Wheat microRNA1023 suppresses invasion of Fusarium graminearum via targeting and silencing FGSG_03101

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ABSTRACT

Fusarium graminearum (F. graminearum) is a destructive pathogenic fungus that causes head blight or scab in wheat, barley and other cereals. To repress pathogen invasion, plants have evolved a sophisticated innate immunity system for pathogen recognition and defense activation. In plant immunity signaling pathways, a lot of small RNAs (sRNAs) have been proved in regulating plant immune response and plant-microbial interaction. In this study, we report that a wheat microRNA (miR1023) can suppress the invasion of F. graminearum by targeting and silencing FGSG_03101 which codes an alpha/beta hydrolase gene in F. graminearum. Transcriptional level evidence indicates that Tae-miR1023 can target FGSG_03101 mRNA and trigger silencing of FGSG_03101 in vitro. F. graminearum PH-1 FGSG_03101 mutant strain displays a weakening ability to invasion and PH-1 Argonaute like gene mutant strains with transferred artificial Tae-miR1023 show enhancing relative transcript level of FGSG_03101, compared with PH-1 wild-type strain. Taken together, our results suggest that wheat miR1023 can target and silence fungal FGSG_03101 to suppress invasion of F. graminearum.

Introduction

Plant pathogens have evolved sophisticated ways to invade their hosts, mainly by adapting to the host environment and by producing pathogenicity related products such as toxic secondary metabolites, effector proteins and/or extracellular enzymes (Jonkers et al. 2012). The expression of genes involved in the adaptation to a host and synthesis of pathogenicity factors are under tight regulation to assure successful infection and survival. The pathogenic fungus Fusarium graminearum, causing Fusarium head blight or scab, is one of the most important cereal killers worldwide, exerting great economic and agronomic losses on global grain production and the grain industry (Goswami and Kistler 2004; Trail 2009).

To resist pathogen invasion, plants have evolved two major layers of innate immunity for pathogen recognition and defense activation (Jones and Dangl 2006). The first layer employs pattern recognition receptors to detect conserved pathogen-associated molecular patterns (PAMP) and to trigger PAMP-triggered immunity (PTI). The second layer, effector-triggered immunity, involves a rapid and robust defense activation triggered by the direct or indirect recognition between a isolate-specific effector and its cognate host resistance (R) protein, often accompanied by a hypersensitive reaction at the attempted pathogen infection sites, which activates a set of innate immunity signaling pathways (Dangl and Jones 2001; Ausubel 2005; Liu et al. 2015). More and more small RNAs (sRNAs) have been proved in regulating plant immune response and plant-microbial interaction (Jones-Rhoades et al. 2006; Ding 2010; Katiyar-Agarwal et al. 2010). sRNAs are a class of short non-coding RNA regulators that control gene expression through mediating mRNA degradation, translational inhibition, or chromatin modification. Plant disease resistance and defense responses are achieved by activation and repression of a large set of genes. Certain endogenous sRNAs in plants, including microRNAs (miRNAs) and small interfering RNAs, are induced or repressed in response to pathogen attack and subsequently regulate the expression of genes involved in disease resistance and defense responses by mediating transcriptional or post-transcriptional gene silencing. Thus, these small RNAs play an important role in gene expression reprogramming in plant disease resistance and defense responses (Mallory and Vaucheret 2006). For example, Arabidopsis miR393 is the first miRNA identified to play a role in plant antibacterial PTI by regulating the auxin signaling pathway (Zhang et al. 2011). In Medicago truncatula, highly abundant miRNA families, e.g. miR2109, miR2118 and miR1507, act as master regulators and target sites encoding highly conserved domains of a large group of NB-LRR receptors (Zhai et al. 2011). In Hordeum vulgare L., Triticaceae-specific miR9863 family differentially regulates a subset of barley Mla alleles at the post-transcriptional level (Liu et al. 2014). In fungi, studies in the fission yeast and Neurospora crassa reveal functions of sRNAs in genome defense, heterochromatin formation, and gene regulation (Liang et al. 2013). Recently, some Botrytis cinerea sRNAs have been proved to hijack the host RNA interference (RNAi) machinery by loading into Arabidopsis Argonaute 1 (AGO1) to selectively silence host immunity genes, demonstrating that a fungal pathogen transfers ‘virulent’ sRNA.

