Glucose-6-Phosphate Isomerase FgGPI, a β_2 Tubulin-Interacting Protein, Is Indispensable for Fungal Development and Deoxynivalenol Biosynthesis in *Fusarium graminearum*

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ABSTRACT

Glucose-6-phosphate isomerase (GPI) is ubiquitous in most organisms, catalyzing the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate. In this study, we investigated biological and genetic functions of *FgGPI* in the phytopathogen *Fusarium graminearum*. We found that hyphal growth, conidial germination, and septa formation were significantly inhibited in *FgGPI* deletion mutant $\Delta FgGPI$. *FgGPI* was also positively associated with glucose metabolism, ATP biosynthesis, and carbon source utilization. In addition, pyruvate production, deoxynivalenol (DON) biosynthesis, and virulence were reduced in $\Delta FgGPI$. A coimmunoprecipitation assay demonstrated that

FgGPI interacts with *Fg* β_2 . More importantly, the coimmunoprecipitation assay showed that carbendazim-resistant substitutions in β_2 tubulin could reduce the interaction intensity between *FgGPI* and *Fg* β_2 , thereby increasing *FgGPI* expression and accelerating DON biosynthesis in carbendazim-resistant strains. Taken together, our work revealed the indispensable role of *FgGPI* in fungal developmental processes, DON biosynthesis, and pathogenicity in *F. graminearum*.

Keywords: developmental processes, DON, *FgGPI*, fungal pathogens, *Fusarium graminearum*, genetics

Fusarium head blight (FHB), a major disease of wheat and several small grain cereals worldwide, is caused by the filamentous fungus *Fusarium graminearum* (Goswami and Kistler 2004). Infection by *F. graminearum* in wheat not only causes huge economic and yield losses, but the trichothecenes secreted by the pathogen, including deoxynivalenol (DON), zearalenone, and other type B trichothecenes, also endanger human and animal health (Alexander et al. 2009; Goswami and Kistler 2004). Because trichothecenes have been demonstrated as an important virulence factor of *F. graminearum* (Alexander et al. 2009; Goswami and Kistler 2004), a comprehensive understanding of trichothecene regulation and biosynthesis is essential for the development of effective control strategies, not only for FHB control but for grain storage management as well.

In China, carbendazim and other benzimidazole fungicides have been used extensively to control FHB since the 1970s (Zhou et al. 1994). However, the effectiveness of carbendazim and other benzimidazole fungicides has been threatened by the emergence of resistant populations in the field since the 1990s (Zhou and Wang 2001). Our previous studies demonstrated that amino acid substitutions in β_2 tubulin protein at codons 167, 198, or 200 are responsible for carbendazim resistance (Chen et al. 2008; Liu et al. 2019). Among these substitutions, the F167Y substitution is dominant in the field and occupied >90% (Duan et al. 2015; Yang et al. 2018). Notably, carbendazim resistance can enhance DON biosynthesis of *F. graminearum* (Zhang et al. 2009; Zhou et al.

2020b). Our previous quantitative reverse transcription PCR (qRT-PCR) assays showed that the F167Y substitution in β_2 tubulin is involved in the regulation of multiple metabolism processes, such as glycolysis and the tricarboxylic acid (TCA) cycle, which are closely associated with secondary metabolism and pathogenicity in *F. graminearum* (Zhang et al. 2016; Zhou et al. 2020b).

Farnesyl pyrophosphate is the precursor of DON biosynthesis, which is synthesized by the isoprenoid biosynthetic pathway (Kimura et al. 2007). Pyruvate, the precursor of the isoprenoid biosynthetic pathway, is synthesized by glycolysis. Glucose-6-phosphate isomerase (GPI) controls the second step of glycolysis and catalyzes the reversible isomerization of glucose-6-phosphate and fructose-6-phosphate (Haller et al. 2011; Lin et al. 2009). *GPI* deficiency results in temperature-sensitive growth and significantly decreases glycerolipid biosynthesis in Chinese hamster cell lines (Haller et al. 2010). Similarly, *GPI*-deficient mutants exhibit reduced phosphatidate phosphatase 1 activity and glycerolipid biosynthesis in animal cells (Haller et al. 2010). The knockout of *GPI* severely reduces glucose consumption and lactic acid secretion, which fully suppresses the “Warburg effect” in tumor cells (de Padua et al. 2017). In addition, Sierkstra et al. (1993) found that *GPI* is necessary for glucose repression in *Saccharomyces cerevisiae*. In human pathogen *Cryptococcus neoformans*, *GPI* plays crucial roles in virulence factor production, stress resistance, and cell wall integrity (Zhang et al. 2015). In addition, GPI protein has interesting physiological functions when it is secreted to the extracellular matrix through yet-to-be-determined secretory pathways. For instance, GPI was identified as an autocrine motility factor in tumor cells (Faik et al. 1988; Watanabe et al. 1996). Although the biological functions of *GPI* are extensively studied in cells, mammals, human pathogens, and yeast, little is known regarding its function in phytopathogens, including *F. graminearum*. Therefore, it is worth studying the functions of *GPI* in fungal development, secondary metabolism, and pathogenicity in phytopathogens.

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To investigate the biological functions of *GPI*, deletion and complementation mutants were constructed in this study. Our results showed that the disruption of *FgGPI* resulted in reduced hyphal growth and abnormal conidial septa. The *FgGPI* deletion mutants ($\Delta FgGPI$) exhibited increased sensitivity to osmotic stress, but they showed decreased sensitivity to oxidative stress. In addition, $\Delta FgGPI$ could not utilize fructose and xylose as sole carbon sources. Pathogenicity assays showed that $\Delta FgGPI$ exhibited attenuated virulence in plants, which might be associated with reduced pyruvate production and DON biosynthesis. Furthermore, the substitutions with carbendazim resistance in β_2 tubulin could reduce the interaction between *GPI* and β_2 tubulin, thereby increasing *GPI* expression and accelerating DON biosynthesis in carbendazim-resistant strains.

MATERIALS AND METHODS

Strains and culture conditions. *F. graminearum* wild-type strain 2021 was used to construct the derived mutants used in this study (Supplementary Table S2). Growth rate and colony morphology assays for all strains were grown on potato dextrose agar (PDA), complete medium (CM), and minimal medium (MM). Mung bean broth (MBB) was used for the conidiation assay as described previously (Zhang et al. 2016). For DON production analysis, all mutants were cultured in trichothecene biosynthesis induction (TBI) medium at 28°C in the dark for 7 days.

