delta Fggda1$  mutant showed ~twofold higher transcript levels of the *O*-mannosyltransferase *FgPMT1* and *FgPMT2* (Fig. 5d). We next examined whether loss of *FgGDA1* affects mannosylation of *O*-glycoproteins in the fungal cell wall. However, *O*-linked glycan profiles did not differ significantly among the wild type,  $\Delta Fggda1$ , and  $\Delta Fgynd1$  mutants (Fig. 5e). Together, these results suggest that *FgGDA1* broadly influences protein



**Fig. 3** Virulence of *F. graminearum* strains. **a** Wheat head virulence assay. The center spikelet of each wheat head was inoculated with 10  $\mu$ L of conidial suspension. 0.01% Tween 20 was used for mock inoculation. The disease index (diseased spikelets per wheat head) is denoted below the picture. The pictures were taken 21 days after inoculation. **b** Disease index of each fungal strain. The infected spikelets per wheat head were counted. Error bars represent standard deviations of biological replicates ( $n=10$  for wild-type,  $n=20$  for  $\Delta Fggda1$ ,  $\Delta Fgynd1$ , FgGDA1c, FgYND1c, FgGDA1oe, and FgYND1oe). **c** Microscopic images of the cross-sections of the wheat spikes after inoculation with fungal strains. The conidial suspension of the strains expressing green fluorescent protein (GFP) in the cytosolic region was injected into the center spikelet. Infected wheat heads were harvested 6 days after inoculation and were longitudinally sectioned. GFP fluorescence signal indicates fungal hyphal growth spreading from the inoculation sites. Arrowheads indicate the inoculated spikelets. “Reflected” means reflected light

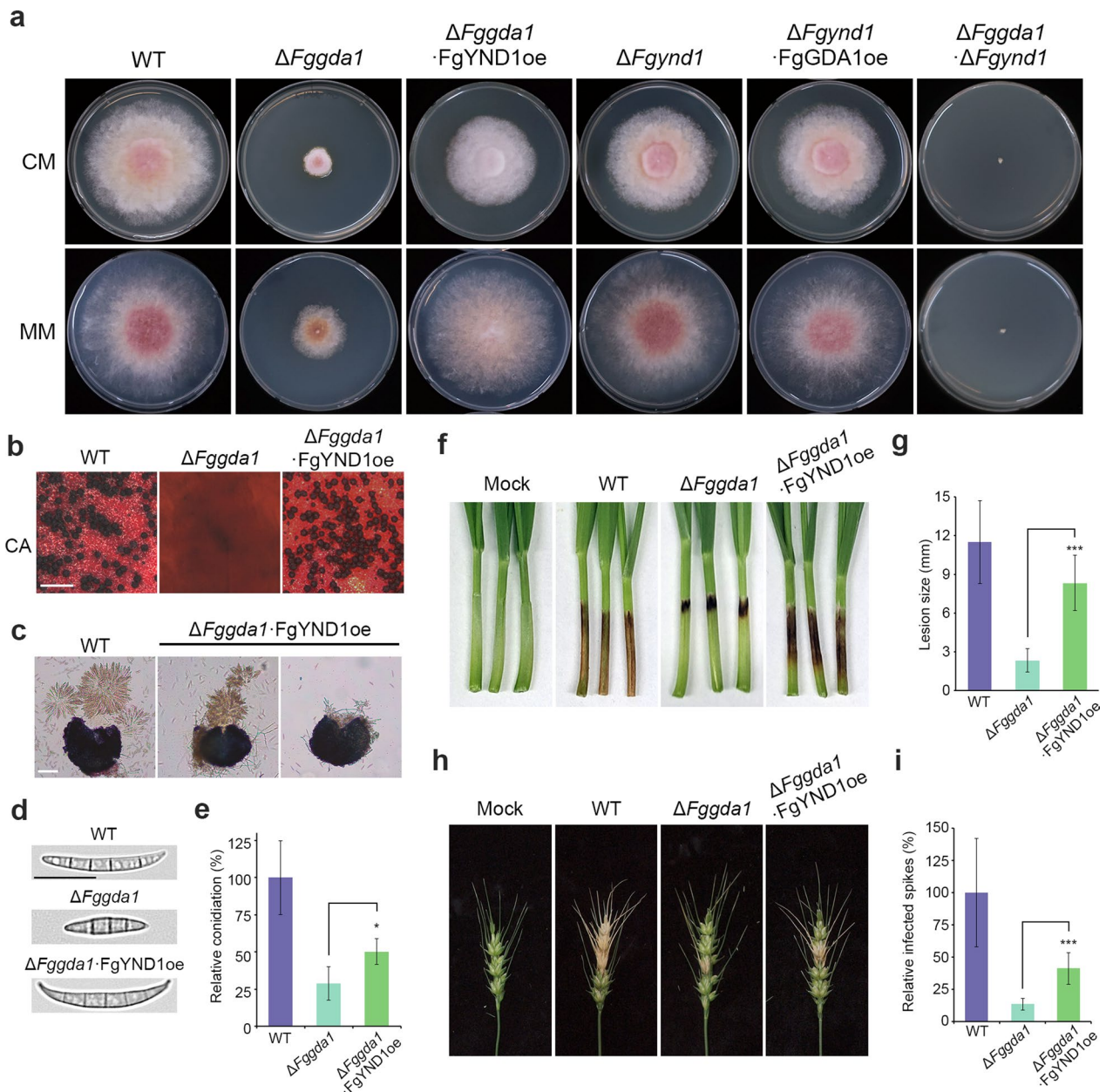
glycosylation but has only a minor effect on the *O*-linked glycan structures of cell wall proteins in *F. graminearum*.