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effectors into host cells to achieve infection (Weiberg et al. 2013). And Arabidopsis cells also have been proved to secrete exosome-like extracellular vesicles to deliver sRNAs into fungal pathogen Botrytis cinerea. Transferred host sRNAs induce silencing of fungal genes critical for pathogenicity. Thus, Arabidopsis has adapted exosome-mediated cross-kingdom RNA interference as part of its immune responses during the evolutionary arms race with the pathogen (Cai et al. 2018).

In order to detect whether common wheat endogenous sRNAs can be transferred into pathogens to exert biological function, we decided to screen wheat sRNAs, which could target F. graminearum genome and investigated the effect of silencing of target candidate genes. Fortunately, we found wheat miR1023 (Tae-miR1023) could target and silence an Alpha/Beta 1,3-β-glucanase gene (FGSG_03101), and suppress the invasion of F. graminearum.

Materials and methods

Plant materials

N. benthamiana plants are grown in a controlled environment at 25°C with a 14-h-light/ 8-h-darkness photoperiod. Wheat plants (Chinese spring) used for BSMV-based miRNA silencing experiment and F. graminearum (strain PH-1) punch inoculation experiment are grown in pots in a green house with 16-h-light/ 8-h-darkness cycle until the two-leaf stage. After inoculated with BSMV, wheat plants are transferred to a climate chamber at 23–25°C for the evaluation. For each biological replicates, six wheat seeds are sown in one pot of 12 centimeter (cm) diameter, and 2 pots per per BSMV construct. Totally, 10–12 wheat plants of two-leaf stage are prepared for BSMV inoculation. Twenty segments of 4th wheat leaves displaying BSMV-infected symptom are collected from three biological replicates for F. graminearum punch inoculation experiment.

Small RNA isolation and deep sequencing

Fifteen-day-old wheat leaves of wheat were inoculated with F. graminearum strain PH-1 for 0 and 40 h, and total RNAs were isolated using TRIzol solution (Invitrogen 15596-026) according to the manufacturer's instructions. Small RNAs of 18–30 nt were excised and isolated from 5 to 10 mg total RNAs electrophoresed on 15% polyacrylamide denaturing gel, and then were ligated with 59 and 39 adapters. The ligated small RNAs were used as templates for cDNA synthesis followed by PCR amplification. The obtained libraries were sequenced using the Solexa sequencing platform (BGI, Beijing).

Fungal strains, culture conditions and punch inoculation experiment

F. graminearum strain PH-1 is used as the wild-type (WT) strain for constructing various genes deletion mutant in this study. The WT strain, resulting mutants strains are routinely cultured on potato dextrose agar (200 g potato, 20 g dextrose, 20 g agar and 1 L water) at 25°C with a 12-h-light/ 12-h-darkness cycle. The WT strain and its derived mutants are grown on carrot agar for induction of sexual development near-UV light (wavelength, 365 nm; HKiv Co., Ltd, Xiamen, China), and in mung bean broth for conidiation assays under continuous light. Assays for F. graminearum punch inoculation are performed as described previously (Chen et al. 2016). For all leaf inoculation assays, F. graminearum conidia concentration is adjusted to 5 × 104 conidia per mL-1. Inoculations of 4th wheat leaves displaying BSMV symptom, or non-BSMV-infected wheat leaves, are done by wound inoculation of detached leaves segments with F. graminearum strains. Fifteen leaves are detached for each biological replicate and transferred in 1% agar plates supplemented with 85 μM Benzimidazole. For assessing the progression of F. graminearum disease symptoms, the lesion size is measured from the digital images using the free software ImageJ program (http://rsb.info.nih.gov/ij/index.html). Each experiment is repeated three times.