Strain construction. The *FgGPI* deletion mutant was constructed using the homologous recombination strategy described previously (Liu et al. 2013; Qiu et al. 2011; Zhang et al. 2016). The open reading frame (ORF) of *FgGPI* was replaced with *HPH-HSV-tk*. All transformants were analyzed by PCR (*data not shown*), Southern blot, and qRT-PCR assays (Supplementary Fig. S1). In addition, we performed *FgGPI* complementation by reintroducing the entire *FgGPI* gene with its native promoter into the $\Delta FgGPI$ -1 mutant as described previously (Liu et al. 2013; Qiu et al. 2011; Zhang et al. 2016). All complementary transformants were analyzed by PCR and by qRT-PCR assays (*data not shown*).

To construct the *FgGPI*-green fluorescent protein (GFP) cassette, *FgGPI* containing its native promoter region and ORF (excluding

the stop codon) was amplified. Then the targeted fragment and the *Xho*I-digested plasmid pYF11 were cotransformed into yeast strain XK1-25 as described previously (Bruno et al. 2004). Subsequently, the *FgGPI*-GFP fusion vector was recovered from the yeast transformants via the Yeast Plasmid Kit (Solarbio, Beijing, China) and then transferred into *Escherichia coli* strain DH5 α for amplification. The *FgGPI*-GFP constructs were verified by DNA sequencing and transformed into wild-type strain 2021 and derived mutants.

Sensitivity of mycelial growth to various stresses and carbon source. To test the sensitivity of wild-type strain 2021 and $\Delta FgGPI$ to various environmental stresses, mycelial plugs taken from a 3-day-old colony of each strain were inoculated on a PDA plate containing the following compounds: 0.05% Congo red (wt/vol), hydrogen peroxide (H_2O_2 ; 10 and 20 mM), sodium chloride (NaCl; 1 and 1.5 M), and fungicides fludioxonil or carbendazim. After incubation at 25°C for 3 days, the colony diameter in each plate was determined. Carbon source utilization assays were performed on MM supplemented with different carbon sources (glucose, fructose, galactose, lactose, and xylose) at concentrations of 2% (wt/vol). All experiments were repeated three times.

Western blot assays. All tested strains were incubated in yeast extract peptone dextrose (YEPD) at 25°C for 2 days, then mycelia were harvested and washed for protein extraction as described previously (Zhou et al. 2020b). Western blot analysis was performed as previously described (Zhou et al. 2020b). Polyclonal anti- β_2 antibody IF11 (Zhou et al. 2016) and monoclonal anti-GFP antibody 300943 (Zenbio, Sichuan, China) were used at a 1:20,000 and 1:1,000 dilution for immunoblot analyses, respectively. All samples were further detected with monoclonal anti- β -actin antibody 700068 (Zenbio) as a reference. Incubation with a secondary antibody and chemiluminescent detection were performed as described previously (Zhou et al. 2016).

Coimmunoprecipitation assays. Transformants expressing *FgGPI*-GFP and β_2 tubulin protein were confirmed by Western blot analysis. In addition, the strains expressing a single *FgGPI*-GFP or β_2 tubulin protein were used as negative controls. For coimmunoprecipitation assays, total protein was extracted and incubated with the anti-GFP antibody as described previously (Zhou et al. 2020b).

Fig. 1. Subcellular localization of glucose-6-phosphate isomerase in *Fusarium graminearum* (*FgGPI*). **A**, Conidia, 8-h germinated conidia, and hyphae of the *FgGPI*-green fluorescent protein (GFP) strain were examined by confocal microscopy. **B**, Hyphae of the *FgGPI*-GFP strain were stained with 7-amino-4-chloromethylcoumarin (CMAC) and examined by confocal microscopy. Bar = 10 μ m. DIC = differential interference contrast.

Protein eluted from SureBeads Protein G magnetic beads (Bio-Rad, Hercules, CA) was analyzed by Western blot assay with anti-GFP and anti- β_2 antibodies. The protein samples were further detected with anti- β -actin antibody as a control.

For carbendazim treatment, the wild-type 2021 and the derived β_2 tubulin substitution mutants (Fg β_2^{F167Y} , Fg β_2^{E198L} , and Fg β_2^{F200Y}) labeled with FgGPI-GFP were grown in TBI medium for 24 h before carbendazim (half-maximal effective concentration of each strain) was added. After incubation for another 24 h, mycelia of each strain were harvested and the interaction intensity between GPI and β_2 tubulin was measured.

RNA extraction and qRT-PCR assays. To analyze gene expression, mycelia were harvested from 2-day-old YEPD cultures and used for RNA isolation with the RNAsimple Total RNA Kit (Tiangen, Beijing, China). First-strand cDNA was synthesized with the HiScript II Q RT SuperMix for qPCR kit (Vazyme, Nanjing, China) following the manufacturer's protocols and was then used for qPCR assays with the ChamQ SYBR qPCR Master Mix kit (Vazyme, Nanjing, China) following the manufacturer's instructions. All qRT-PCR primers were designed with Integrated DNA Technologies (<https://www.idtdna.com/Primerquest/Home/Index>) and tested by uMelt HETSSM (<https://www.dna-utah.org/hets/>).

Fig. 2. Colonies of wild-type strain 2021, the *Fusarium graminearum* glucose-6-phosphate isomerase deletion mutant (Δ FgGPI), and the complementation strain were cultured on potato dextrose agar (PDA), complete medium (CM), and minimal medium (MM) agar plates. Photographs were taken after growth for 3 days at 25°C.