## Discussion

Golgi-localized nucleoside diphosphatases are essential for key Golgi functions, such as glycosylation of lipids and proteins. Since over half of cellular proteins are affected by protein glycosylation, one of the most prevalent PTMs, its precise regulation is essential for cellular function (He et al. 2024). Importantly, protein glycosylation can contribute to fungal virulence in plant pathogenic fungi. In the rice blast fungus *Magnaporthe oryzae* and the corn smut fungus *Ustilago maydis*, genes in the *N*/*O*-glycosylation pathways affect the formation of infection structures, ultimately influencing appressorium-mediated penetration (Fernández-Álvarez et al. 2009; Fernandez-Alvarez et al. 2012; Chen et al. 2014, 2020). Furthermore, previous studies have shown that the *N*-glycoproteome of *F. graminearum* is dramatically altered in response to antifungal volatile treatments (Yu

et al. 2016). Collectively, these findings suggest that protein glycosylation is strongly involved in sensing environmental cues, stress responses, and subsequent fungal developmental processes.

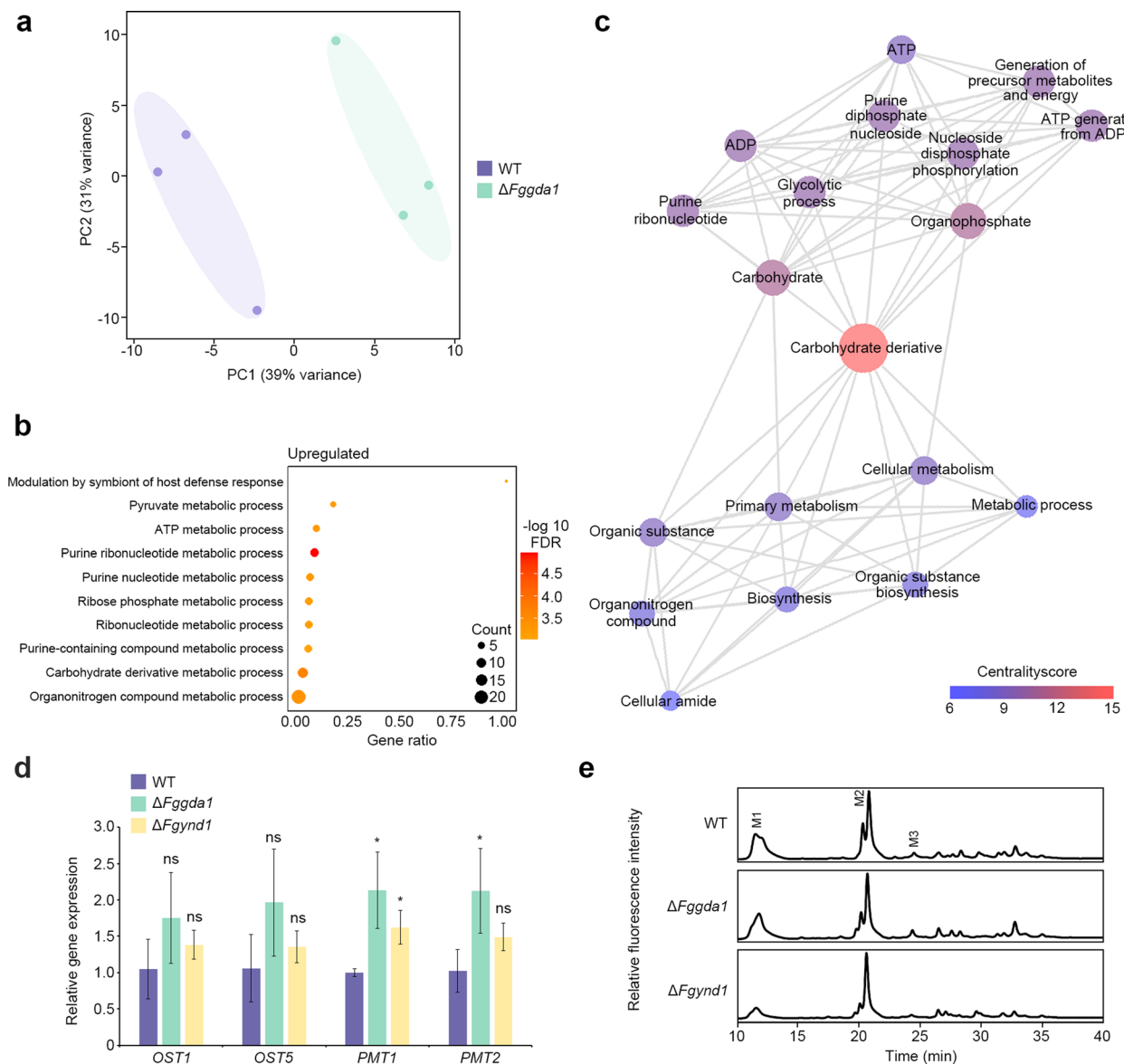
In this study, we identified two Golgi-localized GDPases, Gda1 and Ynd1, as important regulators of protein glycosylation in *F. graminearum*. Both Gda1 and Ynd1 have been reported to function as GDPases, with Ynd1 exhibiting broader substrate specificity, as it can also utilize UDP as a substrate (Gao et al. 1999). Despite its broader substrate range, gene knockout studies in *S. cerevisiae* and *C. neoformans* consistently indicate that GDA1 plays a more crucial role in fungal development (Gao et al. 1999; Jin et al. 2020). In line with this, we observed that deletion of FgGDA1 caused severe growth defects, whereas deletion of FgYND1 resulted in only mild growth impairment, indicating distinct functional contributions of these enzymes. Furthermore, overexpression of FgYND1 partially rescued both the growth defect and virulence of the  $\Delta Fggda1$  mutant, suggesting