Construction for F. graminearum gene deletion mutant

Constructs for gene deletion of F. graminearum are carried out as described previously (Jiang et al. 2011). PCR products are transformed into PH-1 protoplasts by employing polyethylene glycol-mediated protoplast transformation. All mutants generated in this study are preserved in 15% glycerol at −80°C.

Vector constructions

For construct BSMV: OE-miR1023, Tae-miR1023 is engineered into Osa-miR528 precursor backbone using overlap PCR to replace endogenous miRNA sequence. Both miRNA and the partially complementary miRNA* sequences in Osa-miR528 precursor are substituted by Tae-miR1023 and Tae-miR1023*, respectively. The reconstructed precursor is added LIC adaptors for linking with BSMV vector. For construct BSMV: STTM-miR1023 (for silencing of Tae-miR1023 using STTM strategy) is constructed as follows. Primers with LIC adaptor, corresponding target mimic of Tae-miR1023, and STTM 48nt spacer (5′-GTTGTTGTTGT TATGTCCTAAATTAAATGTCCTAAAGAAGATA T-3′) are employed to PCR amplify STTM-miR1023 molecules. STTM-miR1023 is added LIC adaptors for linking with BSMV vector. The Osa-miR528 precursor harboring Tae-miR1023 fragment or STTM fragment with LIC adaptors are cloned into BSMV-γyb using the LIC protocol as described (Jiao et al. 2015).

The plasmids in Western-blotting experiment and YFP observation assay are constructed by Gateway technology (Invitrogen) following the instructions of the manufacturer (http://www.invitrogen.com/content/sfs/manuals/gatewaymanual.pdf). The two 35S: pKANNIBAL-miR1023 or -miR159 expressing vectors are constructed by PCR amplification of Osa-miR528 precursor harboring Tae-miR1023, or Tae- miR159 precursor, followed by sequential digestion with HindIII and KpnI and subsequent cloning into the pKANNIBAL destination vector. All constructs are confirmed by DNA sequencing.

BSMV-based experiments

BSMV-based miRNA overexpression and silencing experiments are performed as described (Jiao et al. 2015). Constructs of pCaBS-α, pCaBS-β, pCaBS-γ-LIC derivatives (OE-miR1023 and STTM-miR1023) are transformed into
Agrobacterium (A. tumefaciens strain EHA105). The Agrobacterium suspensions of OD$_{600} = 0.8$ are mixed at 1:1 ratio (pCaBS-αCaBS-β-each pCaBS-γ-LIC derivative) and infiltrated in N. benthamiana leaves. Agroinfiltrated N. benthamiana leaves can provide excellent sources of virus for secondary BSMV infections in wheat plants. The N. benthamiana sap is extracted from leaves with BSMV symptom at about 12 days post infiltration, ground in 20 mM Na-phosphate buffer (pH 7.2) containing 1% celite, and the sap is mechanically inoculated onto the first two emerging leaves of wheat. Infected wheat plants are further grown for 14–21 d to allow emergence of new leaves displaying viral symptoms. Segments of the fourth leaves of BSMV-infected wheat plants are collected for further experiments from three biological replicates per construct.

### Protein analyses

Western-blotting experiment is performed in order to analyze whether Tae-miR1023 or Tae-miR159 can cleavage Fg-Hydrolase, respectively. First, 35S: pKANNIBAL-miR1023 or -miR159 expressing vectors are transiently transformed into N. benthamiana leaves by Agrobacterium-mediated transformation. After 24 hpi, 35S: CTAPI- Fg-Hydrolase protein expressing vector is agroinfiltrated into the same N. benthamiana leaves. For Fg-Hydrolase protein accumulation analysis, leaf samples are collected at 24–36 h post the 2nd agroinoculation. HA-tagged protein extraction, separation and fraction are detected by immunoblotting using rat anti-HA antibody (Roche) and anti-rat IgG conjugated with horseradish peroxidase (Sigma). For YFP observation assay, cell suspensions of A. tumefaciens strain GV3101 containing the indicated constructs were infiltrated in N. benthamiana leaves. Confocal images were taken using a confocal laser-scanning microscope Zeiss LSM 710 (Carl-Zeiss).