TABLE 1. Hyphal growth, conidial germination, and septa formation of the *Fusarium graminearum* glucose-6-phosphate isomerase (FgGPI) deletion mutants (Δ FgGPI)^w

Strain	Colony diameter (mm) ^x			Conidiation ($\times 10^5$ /ml) ^y	Conidial germination (%) ^z
	PDA	CM	MM		
2021	70.7 $\pm$ 2.5 a	83.3 $\pm$ 0.6 a	55.7 $\pm$ 2.1 a	28.8 $\pm$ 4.4 a	87.2 $\pm$ 2.9 a
Δ FgGPI-1	51.0 $\pm$ 2.6 b	77.7 $\pm$ 2.1 b	40.6 $\pm$ 1.5 b	27.3 $\pm$ 3.5 a	62.7 $\pm$ 6.2 b
Δ FgGPI-2	49.3 $\pm$ 2.1 b	79.7 $\pm$ 2.5 b	40.1 $\pm$ 1.7 b	28 $\pm$ 3.3 a	66.9 $\pm$ 8.1 b
Δ FgGPI-C	77.7 $\pm$ 2.1 a	83.7 $\pm$ 0.6 a	57.0 $\pm$ 2.6 a	28 $\pm$ 4.4 a	83.3 $\pm$ 4.4 a

^w Means and standard deviations were calculated from three independent experiments. Values followed by the same letter are not significantly different ($P < 0.05$).
^x Colonies were cultured on potato dextrose agar (PDA), complete medium (CM), or minimal medium (MM) plates for 3 days at 25°C.
^y Conidiation in 20 ml of mung bean broth cultures was examined after incubation for 5 days.
^z Conidial germination was assayed on water agar after 8 h of incubation at 25°C.

Actin of *F. graminearum* was used as the internal control. The relative quantification of each gene was calculated with the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001). All qRT-PCR assays were performed with a Bio-Rad CFX-96 real-time detection system. All results were calculated with the data from three biological replicates.

Analysis of DON production. To determine DON production, all strains were grown in liquid TBI medium. After incubation at 28°C for 7 days in the dark, 1 ml of TBI medium and all mycelia were harvested, respectively. Subsequently, DON production was assayed with a competitive enzyme-linked immunosorbent assay detection plate kit (Wise, Zhenjiang, China) as described previously (Duan et al. 2018; Li et al. 2019).

Plant infection. After incubation in MBB medium for 5 days, conidia of all strains were collected and resuspended in sterile distilled water to a concentration of $1 \times 10^5 \text{ ml}^{-1}$. Wheat heads of cultivar Huaimai 33 were inoculated with conidia suspension (10 μl) as described previously (Qiu et al. 2012). All mutants were

represented by 20 replicate wheat heads. After a 21-day inoculation, the infected spikelets in each inoculated wheat head were recorded. Wheat germ (20 for each mutant) was inoculated with 2.5- μl conidial suspensions ($1 \times 10^5 \text{ ml}^{-1}$) and examined after a 10-day inoculation.

Microscopy examinations. Hyphal morphology was examined with an Olympus IX-71 microscope (Tokyo, Japan) using fresh mycelia taken from 1-day-old colonies grown on PDA plates. The localization of FgGPI-GFP protein was observed with a Leica TCS SP5 confocal microscope (Wetzlar, Hessen, Germany). The strain labeled with FgGPI-GFP was cultured in YEPD for 24 h before staining with vacuole-labeling dye, 7-amino-4-chloromethylcoumarin (CMAC). The following filter sets of the confocal microscope were used: laser at 353 nm at 30% power for blue fluorescence or 488 nm at 30% power for green fluorescence; Plan-Neofluar 100 $\times$ /1.30 oil differential interference contrast objective; pinhole, 100 μm ; and digital gain, 1.00.

Statistical analysis. Data processing was performed using DPS software. The results for hyphal growth rate, conidia production, conidial germination, relative gene expression level, fungicide sensitivity, DON production, pyruvate production, and relative FgGPI-GFP protein expression level are expressed as means $\pm$ standard deviations from three independent experiments. Means followed by the same letter in the figures and table are not significantly different ($P < 0.05$).

RESULTS

FgGPI localizes in the cytoplasm. To determine the subcellular localization of FgGPI in *F. graminearum*, we constructed FgGPI-GFP fusion protein with its native promoter region into PYF11 plasmid and transformed it into wild-type strain 2021. All transformants were observed under confocal microscopy and further verified by Western blot assay. As indicated, GFP signals were observed and distributed throughout conidia, 8-h germlings, and hyphae (Fig. 1A). When we stained the strain FgGPI-GFP with the vacuole-labelling dye CMAC, no colocalization of blue fluorescent and FgGPI-GFP signals were observed in hyphae (Fig. 1B). Thus, our results showed that FgGPI localized in the cytoplasm rather than in the vacuoles in *F. graminearum*.

FgGPI is responsible for hyphal growth, conidial germination, and septa formation. To determine *FgGPI* function in *F. graminearum*, we generated *FgGPI* deletion mutant ΔFgGPI from wild-type strain 2021. The growth rate of the deletion mutants was evaluated on PDA, CM, and MM plates, respectively. As indicated, ΔFgGPI grew much slower than wild-type 2021 and the complementary strain on PDA, CM, and MM plates (Fig. 2; Table 1). However, there was no difference between ΔFgGPI and strain 2021 in hyphal morphology (Supplementary Fig. S2). In addition, there was no difference in conidiation between strain 2021 and ΔFgGPI after 5 days of incubation in MBB. However, conidia germination was significantly inhibited in ΔFgGPI (Fig. 3A; Table 1). In ΔFgGPI , 62% of conidia had three septa or fewer, whereas 31% of conidia produced by strain 2021 formed three septa or fewer (Fig. 3C). Collectively, our results suggested that *FgGPI* is involved in hyphal growth, conidial germination, and septa formation in *F. graminearum*.

Sensitivity of ΔFgGPI to various stresses. To investigate the roles of *FgGPI* in environmental stress response, we tested the sensitivity of ΔFgGPI to various stresses, including osmotic and oxidative stresses (NaCl and H_2O_2), cell wall-damaging agent (Congo red), and fungicides (carbendazim and fludioxonil). As the results show, ΔFgGPI exhibited significantly increased sensitivity to 1.0 or 1.5 M NaCl (Fig. 4A; Supplementary Table S1). Previous studies showed that osmotic stress triggers glycerol accumulation in filamentous fungus (De Vries et al. 2003; Fillinger et al. 2001). Therefore, we measured the glycerol accumulation in ΔFgGPI .