**Fig. 4** Overexpression of *FgYND1* partially rescues the  $\Delta Fggda1$  mutant phenotype. **a** Mycelial growth of *F. graminearum* strains on complete medium (CM) and minimal medium (MM). Pictures were taken 4 days after inoculation. **b** Perithecia formation. The perithecia were imaged 7 days after sexual induction on carrot agar. Scale bar = 1000  $\mu$ m. **c** Asci and ascospores formed in a perithecium of the wild-type,  $\Delta Fggda1$ , and  $\Delta Fggda1$ -FgYND1oe mutant strains. Scale bar = 25  $\mu$ m. **d** Macroconidia morphology. Scale bar = 20  $\mu$ m. Macroconidia were induced in CMC medium for 5 days and subsequently observed by bright field (BF) microscopy. Scale bar = 20  $\mu$ m. **e** Conidial production. Error bars represent standard deviations of biological replicates ( $n=3$  for wild-type,  $n=6$  for  $\Delta Fggda1$ ,  $\Delta FgGda1$ -FgYND1oe). **f** Virulence on coleoptiles and **g** lesion size. Conidial suspension was inoculated into fresh wheat coleoptile wounds. Distilled water was used as a mock control. The pictures were taken 5 days after inoculation. Error bars represent standard deviations of biological replicates ( $n=12$  for wild-type,  $n=24$  for  $\Delta Fggda1$ , and  $\Delta FgGda1$ -FgYND1oe). **h** Virulence on wheat heads and **i** disease index (diseased spikelets per wheat head). Error bars represent standard deviations of biological replicates ( $n=10$  for wild-type,  $n=20$  for  $\Delta Fggda1$ , and  $\Delta FgGda1$ -FgYND1oe). Significant differences (\* $P < 0.05$ ; \*\*\* $P < 0.001$ ) are indicated by asterisks

a partially redundant role for these two genes in fungal development and virulence, consistent with previous reports (Gao et al. 1999). In contrast, double deletion

of *GDA1* and *YND1* has produced variable outcomes in different fungal species: in *S. cerevisiae*, the double deletion resulted in more severe defects than single deletions,



**Fig. 5** Glycoproteomic analysis of *F. graminearum* strains. **a** Principal Component Analysis (PCA) of glycoproteome samples from the wild-type and  $\Delta Fggda1$  mutant strains. The glycoproteome profiles show clear separation based on the genotypes of the strains. **b** Gene ontology (GO) enrichment analysis of the upregulated proteins in the  $\Delta Fggda1$  mutant strain. Top 10 upregulated biological processes were selected and visualized as a scatterplot. **c** Protein-protein interactions (PPIs) associated with enriched GO biological processes in the glycoproteome of the  $\Delta Fggda1$  mutant strain were analyzed. A total of 19 GO biological processes were visualized using the R program. **d** Transcript levels of glycosylation-related genes in *F. graminearum* strains relative to ubiquitin C-terminal hydrolase (*UBH1*) were measured by quantitative reverse transcription-polymerase chain reaction (qRT-PCR). A total of  $5 \times 10^6$  conidia were inoculated into CM medium, incubated for two days, and then the resulting mycelia were harvested for use. The relative transcript abundances of the indicated gene in the wild-type strain were arbitrarily set to 1. Two deletion mutant strains were utilized in this experiment. Significant differences ( $*P < 0.05$ ) in the relative transcript levels of each gene compared with the wild-type strain are indicated with asterisks. **e** HPLC profiles of O-linked glycans assembled on the cell wall mannoproteins of *F. graminearum* strains. M1–M3 peaks correspond to mannose residues

whereas in *C. neoformans* and *F. graminearum*, the double deletion resulted in lethal or nearly lethal developmental defects (Gao et al. 1999; Jin et al. 2020). Unlike in other fungi, disruption of *FgGDA1* and *FgYND1* did not markedly alter O-linked glycan structures in the cell wall,

highlighting the importance of species-specific genetic analyses.

A key finding of this study is the strong influence of *FgGDA1* on global protein glycosylation. Unlike *C. albicans*, where *GDA1* disruption alters O-glycan structures,

*FgGDA1* had only minimal impact on cell wall O-glycans but profoundly affected overall glycoprotein composition. This is evident from ConA-captured glycoproteome analysis, where the glycoprotein profile of the  $\Delta FgGda1$  mutant was distinctly different from that of the wild-type strain. Notably, the enriched glycoproteins are associated with carbon metabolism, including citrate synthase, pyruvate dehydrogenase subunits, and pyruvate decarboxylase.

Furthermore, loss of *FgGDA1* led not only to increased levels of glycosylated proteins but also to elevated expression of several glycosylation-related enzymes. These observations suggest that protein glycosylation activity is upregulated to compensate for reduced mannosylation capacity caused by a decreased supply of the GDP-mannose precursor in the absence of *FgGDA1*. Given that hyperglycosylation of enzymes can reduce substrate affinity, such modifications may disrupt normal cellular processes, ultimately leading to growth deficiencies (Kruszewska et al. 2008; Adav et al. 2014). Collectively, these results highlight that *FgGDA1* participates in a negative feedback response to Golgi mannosylation defects, influencing the expression of glycosylation-related genes and the overall glycoprotein profiles.

## Conclusions

In conclusion, we performed a functional characterization of the *GDA1* and *YND1* orthologs in *F. graminearum*, revealing extensive pleiotropic phenotypic defects upon deletion of *FgGDA1*. Specifically, *FgGDA1* contributes substantially to overall fungal development and virulence, with overexpression of *FgYND1* partially rescuing the developmental defects caused by *FgGDA1* deletion. Although *FgGDA1* does not significantly alter cell wall glycan structures, we demonstrated distinct glycoproteome profiles between the wild-type and  $\Delta FgGda1$  mutant. Collectively, these findings indicate that *FgGDA1* serves as a pivotal regulator of protein glycosylation in *F. graminearum*. Further investigation of its specific glycoprotein targets will advance our understanding of how this enzyme governs fungal development and virulence.

## Methods

### Fungal strains and culture conditions

The *F. graminearum* wild-type strain Z-3639 was used as parental strains for transformation experiments. All strains were stored as mycelial suspensions in a 20% glycerol solution at  $-80^{\circ}\text{C}$ . The culture media were prepared following the *Fusarium* laboratory manual (Leslie and Summerell 2008) and the fungal strains were grown at a temperature of  $25^{\circ}\text{C}$ .