### RNA, DNA and PCR analysis

Plant total RNAs are extracted from three independent biological replicates, BSMV-infected leaves and F. graminearum-infected lesion area of wheat leaves with TRizol reagent as described by the manufacturer (Invitrogen), and treated with Dnase I. DNA and Total RNA are extracted from cultured F. graminearum strains using fungal DNA or Total RNA extraction kits. About 2 mg of total RNA and M-MLV Reverse Transcriptase (Promega) are further used for reverse transcription. For coding genes reverse transcription, first-strand cDNA is synthesized using Oligo (dT)$_{18}$. For miRNA reverse transcription, specifically designed end-point stem-loop reverse transcription primers are used, and follow the procedures described by Liu (Liu et al. 2014). Real-time RT–PCR assays with three technical replicates are performed using StepOne real-time system (Applied Biosystems) and GoTaq qPCR Master Mix (Promega, A6001). MiRNA forward primer is respectively used with miRNA universal reverse primer to quantify the relative transcript levels of mature Tae-miR1023 and Tae-miR159. Real-time RT–PCR components for miRNA are as follows: 2× GoTaq qPCR Master Mix 5 µL, diluted cDNA 1 µL, miRNA forward primer 0.2 µL, miRNA universal reverse primer 0.2 µL, ddH$_2$O up to 10 µL. Real-time RT–PCR conditions are as follows: 95°C for 5 min, followed by 35–40 cycles of 95°C for 5 s, 60°C for 10 s, and 72°C for 1 s. For melting curve analysis, denature samples at 95°C, then cool to 65°C at 20°C per second (Liu et al. 2014). For the determination of target genes FGSG\_03101, gene-specific primer pairs spanning the miRNA-guided cleavage site are used. Tae-U6 and Tae-Actin, Fg-Actin which served as internal reference gene for miRNAs and protein-coding genes are detected, respectively. Error bars representing standard error (SE) are calculated from three biological replicates per construct.

Sequence data for genes used in this article can be found under GenBank accession numbers KC775781 (Fg-Actin), X63066 (Tae-U6), XM\_011324370.1 (alpha/beta Hydrolase, FGSG\_03101), XM\_011321673.1 (Fg-AGO like 1), XM\_011317703.1 (Fg-AGO like 2), XM\_011328784.1 (Fg-Actin).

### Results

#### Construction of wheat sRNA library and alignment with F. graminearum genome

To explore the role of wheat sRNAs in regulation of host–pathogen interaction, we profiled sRNA libraries prepared from F. graminearum (strain PH-1)-infected wheat leaves collected at 0 and 40 h after inoculation. sRNA library prepared from F. graminearum total biomass after three days of culture were used as controls. By using 100 normalized reads per million sRNA reads as a cutoff, we identified a total of 3102 sRNAs from wheat libraries could target F. graminearum genes with 0–3 nucleotide mismatch, compared with the cultured F. graminearum library. Among them, one predicted F. graminearum target of 21 nucleotide (nt) length Tae-miR1023 (Figure 1(A)), coding an alpha/beta Hydrolase (FGSG\_03101), is likely to function in pathogenicity. To test whether Tae-sRNAs could indeed suppress fungal genes during infection, Tae-miR1023 was selected for further characterization. We conducted real-time RT–PCR analysis to detect the relative transcript level of Tae-miR1023 and FGSG\_03101 during F. graminearum infection, and found FGSG\_03101 was enriched after F. graminearum infection and Tae-miR1023 expression increased gradually until three days post inoculation (dpi) and then decreased (Figure 1(B)), accompanied by gradually increasing lesion size of wheat leaves (Figure 1(C)). This result showed that Tae-miR1023 and FGSG\_03101 were involved in process of F. graminearum infection.