Fig. 3. Conidial germination and septa formation of the *Fusarium graminearum* glucose-6-phosphate isomerase (*FgGPI*) deletion mutants (ΔFgGPI). **A**, Conidia of all strains were cultivated in water agar medium. After incubation at 25°C for 8 h, conidial germination of 150 conidia was examined. **B**, ΔFgGPI deletion mutants produced smaller conidia in mung bean broth after 3 days compared with wild-type strain 2021. Bar = 20 μm . **C**, Comparisons in septa numbers of conidia among the 2021 strain, *FgGPI* deletion mutants, and $\Delta\text{FgGPI-C}$ mutant. Conidia ($n = 150$) were examined for each strain. Bars denote standard errors from three repeated experiments. Values on the bars followed by the same letter are not significantly different ($P < 0.05$).

Results showed that glycerol accumulation significantly decreased in Δ FgGPI compared with strain 2021 in the absence of NaCl. Conversely, NaCl treatment could not induce glycerol accumulation in Δ FgGPI (Fig. 4B). Additionally, Δ FgGPI showed decreased sensitivity to oxidative stress generated by 10 or 20 mM of H_2O_2 (Fig. 4A). We further measured the intracellular H_2O_2 concentration and found that it was significantly reduced in Δ FgGPI (Fig. 4C). In addition, there was no sensitivity change between Δ FgGPI and strain 2021 to Congo red, fludioxonil, and carbendazim (Supplementary Table S1).

FgGPI is indispensable for glucose metabolism and common carbon source utilization. Glycolysis and the TCA cycle are important metabolic pathways involved in ATP biosynthesis in eukaryotic cells. In addition, GPI is an essential enzyme that participates in glycolysis and the pentose phosphate pathway (PPP), occupying an indispensable role in glucose metabolism (de Padua et al. 2017; Limón et al. 2011). Therefore, we performed qRT-PCR assays to measure the transcriptional levels of 14 key genes involved in glycolysis, the TCA cycle, and the PPP in strain 2021 and Δ FgGPI. Results showed that expression levels of eight

Fig. 4. Sensitivity of wild-type strain 2021 and the *Fusarium graminearum* glucose-6-phosphate isomerase deletion mutant (Δ FgGPI) to osmotic and oxidative stresses. **A**, Sensitivity of all strains to osmotic and oxidative stresses. The left image shows comparisons made on potato dextrose agar amended with or without 1 and 1.5 M of sodium chloride (NaCl) and 10 and 20 mM of hydrogen peroxide (H_2O_2). Hyphal growth inhibition was examined after all strains were incubated for 3 days as indicated in the graph on the right. **B**, Glycerol accumulation of each mutant. Intracellular glycerol concentrations (in micromoles per gram of mycelia) of strain 2021, Δ FgGPI-1, Δ FgGPI-2, and Δ FgGPI-C were analyzed after 1.5 M of NaCl treatment for 2 h. **C**, H_2O_2 concentration in each strain. Intracellular H_2O_2 concentrations (in micrograms per gram of mycelia) of each strain were analyzed in trichothecene biosynthesis induction (TBI) and yeast extract peptone dextrose (YEPD) media. Bars denote standard errors from three repeated experiments. Values on the bars followed by the same letter are not significantly different ($P < 0.05$). CK = control check.

selected genes involved in glycolysis and the PPP were significantly decreased in $\Delta FgGPI$ compared with strain 2021 (Fig. 5A). In addition, expression levels of five regulatory genes in the TCA cycle were significantly increased (Fig. 5B). Furthermore, we measured ATP biosynthesis in strain 2021 and $\Delta FgGPI$. As shown in Figure 5D, $\Delta FgGPI$ had a significant reduction in ATP production.

To test whether *FgGPI* is involved in carbon utilization, all mutants were cultured on MM with different carbon sources. As shown in Figure 5C, the growth of $\Delta FgGPI$ was significantly reduced under the sole carbon source condition (i.e., glucose, lactose, and galactose). To note, growth of $\Delta FgGPI$ was completely inhibited when fructose or xylose was the sole carbon source. Taken together, these data suggest that *FgGPI* is critical for glucose metabolism and common carbon source utilization in *F. graminearum*.

***FgGPI* is indispensable for DON biosynthesis and full virulence in *F. graminearum*.** Because DON is a vital virulence factor of *F. graminearum* (Alexander et al. 2009; Goswami and Kistler 2004), we assayed DON biosynthesis in $\Delta FgGPI$. After 7 days of incubation in TBI medium, DON production was significantly decreased in $\Delta FgGPI$ compared with the wild-type progenitor. In addition, we measured DON production with sterilized wheat kernels after a 14-day incubation. As expected, DON production in $\Delta FgGPI$ was drastically reduced (Fig. 6A). Our research reported that pyruvate is not only the product of glycolysis but also the precursor of the DON biosynthesis (Zhou et al. 2020b). Thus, we measured pyruvate biosynthesis in $\Delta FgGPI$ and strain 2021 using a Pyruvate Assay Kit (BC2205; Solarbio). After

incubation in TBI medium for 2 days, pyruvate biosynthesis in $\Delta FgGPI$ was 30% lower than in strain 2021 (Fig. 6B). Taking this into consideration, we assayed whether exogenous pyruvate could partially restore DON biosynthesis in $\Delta FgGPI$. DON production was significantly increased in $\Delta FgGPI$ and the wild-type progenitor after incubation in TBI medium with 0.4 M of pyruvate for 7 days (Fig. 6C). The results suggested that exogenous pyruvate could partly restore the ability for DON biosynthesis in $\Delta FgGPI$.

Pathogenicity of $\Delta FgGPI$ was evaluated on flowering wheat heads. After a 21-day inoculation, no FHB symptoms generated by $\Delta FgGPI$ were observed on inoculated spikelets, whereas wild-type strain 2021 and the complementary strain caused severe FHB symptoms on wheat heads (Fig. 7A). We further assayed the virulence of $\Delta FgGPI$ by droplet inoculation on wheat germ. After 10 days of incubation at 25°C, the $\Delta FgGPI$ mutants significantly reduced disease symptoms on the wheat coleoptiles. Meanwhile, serious disease symptoms were observed from the wild-type strain and the complementary strain under the same conditions (Fig. 7B). Taken together, these results indicated that *FgGPI* was indispensable for DON biosynthesis and full virulence in *F. graminearum*.