### Vegetative growth, conidiation, and sexual development

Colony morphology was assessed on complete medium (CM;  $\text{NaNO}_3$ , 2.0 g;  $\text{KH}_2\text{PO}_4$ , 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g; KCl, 0.5 g; sucrose, 30.0 g; peptone, 2.5 g; yeast extract, 1.0 g; trace element solution, 0.2 mL; vitamin stock, 10.0 mL; and agar, 20.0 g per liter of medium) and minimal medium (MM;  $\text{NaNO}_3$ , 2.0 g;  $\text{KH}_2\text{PO}_4$ , 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g; KCl, 0.5 g; sucrose, 30.0 g; trace element solution, 0.2 mL; and agar, 20.0 g per liter of medium) 4–5 days after inoculation. For conidiation assays, five fresh mycelial plugs from CM were inoculated in 50 mL of carboxymethylcellulose (CMC) medium ( $\text{NH}_4\text{NO}_3$ , 1.0 g;  $\text{KH}_2\text{PO}_4$ , 1.0 g;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.5 g; yeast extract, 1.0 g; and carboxymethyl cellulose, 15.0 g per liter of medium) medium for *F. graminearum* for 5 days on a rotary shaker (150 rpm) and the number of conidia was measured with a hemocytometer. The spore structure was observed under a light microscope at  $400\times$  magnification (Leica DM500, Jena, Germany). The conidial lengths were examined based on measurements from 100 spores per isolate using ImageJ (Schneider et al. 2012) and the dot plots were drawn with GraphPad Prism 8.

For sexual development of *F. graminearum*, fungal strains were grown on carrot agar (carrot, 20.0 g and agar, 20.0 g per liter of medium) for 7 days, and aerial mycelia were removed with 0.4 mL of a sterile 2.5% Tween 60 solution (Leslie and Summerell 2008). The plates were then incubated under near-UV light (wavelength: 365 nm; Sankyo Denki, Tokyo, Japan) for 7 days.

### Nucleic acid manipulations, Southern blotting, and PCR

The genomic DNA was extracted from freeze-dried mycelia powder according to the *Fusarium* laboratory manual (Leslie and Summerell 2008). Total RNA was extracted from mycelia ground in liquid nitrogen using the Easy-Spin Total RNA Extraction Kit (Intron Biotech, Seongnam, Republic of Korea). Standard protocols were used for restriction endonuclease digestion and agarose gel electrophoresis (Sambrook 2001). Southern blot hybridization was performed using the North2South Biotin Random Prime Labeling Kit and the North2South Chemiluminescent Hybridization and Detection Kit (Thermo Scientific, USA). The PCR primers (Additional file 2: Table S1) were synthesized by an oligonucleotide synthesis facility (Bioneer, Seoul, Republic of Korea).

### Genetic manipulations and fungal transformations

The double-joint (DJ) PCR method was used to generate the fusion PCR products required for gene deletion and

complementation (Yu et al. 2004). Fungal transformation was performed as previously described (Son et al. 2011b).

To construct deletion mutants, the 5' and 3' flanking regions of the target genes were amplified from the genomic DNA, and the geneticin resistance gene cassette (*GEN*) was amplified from pII99 (Namiki et al. 2001). Three fragments were fused by the DJ PCR method, and the final constructs were amplified using the nested primers. The resulting amplicons were transformed into the fungal wild-type protoplasts, and the mutants were confirmed by Southern hybridization with a flanking-region probe (Additional file 1: Figure S1).

For complementation and overexpression, the *FgGDA1-GFP* and *FgYND1-GFP* fusion constructs were generated via a yeast gap repair approach (Bruno et al. 2004). For complementation, the ORF of *FgGda1/FgYnd1* and its native promoter were amplified from the genomic DNA of the wild-type strain. The resulting construct and *XhoI*-digested pDL2 were co-transformed into the yeast strain PJ69-4A (Zhou et al. 2011) using the Alkali-Cation Yeast Transformation Kit (MP Bio, Seoul, Republic of Korea). The *FgGDA1-GFP/FgYND1-GFP* fusion vector obtained from the yeast transformants was transformed into *Escherichia coli* DH10B. After verification by sequencing, the plasmid DNA was extracted with the DNA-spin Plasmid DNA Purification Kit (Intron Biotech, Seongnam, Republic of Korea) and used to transform the deletion mutant strain. To overexpress *FgGDA1* and *FgYND1*, the ORF was amplified and cotransformed with *XhoI*-digested pDL2 into the yeast strain PJ69-4A, as described above. The RP27-*FgGDA1-GFP/RP27-FgYND1-GFP* fusion vector carrying the *Magnaporthe oryzae* ribosomal protein 27 promoter (Bourett et al. 2002) was obtained from the yeast transformants. The subsequent process was carried out in the same way.

To generate the cytosolic GFP-expressed mutant, the pIGPAPA vector was introduced to the  $\Delta Fggda1$  mutant strains. Fungal transformants showing the brightest fluorescence were used in subsequent microscopy analysis.

### Microscopic observation

Microscopic observation was performed with a Leica DM6 B microscope (Leica Microsystems, Wetzlar, Germany) equipped with a Leica DMC6200 camera using the fluorescent filter GFP (part no. 11504166). The perithecia were imaged 7 days after sexual induction using a Zeiss SteREO Lumar.V12 microscope (Carl Zeiss, Germany).

To observe the spread of mycelia on wheat heads, wheat heads were inoculated with the cytosolic GFP-expressing strains and harvested at 6 days post-inoculation. Free-hand longitudinal sections across the center of infected spikelets were prepared using a clean razor blade. Sectioned wheat heads were imaged under reflected light

and GFP fluorescence settings (470-nm-excitation and 525-nm-emission wavelength filters) using a SteREO Lumar V12 microscope (Carl Zeiss, Oberkochen, Germany).