#### Tae-miR1023 triggers silencing of FGSG\_03101 that is involved in F. graminearum pathogenicity

The technology about host-induced gene silencing (HIGS) targeting fungal genes has been developed in several plant–microbial interactions (Nowara et al. 2010). In addition, Barley stripe mosaic virus (BSMV) is a hordeivirus with a tripartite genome, composed of α, β, and γ RNAs (Jackson et al. 2009). BSMV has become a popular vector for virus-induced gene silencing (VIGS), virus-mediated overexpression of heterologous protein and HIGS in barley and wheat. In wheat, BSMV vectors have been used to assess the possibility of controlling devastating Fusarium diseases via HIGS of the fungal CYP51 genes and demonstrated that silencing of an azole fungicide target was highly efficient in controlling fungal growth (Koch et al. 2013). To determine whether Tae-miR1023 could trigger silencing of FGSG\_03101, we examined the transcript level of...
after BSMV-induced Tae-miR1023 overexpression or silencing. The previous study found that BSMV expressing Tae-miR159a precursor could produce virus small interfering RNA from the same miRNAs generating sites of Tae-miR159a precursor and down regulate its target gene TaMYB3 in vivo (Feng et al. 2013). Here, modified BSMV vectors were employed for expression of Tae-miR1023 using Oryza sativa miR528 (Osa-miR528) precursor as stem-loop backbone to replace miRNA and miRNA* regions with Tae-miR1023 sequences, and BSMV-based miRNA silencing assay was performed through BSMV-based expression of miRNA target mimics to suppress Tae-miR1023. We used the modified BSMV vector to express Osa-miR528 precursor harboring Tae-miR1023 and Tae-miR1023*, and short tandem target mimic (STTM) against Tae-miR1023, and then cloned it into a pCaBS-γ-LIC vector to generate BSMV: OE-miR1023 and BSMV: STTM-miR1023 constructs, respectively (Figure S1). BSMV-based experiment procedures can be found in the Materials and Methods section. After 15th-day post BSMV constructs inoculation of wheat plants, segments of the 4th leaves of BSMV-infected wheat plants were collected and inoculated with F. graminearum (strain PH-1) by the punch inoculation method. Relative transcript levels of Tae-miR1023 and FGSG_03101 were detected by stem-loop RT-PCR and real-time qPCR assay at 1 and 3 dpi, respectively. Error bars represented a standard error (SE) of three representing experiments from four replicates.

FGSG_03101 after BSMV-induced Tae-miR1023 overexpression or silencing. The previous study found that BSMV expressing Tae-miR159a precursor could produce virus small interfering RNA from the same miRNAs generating sites of Tae-miR159a precursor and down regulate its target gene TaMYB3 in vivo (Feng et al. 2013). Here, modified BSMV vectors were employed for expression of Tae-miR1023 using Oryza sativa miR528 (Osa-miR528) precursor as stem-loop backbone to replace miRNA and miRNA* regions with Tae-miR1023 sequences, and BSMV-based miRNA silencing assay was performed through BSMV-based expression of miRNA target mimics to suppress Tae-miR1023. We used the modified BSMV vector to express Osa-miR528 precursor harboring Tae-miR1023 and Tae-miR1023*, and short tandem target mimic (STTM) against Tae-miR1023, and then cloned it into a pCaBS-γ-LIC vector to generate BSMV: OE-miR1023 and BSMV: STTM-miR1023 constructs, respectively (Figure S1). BSMV-based experiment procedures can be found in the Materials and Methods section. After 15th-day post BSMV constructs inoculation of wheat plants, segments of the 4th leaves of BSMV-infected wheat plants were collected and inoculated with F. graminearum (strain PH-1) by the punch inoculation method. Relative transcript levels of Tae-miR1023 and FGSG_03101 were detected by stem-loop RT-PCR and real-time qPCR assay at 1 and 3 dpi, respectively. Error bars represented a standard error (SE) of three representing experiments from four replicates.