Substitutions in β_2 tubulin conferring carbendazim resistance reduce the interaction between GPI and β_2 tubulin. Researchers showed that microtubules (MTs) bind with most glycolytic enzymes, serving as the scaffold and thereby regulating their activity (Orosz et al. 2000; Volker et al. 1995; Walsh et al. 1989). Furthermore, our studies found that β_2 tubulin has a profound effect on fungal growth and metabolic processes (Zhou

Fig. 5. *Fusarium graminearum* glucose-6-phosphate isomerase (*FgGPI*) is indispensable for common carbon source utilization and ATP biosynthesis in *F. graminearum*. **A** and **B**, Assays for the expression of key genes involved in glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle of wild-type strain 2021 and *FgGPI* deletion mutants by quantitative reverse transcription PCR. **C**, Carbon utilization of each strain on different carbon sources. **D**, Production of ATP in strain 2021, the *FgGPI* deletion mutant ($\Delta FgGPI$), and the complementation strain. Bars denote standard errors from three repeated experiments. Values on the bars followed by the same letter are not significantly different ($P < 0.05$). TBI = trichothecene biosynthesis induction and YEPD = yeast extract peptone dextrose.

et al. 2020a). However, little is known about MT–enzyme interaction in phytopathogens. In this study, we tested the interaction between β_2 tubulin and GPI by coimmunoprecipitation assays, and the results showed that GPI could interact with β_2 tubulin in *F. graminearum* (Fig. 8A).

In a 2020 study, we found that carbendazim-resistant substitutions in β_2 tubulin could alter the interaction intensity between β_2 tubulin and some metabolic enzymes, thereby regulating the expression of these enzymes (Zhou et al. 2020b). Thus, we assayed the interaction intensity between FgGPI and Fg β_2 in different carbendazim-resistant substitution mutants (Fg β_2^{F167Y} , Fg β_2^{E198L} , and Fg β_2^{F200Y}). Our results showed that carbendazim-resistant substitutions in β_2 tubulin could reduce the interaction intensity between FgGPI and Fg β_2 , which resulted in increased protein expression levels of FgGPI in carbendazim-resistant mutants (Fig. 8B, C, and D). Although slight but not significantly increased expression of FgGPI was observed in Fg β_2^{E198L} , we think this is consistent with the slightly increased DON biosynthesis in the Fg β_2^{E198L} mutant (Zhou et al. 2020b). Furthermore, we investigated whether carbendazim could disrupt the interaction of GPI and β_2 tubulin. The results showed that carbendazim treatment could not disrupt the interaction between GPI and β_2 tubulin in all strains (Supplementary Fig. S3), which was different from the results discovered previously (Zhou et al. 2020b) and suggested that the interacting domains or sites between β_2 tubulin and GPI are different from isocitrate dehydrogenase subunit 2 (IDH3).

DISCUSSION

To date, chemical control strategies aimed at restricting *F. graminearum* growth and reducing mycotoxin accumulation in wheat have failed to control FHB effectively. Furthermore, the improper use of some fungicides, such as carbendazim, not only gives rise to carbendazim resistance in the field but also triggers certain cellular metabolic changes in fungi, including the enhancement of DON biosynthesis (Audenaert et al. 2010; Tang et al. 2018; Zhang et al. 2009; Zhou et al. 2020b). In our previous studies, we found that production of pyruvate and acetyl-coenzyme A (CoA) was increased in carbendazim-resistant strains. In addition, exogenous acetyl-CoA could accelerate DON biosynthesis in *F. graminearum* (Zhou et al. 2020b). Pyruvate, the precursor of various secondary metabolites including DON, penicillin, and aflatoxin, is synthesized by glycolysis. GPI, the enzyme that catalyzes the second step of glycolysis, is involved in multiple metabolic pathways. Thus, comprehensive understanding of GPI functions is necessary for *Fusarium*-incited disease control.

Previous studies showed that the *Aspergillus nidulans* GPI mutant causes defects in vegetative growth and conidiation (Upadhyay and Shaw 2006). In addition, the lack of GPI attenuates the virulence of *Xanthomonas campestris* pv. *citri* on citrus leaves (Tung and Kuo 1999). In our study, we generated FgGPI deletion mutants and found that they displayed similar biochemical defects in vegetative growth and plant infection, including a reduced growth rate, conidial germination, abnormal septa formation, and attenuated virulence. Taken together, these results suggest that FgGPI is indispensable for development processes and plant infection in *F. graminearum*.

In *C. neoformans*, the Δ GPI mutant exhibits increased sensitivity to cell wall-damaging agents (Congo red and sodium dodecyl sulfate) and osmotic stresses (NaCl and potassium chloride) (Zhang et al. 2015). In our studies, we found that Δ FgGPI growth was significantly inhibited by NaCl compared with the wild-type progenitor. In addition, glycerol accumulation was significantly decreased in Δ FgGPI. The mutants also showed increased tolerance to oxidative stress generated by H_2O_2 . However, no sensibility difference was found between Δ FgGPI and strain 2021 with regard to the cell wall-damaging agent. These results indicate that GPI is

ubiquitous in most organisms, and each has species-specific functions on certain aspects.

Phytopathogens meet various nutritional conditions during their life cycles and coordinate the related metabolic pathways to survive in different conditions (Lee et al. 2009). Although glucose is the preferred monosaccharide to almost all fungi, a variety of other carbon sources such as fructose, lactose, galactose, and xylose can be utilized through different metabolic pathways, including glycolysis and the PPP (de Padua et al. 2017; Limón et al. 2011; Sanfeliix-Haywood et al. 2011). In *Lactobacillus casei*, the

Fig. 6. *Fusarium graminearum* glucose-6-phosphate isomerase (FgGPI) is required for deoxynivalenol (DON) biosynthesis in *F. graminearum*. **A**, DON production of each strain in trichothecene biosynthesis induction (TBI) medium and infected wheat kernels. **B**, Pyruvate production of each strain in TBI medium. **C**, Exogenous pyruvate could partially restore the defect of the FgGPI deletion mutant (Δ FgGPI) in DON biosynthesis. Bars denote standard errors from three repeated experiments. Values on the bars followed by the same letter are not significantly different ($P < 0.05$). PA = pyruvate.