### Virulence test

The point inoculation method of wheat heads with *F. graminearum* was performed to assay fungal virulence as previously described (Son et al. 2011a). Conidia were harvested from CMC cultures, and 10  $\mu$ L of each suspension ( $10^5$  conidia/mL) was injected into the center spikelet of a wheat head. The inoculated wheat plants were incubated in a humid chamber for 3 days and grown in a greenhouse for an additional 18 days.

For the wheat coleoptile virulence assay, two-day-old seedlings of wheat were used for the coleoptile infection assay according to previous study with modifications (Wu et al. 2005). Two days after seed sowing, the top 2 to 3 mm of the coleoptiles were removed, and conidial suspension (10  $\mu$ L,  $1 \times 10^6$  conidia/mL) obtained from each strain was inoculated into fresh wheat coleoptile wounds. Distilled water-inoculated coleoptiles served as a mock control. After inoculation, the seedlings were grown in a growth chamber at 25°C and 95% humidity.

### Mycotoxin analysis

The DON extraction was performed as previously described (Park et al. 2012). For DON induction in rice media, a fresh mycelial plug was inoculated on 1 g of rice substrate for 3 weeks. The wheat or rice culture was harvested, ground, and mixed vigorously with 6 mL of 84% acetonitrile for 30 min. After phase separation, the upper phase was filtered through a 0.45  $\mu$ m syringe filter. Reverse-phase HPLC on a Prominence HPLC system (Shimadzu, Japan) with a C18 column was used for the analysis, with a simple modification of the previous methods (Kim et al. 2005; Ok et al. 2018). For DON detection, the mobile phase was 10% aqueous acetonitrile (ACN), and the flow rate was 1 mL/min. A gradient elution program was applied as follows: after 10% ACN was maintained for 11 min, it linearly increased to 30% ACN at 12 min. It was linearly decreased from 30% ACN at 12 min to 10% ACN at 18 min. Subsequently, 10% ACN was held for 17 min to re-equilibrate the column before injection of the next sample, giving a total run time of 35 min. The diode-array detection was applied (DON was detected at wavelength 237 nm).

### Gene expression analysis by qRT-PCR

For qRT-PCR, total RNA was prepared using the Easy-Spin Total RNA Extraction Kit (Intron Biotech, Seongnam, Republic of Korea). First-strand cDNA was synthesized from total RNA with SuperScript III reverse

transcriptase (Invitrogen, Carlsbad, CA, USA). qRT-PCR was carried out with SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) using the corresponding primers (Table S1). Cycling conditions were 10 min at 95°C followed by 40 cycles of 10 s at 95°C, 10 s at 62°C, and 20 s at 72°C. Data were analyzed using the  $\Delta\Delta C_t$  method (Livak and Schmittgen 2001). The endogenous housekeeping gene ubiquitin C-terminal hydrolase (*UBH1*) was used as a reference gene for *F. graminearum*. The PCR was repeated three times with three biological replicates. The relative transcript levels of target genes were calculated as previously described (Seong et al. 2008).

#### Western blot analysis

For total protein extraction, fresh mycelia were ground in liquid nitrogen and re-suspended in 250  $\mu$ L extraction buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 5% glycerol, and 1% NP-40) containing protease inhibitors. Lysates were sonicated and centrifuged at 5000 $\times g$  for 20 min. The resulting supernatants were quantified with Pierce 660 nm Protein Assay Reagent (Thermo Scientific, Waltham, USA) and were used for western blot analysis. After boiling the sample, the denatured proteins were separated on 10% SDS polyacrylamide gels and transferred to nitrocellulose membranes using the Trans-Blot Turbo Transfer System (Bio-Rad). Glycosylated proteins were detected using the ConA-HRP antibody. The procedure was performed according to the manufacturer's protocols, and the signals were visualized using a Chemi-Doc imaging system (Bio-Rad, Hercules, CA, USA).

#### Glycoproteomics analysis

The glycoproteins were obtained as described previously with minor modifications (Yu et al. 2016; Thak et al. 2018). The total proteins were dissolved with 10 mL of Concanavalin A (ConA) binding buffer (20 mM Tris-HCl, pH 7.4, 0.5 M NaCl, 1 mM  $\text{CaCl}_2$ , 1 mM  $\text{MnCl}_2$ ) and incubated with 1 mL of ConA-Sepharose beads (GE Healthcare, Chicago, IL) in a column for 2 h with slow rotation. The beads were then washed with 10 mL of ConA binding buffer, and glycoproteins were eluted by the addition of 5 mL of 0.5 M methyl- $\alpha$ -D-mannopyranoside and 0.5 M methyl  $\alpha$ -glucopyranoside. The glycoproteins were quantified with Pierce 660 nm Protein Assay Reagent (Thermo Scientific, Waltham, USA). After boiling sample, the denatured proteins were separated on SDS polyacrylamide gels and stained with Coomassie Brilliant Blue. Bands of interest were excised and trypsin digested. Tryptic peptides were analyzed using a Q Exactive system equipped with

EASY-nLC-1000 nano flow liquid chromatography system Reagent (Thermo Scientific, Waltham, USA). Samples were trapped in an Acclaim<sup>TM</sup> PepMap<sup>TM</sup> 100 C18 column (75  $\mu$ m $\times$ 2 cm, Thermo Scientific, San Jose, CA) and separated on an EASY-Spray column (75  $\mu$ m $\times$ 15 cm, particle size  $\leq$  3  $\mu$ m, pore size 100 Å, PepMap<sup>TM</sup> RSLC C18, Thermo Scientific, San Jose, CA) using a 90 min gradient of solvent A and B (0.1% FA in ACN). The gradient included 5% to 40% solvent B at a flow rate of 300 nL/min and a column temperature of 35°C. The eluted peptides were ionized at 2 kV. A full MS scan was performed from 400 to 2000 m/z with a full width at half maximum (FWHM) resolution of 70,000, followed by a data-dependent HCD MS/MS scan of the top 10 ions. Parameters used for MS/MS scan were: resolution=17,500, loop count=10 (Top 10), isolation window=m/z 2.0, normalized collision energy=27.