To confirm whether FGSG_03101 is involved in F. graminearum pathogenicity, we obtained an FGSG_03101 knockout mutant strain (ΔFGSG_03101). F. graminearum (PH-1) WT strain and ΔFGSG_03101 were inoculated on wheat leaves, and a lesion size of wheat leaves and F. graminearum spores number were counted at 1, 3 and 5 dpi. Lesion size of wheat leaves and F. graminearum spores number were significantly lower in BSMV: STTM-miR1023 infected plants, at 1, 3 and 5 dpi, respectively (Figure 3).

Figure 1. Tae-miR1023 and FGSG_03101 respond to the invasion of F. graminearum. (A) Schematic diagram of Tae-miR1023 (red color) and its target site (black color) in FGSG_03101 of F. graminearum. (B) Detection of relative transcript level of Tae-miR1023 and FGSG_03101 from F. graminearum-infected lesion area in wheat leaves by real-time quantitative PCR (qPCR) assay after inoculation of F. graminearum at 0, 0.5, 1, 2, 3, 4, 5 and 6 dpi, respectively. (C) Corresponding F. graminearum-infected symptom in wheat leaves at different time courses.

Figure 2. Detection of relative transcript level of Tae-miR1023 and FGSG_03101 from F. graminearum-infected lesion area in BSMV pre-inoculated wheat leaves. BSMV-based experiment procedures can be found in the “Materials and Methods” section. After 15th-day post BSMV constructs, inoculation of wheat plants, segments of the 4th leaves of BSMV-infected wheat plants were collected and then inoculated with F. graminearum (strain PH-1) by the punch inoculation method. Relative transcript levels of Tae-miR1023 and FGSG_03101 were detected by stem-loop RT-PCR and real-time qPCR assay at 1 and 3 dpi, respectively. Error bars represented a standard error (SE) of three representing experiments from four replicates.
Figure 3. Lesion size of wheat leaves and F. graminearum spores number were counted after BSMV-induced overexpression or silencing Tae-miR1023. (A) Lesion area phenotypes of F. graminearum-infected wheat leaves which were pre-inoculated by BSMV: OE-miR1023, BSMV: STTM-miR1023 and BSMV empty vector (BSMV: EV), were photographed at 1, 3 and 5 dpi. BSMV: EV infected wheat plants were used as controls. (B) Lesion-size (mm²) of corresponding different treatments. (C) Number of F. graminearum spores produced by lesions on corresponding treated wheat leaves. Error bars representing SE were calculated from three replicates. Significance was determined at *P < .05 and **P < .01 (n ≥ 3) with a t-test.

Figure 4. Lesion size and fungal spores number of wheat leaves infected by F. graminearum WT or FGSG_03101 knockout mutant strains. (A) Lesion area phenotypes of wheat leaves infected by F. graminearum PH-1 WT or FGSG_03101 knockout mutant strains were photographed at 1, 3 and 5 dpi. (B) Lesion-size (mm²) of corresponding different treatments. (C) Number of F. graminearum spores produced by lesions on corresponding treated wheat leaves. Error bars representing SE were calculated from three replicates. Significance was determined at *P < .05 and **P < .01 (n ≥ 3) with a t-test.
**Discussion**