physiological amounts of GPI activities were limited for *L. casei* growth on lactose and galactose, and the restriction could be overcome by *GPI* gene overexpression (Sanflix-Haywood et al. 2011). In *Trichoderma reesei*, the growth rate of the Δ GPI mutant was significantly reduced in medium with lactose or galactose as the sole carbon source. Furthermore, the mutant could not grow in medium with xylose or fructose as the sole carbon source (Limn et al. 2011). In our current studies, qRT-PCR assays showed that glucose metabolism was severely impaired and ATP biosynthesis was significantly reduced in Δ FgGPI. In addition, Δ FgGPI growth

was significantly inhibited with lactose or galactose, and it was completely inhibited with fructose or xylose as the sole carbon source. These results suggest that GPI is critical for common carbon utilization and ATP biosynthesis. Furthermore, these findings indicate that carbon sources that participated in glucose metabolism after the GPI-catalyzed step could not support Δ FgGPI growth.

As a participant in glycolysis, we wonder whether GPI plays a role in controlling secondary metabolism. In our current study, we found that pyruvate and DON production was significantly decreased in Δ FgGPI (Fig. 6). In addition, previous studies showed that

Fig. 7. Defects of the *Fusarium graminearum* glucose-6-phosphate isomerase deletion mutant (Δ FgGPI) in plant infection. **A**, Wheat heads were inoculated with conidia of the wild-type 2021 strain, Δ FgGPI, and the complementation strain (NC) and photographed after 21 days of inoculation. **B**, Wheat germ was inoculated with conidial suspensions and recorded after 10 days of inoculation.

polysaccharide capsule biosynthesis was remarkably reduced in the *C. neoformans* GPI deletion mutant. These results indicate that GPI is indispensable for secondary metabolism not only in phytopathogens but also in human pathogens.

Most glycolytic enzymes have been demonstrated to bind tubulin dimers or MT polymers, but binding has different effects on enzyme activity. For phosphofructokinase and triosephosphate isomerase, enzymes show decreased activity after binding to MTs (Lehotzky et al. 1993; Orosz et al. 2000; Vértessy et al. 1997). Other enzymes are reported to have increased activity when bound to MTs including pyruvate kinase and hexokinase (Wágner et al. 2001; Walsh et al. 1989). Our previous study indicated that carbendazim could disrupt the interaction between FgIDH3 and Fg β_2 (Zhou et al. 2020b). In addition, carbendazim-resistant substitutions in β_2 tubulin could reduce binding between Fg β_2 and FgIDH3, thereby accelerating DON biosynthesis in *F. graminearum* (Zhou et al. 2020b). In the current studies, we found that the expression of GPI protein was higher in carbendazim-resistant strains than in the wild type (Fig. 8). In addition, coimmunoprecipitation assays demonstrated that GPI interacted with β_2 tubulin and the carbendazim-resistant substitutions in β_2 tubulin could reduce the interaction intensity between FgGPI and Fg β_2 . However, carbendazim treatment could not disrupt binding between FgGPI and Fg β_2 . These results indicated that the increased expression of GPI in carbendazim-resistant strains (Fg β_2^{F167Y} and Fg β_2^{F200Y}) was associated with

the reduced interaction between FgGPI and Fg β_2 . However, the different orientation on protein interaction intensity transition between IDH3 and GPI with β_2 tubulin under carbendazim treatment might relate to the different dimensional structure of each enzyme and their interaction regions with β_2 tubulin, which needs to be further investigated.

As an essential cytoplasmic enzyme of glycolysis, extracellular functions of GPI have been mentioned in animals, plants, yeast, and prokaryotes, which also play vital roles in various biologically important processes (Chaput et al. 1988; Faik et al. 1988; Kainulainen et al. 2012; Watanabe et al. 1996; Xu et al. 1996). In tumor cells, GPI has been identified as a cytokine, which is secreted to the extracellular matrix and regulates metastasis and invasion of tumor cells (Watanabe et al. 1996). As a novel moonlighting protein, GPI could bind onto the bacterial surface, thereby enhancing bacterium–bacterium interactions and promoting the survival of *L. crispatus* under changing natural environments (Kainulainen et al. 2012). Although similar functionality of GPI has not been reported in filamentous fungi, this is an attractive research field and will deepen our understanding of fungal pathogenesis.

In summary, our study demonstrated that FgGPI plays important roles in the fungal development and secondary metabolism in *F. graminearum*. We further demonstrated that carbendazim-resistant substitutions could reduce the interaction between GPI and β_2 tubulin, resulting in increased FgGPI expression and DON production in carbendazim-resistant strains.

Fig. 8. Carbendazim-resistant substitutions increase *Fusarium graminearum* glucose-6-phosphate isomerase (FgGPI) expression by reducing the interaction intensity between GPI and β_2 tubulin. **A**, Analysis of the interaction between GPI and β_2 tubulin. **B**, The interaction intensity between GPI and β_2 tubulin was tested in wild-type (WT) strain 2021 and derived carbendazim-resistant mutants labeled with FgGPI-green fluorescent protein (GFP) by coimmunoprecipitation assays. Protein samples were eluted from anti-GFP beads and detected with the anti-GFP and anti- β_2 antibodies, respectively. The protein samples were also detected with monoclonal anti- β -actin antibody as a reference. **C** and **D**, FgGPI expression and the interaction intensity between GPI and β_2 tubulin were measured with strain 2021 and derived carbendazim-resistant mutants labeled with FgGPI-GFP, respectively. Bars denote standard errors from three repeated experiments. Values on the bars followed by the same letter are not significantly different ($P < 0.05$).