The raw MS spectra files were searched against the *Fusarium graminearum* protein database (14,183 entries from UniProtKB) using Proteome Discoverer (v2.4.1.15; Thermo Fisher Scientific). The search settings were as follows: Sequest HT search engine, trypsin (full), maximum two missed cleavage sites, MS tolerance (10 ppm), MS/MS tolerance 0.02 Da, carbamidomethylation (C) for fixed modification, and oxidation (M) for variable modification. Peptide/protein identification and quantitation were conducted on Scaffold (v5.0.1, Proteome Software Inc.) with a SEQUEST XCorr threshold of at least 1.8 for charge 1, 2.5 for charge 2, and 3.5 for charges 3 and 4. Total spectrum count was used for semiquantitative analysis. For normalization, the endogenous housekeeping protein Gadph was used as an internal control.

#### HPLC analysis of O-linked glycans from cell wall mannoproteins

Cell wall mannoproteins (cwMPs) were isolated from *Fusarium graminearum* mycelia with minor modifications to a previously described method (Thak et al. 2018). Briefly, 5 $\times$ 10<sup>6</sup> conidia from each strain were inoculated into 50 mL YEPD medium and incubated for 36 h in a rotary shaker at 150 rpm. cwMPs were then extracted as previously reported.

For O-glycan release, completely dried cwMPs (50  $\mu$ g) were resuspended in 100  $\mu$ L hydrazine monohydrate (Tokyo Chemical Industry, Tokyo, Japan) and incubated at 60°C for 4–6 h. The samples were dried to remove residual hydrazine monohydrate, and the resulting pellets were dissolved in 100  $\mu$ L saturated  $\text{NaHCO}_3$  (Sigma, Burlington, MA, USA). Subsequently, 10  $\mu$ L acetic anhydride ( $\text{CH}_3\text{CO}$ )<sub>2</sub>O was added, and the mixture was incubated on ice for 30 min without shaking. O-glycans were purified using Dowex 50WX8–400 resin ( $\text{H}^+$  form; Sigma), labeled with 2-aminobenzoic acid (2-AA; Sigma), and further purified with a cyano

base cartridge (Bond Elut-CN-E, 100 mg; Agilent). HPLC analysis of the 2-AA-labeled O-glycans was performed on a TSKgel Amide-80 column (0.46 × 25 cm, 5 µm; Tosoh, Tokyo, Japan) at a flow rate of 1.0 mL/min. The mobile phases consisted of solvent A (90%; 2% acetic acid and 1% tetrahydrofuran in acetonitrile) and solvent B (10%; 5% acetic acid, 3% triethylamine, and 1% tetrahydrofuran in HPLC-grade water) as previously described (Moon et al. 2025). After sample injection, the proportion of solvent B was maintained at 10% for 5 min, then increased linearly to 90% over 60 min. Fluorescence detection of 2-AA-oligosaccharides was carried out with a 2475 fluorescence detector (Waters, MA, USA) at excitation and emission wavelengths of 360 nm and 425 nm, respectively. Data were processed using Empower 2 chromatography software (Waters, MA, USA).

### Data analysis

Statistical analyses were performed for all continuous variables. Protein phylogeny was constructed using RaxML (Stamatakis 2014). Gene ontology analysis was conducted using the FungiDB server. Functional enrichment network analysis was performed using ShinyGO 0.77 and visualized in R. Principal component analysis (PCA) was carried out in R v4.4.3. Data visualization was also performed using R packages.

### Abbreviations

|         |                           |
|---------|---------------------------|
| ConA    | Concanavalin A            |
| GDP     | Guanosine diphosphate     |
| GDPases | Guanosine diphosphatases  |
| GMP     | Guanosine monophosphate   |
| NDPases | Nucleoside diphosphatases |

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s42483-025-00403-8>.

**Additional file1: Figure S1.** Confirmation of mutant construction. **Figure S2.** Mycotoxin production. **Figure S3.** Transcript levels of *FgGDA1* and *FgYND1* in the mutant strains.

**Additional file2: Table S1.** Primers used in this study.

### Acknowledgements

Not applicable

### Author contributions

SS, YC, DGS, and HS designed the project. SS, YC, TTTP, SS, NL, JP, SK, HJ, JEK, KM, HK, GJC, HAK performed the experiments. SS and HS prepared the figures and manuscript. All authors read and approved the final manuscript.

### Funding

This study was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIT) (No. RS-2025-00553624), the Cooperative Research Program for Agriculture Science & Technology Development (No. RS-2024-00397951), Rural Development Administration, Republic

of Korea, and the intramural grant from the Korea Institute of Science and Technology (KIST, Grand Challenge 2Z07251).

### Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

#### Ethical approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

Received: 2 October 2025 Accepted: 8 December 2025

Published online: 05 February 2026

### References

- Abeijon C, Yanagisawa K, Mandon E, Häusler A, Moremen K, Hirschberg C, et al. Guanosine diphosphatase is required for protein and sphingolipid glycosylation in the Golgi lumen of *Saccharomyces cerevisiae*. *J Cell Biol.* 1993;122:307–23. <https://doi.org/10.1083/jcb.122.2.307>.
- Adav SS, Ravindran A, Sze SK. Study of *Phanerochaete chrysosporium* secretome revealed protein glycosylation as a substrate-dependent post-translational modification. *J Proteome Res.* 2014;13:4272–80. <https://doi.org/10.1021/pr500385y>.
- Berninsone P, Miret JJ, Hirschberg CB. The Golgi guanosine diphosphatase is required for transport of GDP-mannose into the lumen of *Saccharomyces cerevisiae* Golgi vesicles. *J Biol Chem.* 1994;269:207–11. [https://doi.org/10.1016/S0021-9258\(17\)42335-0](https://doi.org/10.1016/S0021-9258(17)42335-0).
- Bourett TM, Sweigard JA, Czymmek KJ, Carroll A, Howard RJ. Reef coral fluorescent proteins for visualizing fungal pathogens. *Fungal Genet Biol.* 2002;37:211–20. [https://doi.org/10.1016/S1087-1845\(02\)00524-8](https://doi.org/10.1016/S1087-1845(02)00524-8).
- Brandizzi F, Barlowe C. Organization of the ER–Golgi interface for membrane traffic control. *Nat Rev Mol Cell Biol.* 2013;14:382–92. <https://doi.org/10.1038/nrm3588>.
- Bruno KS, Tenjo F, Li L, Hamer JE, Xu J-R. Cellular localization and role of kinase activity of *PMK1* in *Magnaporthe grisea*. *Eukaryot Cell.* 2004;3:1525–32. <https://doi.org/10.1128/ec.3.6.1525-1532.2004>.
- Chen XL, Shi T, Yang J, Shi W, Gao X, Chen D, et al. N-glycosylation of effector proteins by an α-1,3-mannosyltransferase is required for the rice blast fungus to evade host innate immunity. *Plant Cell.* 2014;26:1360–76. <https://doi.org/10.1105/tpc.114.123588>.
- Chen Y, Kistler HC, Ma Z. *Fusarium graminearum* trichothecene mycotoxins: biosynthesis, regulation, and management. *Annu Rev Phytopathol.* 2019;57:15–39. <https://doi.org/10.1146/annurev-phyto-082718-100318>.
- Chen XL, Liu C, Tang B, Ren Z, Wang GL, Liu W. Quantitative proteomics analysis reveals important roles of N-glycosylation on ER quality control system for development and pathogenesis in *Magnaporthe oryzae*. *PLoS Pathog.* 2020;16:e1008355. <https://doi.org/10.1371/journal.ppat.1008355>.
- Cui T, Ma Q, Zhang F, Chen S, Zhang C, Hao J, et al. Characterization of PcSTT3B as a key oligosaccharyltransferase subunit involved in N-glycosylation and its role in development and pathogenicity of *Phytophthora capsici*. *Int J Mol Sci.* 2023;24:7500. <https://doi.org/10.3390/ijms24087500>.
- D'Alessio C, Trombetta ES, Parodi AJ. Nucleoside diphosphatase and glycosyltransferase activities can localize to different subcellular compartments in *Schizosaccharomyces pombe*. *J Biol Chem.* 2003;278:22379–87. <https://doi.org/10.1074/jbc.M300892200>.
- Day KJ, Staehelin LA, Glick BS. A three-stage model of Golgi structure and function. *Histochem Cell Biol.* 2013;140:239–49. <https://doi.org/10.1007/s00418-013-1128-3>.
- Fernández-Álvarez A, Elías-Villalobos A, Ibeas II. The O-mannosyltransferase *PMT4* is essential for normal appressorium formation and penetration in