In this study, we report that a wheat microRNA (miR1023) can suppress the invasion of *F. graminearum* by targeting and silencing FGSG_03101 which codes an alpha/beta hydrolase gene in *F. graminearum*. Transcriptional level evidence indicates that Tae-miR1023 can target FGSG_03101 mRNA and trigger silencing of FGSG_03101 in *vivo*, and translation level proof shows that Tae-miR1023 can suppress the accumulation of alpha/beta Hydrolase coding by FGSG_03101 in *vivo*. *F. graminearum* PH-1 FGSG_03101 mutant strain displays a weakening ability to invasion and suppression of stress responses, illustrating the potential of Tae-miR1023 as a weapon against fungal plant pathogens.
PH-1 Argonaute like gene mutant strains with transferred artificial Tae-miR1023 show enhancing relative transcript level of FGSG_03101, compared with PH-1 WT strain. Taken together, our results suggest that wheat miR1023 can target and silence fungal FGSG_03101 to suppress invasion of F. graminearum.

Transient virus-induced gene silencing displays several advantages when constitutive loss of gene function through stable transformation brings about sporophytic or gametophytic lethality (Sha et al. 2014). Moreover, the currently described BSMV-mediated miRNA silencing and overexpression system is efficient and quick, and it can be carried out for miRNAs silencing through intermediary of argoinoculated N. benthamiana, a more simple but effective method without complicated experiment operations or expensive instruments (Yuan et al. 2011).

Overexpression and silencing of miRNAs are two of the most widely used reverse-genetic strategies to study miRNA function (Tang et al. 2010; Eamens et al. 2011). Here, we demonstrate that BSMV-based miRNA silencing and overexpression system could be used to evaluate the functions of miRNA genes by simple agroinfiltration. The modified BSMV vector may facilitate to high-throughput screen the targets of miRNAs and to characterize endogenous miRNA function in wheat crops (Jiao et al. 2015).

Animal and plant pathogens have evolved virulence or effector proteins to counteract host immune responses (Grant et al. 2006). Various protein effectors have been predicted or discovered in fungal or oomycete pathogens from whole-genome sequencing and secretome analysis, although delivery mechanisms are still under active investigation (Mudgett 2005). Jin’ lab shows that sRNAs as well can act

Figure 6. Suppression of YFP-tagged Fg-Hydrolase coexpressed with Tae-miR1023. (A) Coexpression of YFP-Fg-Hydrolase or its synonymously mutated version (YFP-Fg-Hydrolase-m) with Tae-miR1023 was observed with confocal microscopy. (B) Expression of the YFP sensors carrying a Tae-miR1023 target site of Fg-Hydrolase or a Tae-miR1023 target site-m was analyzed after coexpression of Tae-miR1023. Samples were examined at 48 h after transiently Agrobacterium-mediated transformation into N. benthamiana leaves. (Top) YFP. (Bottom) YFP/bright field overlay. Scale bar is 50 μm. Similar results were obtained in three biological replicates.

Figure 7. Relative transcript level of FGSG_03101 in WT, AGO1 and AGO2 mutant strains coexpressing of siRNA with the same sequence as Tae-miR1023. Relative transcript levels of FGSG_03101 were detected by the real-time qPCR assay. Error bars represented a standard error (SE) of three representing experiments from four replicates.
as effectors through a mechanism that silences host genes in order to debilitate plant immunity and achieve infection. They find that sRNAs from *B. cinerea* hijack the plant RNAi machinery by binding to AGO proteins, which in turn direct host gene silencing. The implications of these findings may extend beyond plant gray mold disease caused by *B. cinerea* and suggest an extra mechanism underlying pathogenesis promoted by sophisticated pathogens with the capability to generate and deliver small regulatory RNAs into hosts to suppress host immunity. In order to detect whether plant endogenous sRNAs can be transferred into pathogens to exert biological function, we decided to screen wheat sRNAs, which could target *F. graminearum* genome, and investigated the effect of silencing of target candidate genes. In summary, we found wheat miR1023 (Tae-miR1023) could target and silence an Alpha/Beta Hydrolase gene (*FGSG_03101*), and suppress the invasion of *F. graminearum*.

**Disclosure statement**

No potential conflict of interest was reported by the author.

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