LITERATURE CITED

- Alexander, N. J., Proctor, R. H., and McCormick, S. P. 2009. Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. *Toxin Rev.* 28:198-215.
- Audenaert, K., Callewaert, E., Höfte, M., De Saeger, S., and Haesaert, G. 2010. Hydroxide peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. *BMC Microbiol.* 10:112.
- Bruno, K. S., Tenjo, F., Li, L., Hamer, J. E., and Xu, J. R. 2004. Cellular localization and role of kinase activity of *PMK1* in *Magnaporthe grisea*. *Eukaryot. Cell* 3:1525-1532.
- Chaput, M., Claes, V., Portetelle, D., Cludts, I., Cravador, A., Burny, A., Gras, H., and Tartar, A. 1988. The neurotrophic factor neuroleukin is 90% homologous with phosphohexose isomerase. *Nature* 332:454-455.
- Chen, C. J., Bi, C. W., Yu, J. J., Wang, J. X., Li, H. X., Luo, Q. Q., and Zhou, M. G. 2008. Carbendazim-resistance and its molecular mechanism in *Gibberella zeae*. *J. Plant Pathol.* 90:69.
- de Padua, M. C., Delodi, G., Vučetić, M., Durivault, J., Vial, V., Bayer, P., Noleto, G. R., Mazure, N. M., Ždravčević, M., and Pouyssegur, J. 2017. Disrupting glucose-6-phosphate isomerase fully suppresses the “Warburg effect” and activates OXPHOS with minimal impact on tumor growth except in hypoxia. *Oncotarget* 8:87623-87637.
- De Vries, R. P., Flitter, S. J., van de Vondervoort, P. J., Chaverroche, M. K., Fontaine, T., Fillinger, S., Ruijter, G. J. G., d'Enfert, C., and Visser, J. 2003. Glycerol dehydrogenase, encoded by *gldB* is essential for osmotolerance in *Aspergillus nidulans*. *Mol. Microbiol.* 49:131-141.
- Duan, Y. B., Xiao, X. M., Li, T., Chen, W. W., Wang, J. X., Bart, A. F., and Zhou, M. G. 2018. Impact of epoxiconazole on *Fusarium* head blight control, grain yield and deoxynivalenol accumulation in wheat. *Pestic. Biochem. Physiol.* 152:138-147.
- Duan, Y. B., Yang, Y., Wang, J. X., Liu, C. C., He, L. L., and Zhou, M. G. 2015. Development and application of loop-mediated isothermal amplification for detecting the highly benzimidazole-resistant isolates in *Sclerotinia sclerotiorum*. *Sci. Rep.* 5:17278.
- Faik, P., Walker, J. I. H., Redmill, A. A. M., and Morgan, M. J. 1988. Mouse glucose-6-phosphate isomerase and neuroleukin have identical 3' sequences. *Nature* 332:455-456.
- Fillinger, S., Ruijter, G., Tamás, M. J., Visser, J., Thevelein, J. M., and d'Enfert, C. 2001. Molecular and physiological characterization of the NAD-dependent glycerol 3-phosphate dehydrogenase in the filamentous fungus *Aspergillus nidulans*. *Mol. Microbiol.* 39:145-157.
- Goswami, R. S., and Kistler, H. C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* 5:515-525.
- Haller, J. F., Krawczyk, S. A., Gostilovitch, L., Corkey, B. E., and Zoeller, R. A. 2011. Glucose-6-phosphate isomerase deficiency results in mTOR activation, failed translocation of lipin 1 α to the nucleus and hypersensitivity to glucose: Implications for the inherited glycolytic disease. *Biochim. Biophys. Acta* 1812:1393-1402.
- Haller, J. F., Smith, C., Liu, D., Zheng, H., Tornheim, K., Han, G. S., Carman, G. M., and Zoeller, R. A. 2010. Isolation of novel animal cell lines defective in glycerolipid biosynthesis reveals mutations in glucose-6-phosphate isomerase. *J. Biol. Chem.* 285:866-877.
- Kainulainen, V., Loimaranta, V., Pekkala, A., Edelman, S., Antikainen, J., Kylväjä, R., Laaksonen, M., Laakkonen, L., Finne, J., and Korhonen, T. K. 2012. Glutamine synthetase and glucose-6-phosphate isomerase are adhesive moonlighting proteins of *Lactobacillus crispatus* released by epithelial cathelicidin LL-37. *J. Bacteriol.* 194:2509-2519.
- Kimura, M., Tokai, T., Takahashi-Ando, N., Ohsato, S., and Fujimura, M. 2007. Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: Pathways, genes, and evolution. *Biosci. Biotechnol. Biochem.* 71:2105-2123.
- Lee, S. H., Lee, J. S., Park, E. H., Kim, K. W., Kim, M. D., Yun, S. H., and Lee, Y. W. 2009. *GzSNF1* is required for normal sexual and asexual development in the ascomycete *Gibberella zeae*. *Eukaryot. Cell* 8:116-127.
- Lehotzky, A., Telegdi, M., Liliom, K., and Ovadi, J. 1993. Interaction of phosphofructokinase with tubulin and microtubules. *J. Biol. Chem.* 268:10888-10894.
- Li, J., Duan, Y. B., Bian, C. H., Pan, X. Y., Yao, C. J., Wang, J. X., and Zhou, M. G. 2019. Effects of validamycin in controlling *Fusarium* head blight caused by *Fusarium graminearum*: Inhibition of DON biosynthesis and induction of host resistance. *Pestic. Biochem. Physiol.* 153:152-160.
- Limón, M. C., Pakula, T., Saloheimo, M., and Penttilä, M. 2011. The effects of disruption of phosphoglucose isomerase gene on carbon utilisation and cellulase production in *Trichoderma reesei* Rut-C30. *Microb. Cell Fact.* 10:40.
- Lin, H. Y., Kao, Y. H., Chen, S. T., and Meng, M. 2009. Effects of inherited mutations on catalytic activity and structural stability of human glucose-6-phosphate isomerase expressed in *Escherichia coli*. *Biochim. Biophys. Acta* 1794:315-323.
- Liu, S. M., Duan, Y. B., Ge, C. Y., Chen, C. J., and Zhou, M. G. 2013. Functional analysis of the β -tubulin gene of *Fusarium graminearum* and the β -tubulin gene of *Botrytis cinerea* by homologous replacement. *Pest Manag. Sci.* 69:582-588.