- Ustilago maydis*. Plant Cell. 2009;21:3397–412. <https://doi.org/10.1105/tpc.109.065839>.
- Fernandez-Alvarez A, Marin-Menguiano M, Lanver D, Jimenez-Martin A, Elias-Villalobos A, Perez-Pulido AJ, et al. Identification of O-mannosylated virulence factors in *Ustilago maydis*. PLoS Pathog. 2012;8:e1002563. <https://doi.org/10.1371/journal.ppat.1002563>.
- Gao XD, Kaigorodov V, Jigami Y. YND1, a homologue of GDA1, encodes membrane-bound apyrase required for Golgi N- and O-glycosylation in *Saccharomyces cerevisiae*. J Biol Chem. 1999;274:21450–6. <https://doi.org/10.1074/jbc.274.30.21450>.
- Goswami RS, Kistler HC. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol Plant Pathol. 2004;5:515–25. <https://doi.org/10.1111/j.1364-3703.2004.00252.x>.
- He M, Zhou X, Wang X. Glycosylation: mechanisms, biological functions and clinical implications. Signal Transduct Target Ther. 2024;9:194. <https://doi.org/10.1038/s41392-024-01886-1>.
- Herrero Ana B, Uccelletti D, Hirschberg CB, Dominguez A, Abejón C. The Golgi GDPase of the fungal pathogen *Candida albicans* affects morphogenesis, glycosylation, and cell wall properties. Eukaryot Cell. 2002;1:420–31. <https://doi.org/10.1128/ec.1.3.420-431.2002>.
- Hirschberg CB, Robbins PW, Abejón C. Transporters of nucleotide sugars, ATP, and nucleotide sulfate in the endoplasmic reticulum and Golgi apparatus. Annu Rev Biochem. 1998;67:49–69. <https://doi.org/10.1146/annurev.biochem.67.1.49>.
- Jin JH, Lee KT, Hong J, Lee D, Jang EH, Kim JY, et al. Genome-wide functional analysis of phosphatases in the pathogenic fungus *Cryptococcus neoformans*. Nat Commun. 2020;11:4212. <https://doi.org/10.1038/s41467-020-18028-0>.
- Khan MK, Pandey A, Athar T, Choudhary S, Deval R, Gezgin S, et al. Fusarium head blight in wheat: contemporary status and molecular approaches. 3 Biotech. 2020;10:172. <https://doi.org/10.1007/s13205-020-2158-x>.
- Kim YT, Lee YR, Jin J, Han KH, Kim H, Kim JC, et al. Two different polyketide synthase genes are required for synthesis of zearalenone in *Gibberella zeae*. Mol Microbiol. 2005;58:1102–13. <https://doi.org/10.1111/j.1365-2958.2005.04884.x>.
- Kruszewska J, Perlińska-Lenart U, Górka-Nieć W, Orłowski J, Zembek P, Palamarczyk G. Alterations in protein secretion caused by metabolic engineering of glycosylation pathways in fungi. Acta Biochim Pol. 2008;55:447–56.
- Leslie JF, Summerell BA. The *Fusarium* laboratory manual. Amsterdam: Wiley; 2008.
- Liu Y-W, Huang C-F, Huang K-B, Lee F-JS. Role for Gcs1p in regulation of Arl1p at trans-Golgi compartments. Mol Biol Cell. 2005;16:4024–33. <https://doi.org/10.1091/mbc.e05-01-0023>.
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the  $2^{-\Delta\Delta CT}$  method. Methods. 2001;25:402–8. <https://doi.org/10.1006/meth.2001.1262>.
- Moon H, Thak EJ, Choi Y, Kim S, Park J, Lee N, et al. Integrative glycomic analysis reveals the crucial role of protein glycosylation in fungal pathogenesis. PLoS Pathog. 2025;21:e103325. <https://doi.org/10.1371/journal.ppat.103325>.
- Munkvold GP. Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears. Eur J Plant Pathol. 2003;109:705–13. <https://doi.org/10.1023/A:1026078324268>.
- Namiki F, Matsunaga M, Okuda M, Inoue I, Nishi K, Fujita Y, et al. Mutation of an arginine biosynthesis gene causes reduced pathogenicity in *Fusarium oxysporum* f. sp. *melonis*. Mol Plant Microbe Interact. 2001;14:580–4. <https://doi.org/10.1094/MPMI.2001.14.4.580>.
- Ok HE, Lee SY, Chun HS. Occurrence and simultaneous determination of nivalenol and deoxynivalenol in rice and bran by HPLC-UV detection and immunoaffinity cleanup. Food Control. 2018;87:53. <https://doi.org/10.1016/j.foodcont.2017.12.005>.
- Park AR, Cho AR, Seo JA, Min K, Son H, Lee J, et al. Functional analyses of regulators of G protein signaling in *Gibberella zeae*. Fungal Genet Biol. 2012;49:511–20. <https://doi.org/10.1016/j.fgb.2012.05.006>.
- Potel CM, Burtscher ML, Garrido-Rodriguez M, Brauer-Nikonow A, Becher I, Le Sueur C, et al. Uncovering protein glycosylation dynamics and heterogeneity using deep quantitative glycoproteomics (DQglyco). Nat Struct Mol Biol. 2025;32:1111–26. <https://doi.org/10.1038/s41594-025-01485-w>.
- Sambrook J. Molecular cloning: a laboratory manual. 3rd ed. New York: Cold Spring Harbor Laboratory Press; 2001.
- Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. Nat Methods. 2012;9:671–5.
- Schwabl S, Teis D. Protein quality control at the Golgi. Curr Opin Cell Biol. 2022;75:102074. <https://doi.org/10.1016/j.cceb.2022.02.008>.
- Seong KY, Zhao X, Xu JR, Güldener U, Kistler HC. Conidial germination in the filamentous fungus *Fusarium graminearum*. Fungal Genet Biol. 2008;45:389–99. <https://doi.org/10.1016/j.fgb.2007.09.002>.
- Son H, Lee J, Park AR, Lee YW. ATP citrate lyase is required for normal sexual and asexual development in *Gibberella zeae*. Fungal Genet Biol. 2011a;48:408–17. <https://doi.org/10.1016/j.fgb.2011.01.002>.
- Son H, Seo YS, Min K, Park AR, Lee J, Jin JM, et al. A phenome-based functional analysis of transcription factors in the cereal head blight fungus, *Fusarium graminearum*. PLoS Pathog. 2011b;7:e1002310. <https://doi.org/10.1371/journal.ppat.1002310>.
- Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics. 2014;30:1312–3. <https://doi.org/10.1093/bioinformatics/btu033>.
- Thak EJ, Kim J, Lee DJ, Kim JY, Kang HA. Structural analysis of N-/O-glycans assembled on proteins in yeasts. J Microbiol. 2018;56:11–23. <https://doi.org/10.1007/s12275-018-7468-x>.
- Wilson WW, McKee G, Nganje W, Dahl B, Bangsund D. Economic impact of USWBS's scab initiative to reduce FHB. Agribusiness Appl Econ.; 2017. p. 163.
- Wu AB, Li HP, Zhao CS, Liao YC. Comparative pathogenicity of *Fusarium graminearum* isolates from China revealed by wheat coleoptile and floret inoculations. Mycopathologia. 2005;160:75–83.
- Xu Y, Zhou H, Zhao G, Yang J, Luo Y, Sun S, et al. Genetical and O-glycoproteomic analyses reveal the roles of three protein O-mannosyltransferases in phytopathogen *Fusarium oxysporum* f.sp. *cucumerinum*. Fungal Genet Biol. 2020;134:103285. <https://doi.org/10.1016/j.fgb.2019.103285>.
- Yu JH, Hamari Z, Han KH, Seo JA, Reyes-Domínguez Y, Scazzocchio C. Double-joint PCR: a PCR-based molecular tool for gene manipulations in filamentous fungi. Fungal Genet Biol. 2004;41:973–81. <https://doi.org/10.1016/j.fgb.2004.08.001>.
- Yu L, He H, Hu Z, Ma Z. Comprehensive quantification of N-glycoproteome in *Fusarium graminearum* reveals intensive glycosylation changes against fungicide. J Proteomics. 2016;142:82–90.
- Zhang X, Wang Y. Glycosylation quality control by the Golgi structure. J Mol Biol. 2016;428:3183–93.
- Zhou X, Li G, Xu JR. Efficient approaches for generating GFP fusion and epitope-tagging constructs in filamentous fungi. In: Xu JR, Bluhm BH, editors. Fungal Genomics: Methods and Protocols. Totowa: Humana Press; 2011. p. 199–212.