- Liu, S. M., Fu, L. Y., Chen, J. P., Wang, S., Jiang, J., Che, Z. P., Tian, Y., and Chen, G. Q. 2019. Carbendazim resistance of *Fusarium graminearum* from Henan wheat. *Plant Dis.* 103:2536-2540.
- Livak, K. J., and Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- $\Delta\Delta C_t$} method. *Methods* 25:402-408.
- Orosz, F., Wagner, G., Liliom, K., Kovacs, J., Baroti, K., Horanyi, M., Farkas, T., Hollan, S., and Ovadi, J. 2000. Enhanced association of mutant triosephosphate isomerase to red cell membranes and to brain microtubules. *Proc. Natl. Acad. Sci. U.S.A.* 97:1026-1031.
- Qiu, J. B., Huang, T. T., Xu, J. Q., Bi, C. W., Chen, C. J., and Zhou, M. G. 2012. β -tubulins in *Gibberella zeae*: Their characterization and contribution to carbendazim resistance. *Pest Manag. Sci.* 68:1191-1198.
- Qiu, J. B., Xu, J. Q., Yu, J. J., Bi, C. W., Chen, C. J., and Zhou, M. G. 2011. Localisation of the benzimidazole fungicide binding site of *Gibberella zeae* β -tubulin studied by site-directed mutagenesis. *Pest Manag. Sci.* 67:191-198.
- Sanfeliix-Haywood, N., Coll-Marqués, J. M., and Yebra, M. J. 2011. Role of α -phosphoglucose isomerase and phosphoglucose isomerase activities at the branching point between sugar catabolism and anabolism in *Lactobacillus casei*. *J. Appl. Microbiol.* 111:433-442.
- Sierkstra, L. N., Silljé, H. H., Verbakel, J. M., and Verrips, C. T. 1993. The glucose-6-phosphate-isomerase reaction is essential for normal glucose repression in *Saccharomyces cerevisiae*. *Eur. J. Biochem.* 214:121-127.
- Tang, G. F., Chen, Y., Xu, J. R., Kistler, H. C., and Ma, Z. H. 2018. The fungal myosin I is essential for *Fusarium* toxismone formation. *PLoS Pathog.* 14:e1006827.
- Tung, S. Y., and Kuo, T. T. 1999. Requirement for phosphoglucose isomerase of *Xanthomonas campestris* in pathogenesis of citrus canker. *Appl. Environ. Microbiol.* 65:5564-5573.
- Upadhyay, S., and Shaw, B. D. 2006. A phosphoglucose isomerase mutant in *Aspergillus nidulans* is defective in hyphal polarity and conidiation. *Fungal Genet. Biol.* 43:739-751.
- Vértessy, B., Orosz, F., Kovacs, J., and Ovadi, J. 1997. Alternative binding of two sequential glycolytic enzymes to microtubules. *J. Biol. Chem.* 272:25542-25546.
- Volker, K. W., Reinitz, C. A., and Knull, H. R. 1995. Glycolytic enzymes and assembly of microtubule networks. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* 112:503-514.
- Wagner, G., Kovacs, J., Low, P., Orosz, F., and Ovadi, J. 2001. Tubulin and microtubule are potential targets for brain hexokinase binding. *FEBS Lett.* 509:81-84.
- Walsh, J., Keith, T., and Knull, H. 1989. Glycolytic enzyme interactions with tubulin and microtubules. *Biochim. Biophys. Acta* 999:64-70.
- Watanabe, H., Takehana, K., Date, M., Shinozaki, T., and Raz, A. 1996. Tumor cell autocrine motility factor is the neuroleukin/phosphohexose isomerase polypeptide. *Cancer Res.* 56:2960-2963.
- Xu, W., Seiter, K., Feldman, E., Ahmed, T., and Chiao, J. W. 1996. The differentiation and maturation mediator for human myeloid leukemia cells shares homology with neuroleukin or phosphoglucose isomerase. *Blood* 87:4502-4506.
- Yang, Y., Li, M. X., Duan, Y. B., Li, T., Shi, Y. Y., Zhao, D. L., Zhou, Z. H., Xin, W. J., Wu, J., Pan, X. Y., Li, Y. J., Zhu, Y. Y., and Zhou, M. G. 2018. A new point mutation in β -tubulin confers resistance to carbendazim in *Fusarium asiaticum* [published correction appears in *Pestic. Biochem. Physiol.* 2019;154:97]. *Pestic. Biochem. Physiol.* 145:15-21.
- Zhang, L. G., Li, B. C., Zhang, Y., Jia, X. J., and Zhou, M. G. 2016. Hexokinase plays a critical role in deoxynivalenol (DON) production and fungal development in *Fusarium graminearum*. *Mol. Plant Pathol.* 17:16-28.
- Zhang, P., Wei, D., Li, Z., Sun, Z., Pan, J., and Zhu, X. 2015. Cryptococcal phosphoglucose isomerase is required for virulence factor production, cell wall integrity and stress resistance. *FEMS Yeast Res.* 15:fov072.
- Zhang, Y. J., Yu, J. J., Zhang, Y. N., Zhang, X., Cheng, C. J., Wang, J. X., Hollomon, D. W., Fan, P. S., and Zhou, M. G. 2009. Effect of carbendazim resistance on trichothecene production and aggressiveness of *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 22:1143-1150.
- Zhou, M. G., and Wang, J. X. 2001. Study on sensitivity baseline of *Fusarium graminearum* to carbendazim and biological characters of MBC-resistant strains. *Acta Phytopathol. Sin.* 31:365-370.
- Zhou, M. G., Ye, Z. Y., and Liu, J. F. 1994. Progress of fungicide resistance. *J. Nanjing Agric. Univ.* 17:33-41.
- Zhou, Y. J., Zhu, Y. Y., Li, Y. J., Duan, Y. B., Zhang, R. S., and Zhou, M. G. 2016. β 1 tubulin rather than β 2 tubulin is the preferred binding target for carbendazim in *Fusarium graminearum*. *Phytopathology* 106:978-985.
- Zhou, Z. H., Duan, Y. B., Zhang, J., Lu, F., Zhu, Y. Y., Shim, W. B., and Zhou, M. G. 2020a. Microtubule-assisted mechanism for toxismone assembly in *Fusarium graminearum*. *Mol. Plant Pathol.* doi:10.1111/mp.13015.
- Zhou, Z. H., Duan, Y. B., and Zhou, M. G. 2020b. Carbendazim-resistance associated β 2-tubulin substitutions increase deoxynivalenol biosynthesis by reducing the interaction between β 2-tubulin and IDH3 in *Fusarium graminearum*. *Environ. Microbiol.* 22:598-614.