Phytopathology Research

RESEARCH

Open Access

Cryphonectria hypovirus 1 infection suppresses the pathogenicity but increases the mycotoxin deoxynivalenol production of *Fusarium graminearum*

Ruiling Bian[1†], Huan Ren[1†], Mengge Jiang[1], Zhihao Zhang[1], Shiyu Zhai[1], Tianxing Pang[1], Ida Bagus Andika[1] and Liying Sun[1*]

Abstract

Fusarium graminearum is a prominent pathogen responsible for causing head blight disease in small grain cereals, leading to substantial agricultural damage. A recent study revealed the infectivity of a hypovirus, Cryphonectria hypovirus 1 (CHV1), in *F. graminearum*. However, the effects of CHV1 on the pathogenicity and development of the fungus remain largely unexplored. In this study, we investigated the effects of CHV1 infection on *F. graminearum* and made several interesting discoveries. First, we observed that CHV1 infection attenuated the pathogenicity of *F. graminearum* and reduced its conidiation. CHV1 efficiently spread through various transmission modes, such as hyphal fusion and spores, including conidiospores and ascospores. However, the accumulation of the virus was reduced following transmission through spores. Interestingly, despite causing hypovirulence, CHV1 infection enhanced the production of deoxynivalenol (DON), a mycotoxin known to play a role in *F. graminearum*'s virulence and induction of DON production by a DON-inducing medium elevated CHV1 accumulation. The transcriptional expression of key regulatory genes involved in DON biosynthesis or toxisome formation was upregulated in response to CHV1 infection. Furthermore, our study revealed that CHV1 infection suppressed the formation of lipid droplets. Overall, our study provides insights of how the infection of a hypovirulence-inducing mycovirus affects the physiological and metabolic processes in *F. graminearum*.

Keywords CHV1, Hypovirulence, *F. graminearum*, DON, Lipid droplets

Background

Fungal diseases account for more than 70% of plant diseases, causing significant reductions in crop yield and quality (Giraldo et al., 2013; Li et al. 2023). Currently, the control of fungal diseases in crops mainly relies on chemical pesticides. However, the excessive use of these pesticides poses risks to food safety, environmental pollution, and resistance development among pathogens (Nicolopoulou-Stamati et al. 2016). In recent years, there has been growing interest in the use of mycoviruses for the biological control of pathogenic fungi. Mycoviruses multiply within fungal cells and can attenuate the virulence of phytopathogenic fungi; thus, they are referred to as hypovirulence-associated fungal viruses (Kotta-Loizou 2021). Generally, mycoviruses induce hypovirulence by significantly altering the fungal host's morphology,

[†]Ruiling Bian and Huan Ren contributed equally to this study.

*Correspondence:
Liying Sun
sunliying@nwafu.edu.cn
[1] State Key Laboratory of Crop Stress Biology for Arid Areas and College of Plant Protection, Northwest A&F University, Yangling 712100, China

development, and gene expression. Various effects of mycoviruses on fungal hosts have been observed. For instance, some mycoviruses can hinder fungal growth (Li et al. 2019), while others can reduce the secretion of secondary metabolites (Liu et al. 2022), and some can even transform host fungi into endophytes, promoting the growth and development of host plants (Zhang et al. 2020). Therefore, mycoviruses not only serve as potential biocontrol agents for fungal disease but also as probes for studying their fungal hosts (Myers et al. 2022).

Cryphonectria hypovirus 1 (CHV1) is the first mycovirus to be successfully used as a biological control agent to manage chestnut blight caused by *Cryphonectria parasitica*. It infects the chestnut blight fungus *C. parasitica*, causing hypovirulence with various phenotypic changes (Hillman et al. 1994; Sun et al. 2006). CHV1 is a prototype species in the genus *Hypovirus* in the family *Hypoviridae*. Like other hypoviruses, CHV1 lacks a rigid virion; instead, its genomic RNA is encapsulated within pleomorphic lipid vesicles derived from its fungal host (Dodds 1980). CHV1-EP713 is the first hypovirus to be sequenced. Its genome comprises a positive single-stranded RNA (+ssRNA) with a length of 12.7 kilobases and encodes two open reading frames (ORFs)-ORFA and ORFB (Shapira et al. 1991; Chiba et al. 2023). Notably, CHV1 is one of the most extensively studied mycoviruses. Reverse genetics techniques have been developed for several CHV1 strains, including CHV1-EP713 (Choi et al. 1992; Chen et al. 1999). A series of mutants have been constructed for analyzing the functions of viral factors in regulating host symptom induction, virulence attenuation, and viral replication (Craven et al. 1993; Suzuki et al. 2002; Suzuki et al. 2003). Several studies have extended CHV1's host range to several other phytopathogenic fungi. Previously, CHV1 was artificially introduced into the phytopathogenic fungus *Valsa mali,* which is the causative agent of apple Valsa canker disease (Yang et al. 2021). CHV1 infection could reduce the hyphal growth, sporulation, and pathogenicity of *V. mali*. Previous studies also showed that CHV1 could be transfected into *Fusarium graminearum* by protoplast fusion. CHV1 infection also caused morphological changes in *F. graminearum* during its growth on potato dextrose agar (PDA) plates. Additionally, the cross-kingdom transmission of CHV1 between plant and fungus has been demonstrated (Bian et al. 2020). These studies indicate that CHV1 has a wide host range and the potential to be a biological control agent for a wide range of plant pathogenic fungi.

Apart from serving as a potential biological control agent, mycoviruses could be a valuable tool for studying fungal hosts, enabling researchers to investigate various aspects of fungal biology, pathogenicity, gene regulation, and defense mechanisms (Ghabrial et al. 2015; Myers et al. 2022). Mycoviruses can interact with their fungal hosts at the molecular level, influencing gene expression, protein production, and signaling pathways. Several studies have shown that CHV1 can alter the expression of a wide range of host genes involved in various biological processes (Allen et al. 2004; Deng et al. 2007; Andika et al. 2019; Chun et al. 2020). This alteration in gene expression is a key mechanism by which the virus affects fungal growth, metabolism, stress response, and pathogenicity (McCabe at al. 1999; Wu et al. 2017; Chun et al. 2020; Chun et al. 2022). Therefore, understanding the effects of CHV1 infection on gene expression in fungal hosts can provide valuable information about the regulation of key genes involved in pathogenicity or other biological processes.

F. graminearum is an economically important fungal pathogen that causes various crop diseases. It is primarily known for causing Fusarium head blight (FHB) in grains such as wheat, barley, and corn (Pestka 2010; Zhang et al. 2021). FHB can lead to significant yield losses and reduce grain quality, thus posing a major threat to agricultural productivity and food safety (Machado et al. 2018). Apart from FHB, *F. graminearum* is also associated with other diseases such as Fusarium ear rot, stalk rot, and root rot in different crop species (Walter et al. 2010; Tang et al. 2021). *F. graminearum* infects plants through the flowers or wounds in the stems, leaves, or ears (Reid et al. 1994; Boenisch et al. 2011). Once inside the plant, the fungus produces mycotoxins, particularly deoxynivalenol (DON), which can have detrimental effects on both plants and animals (Chen et al. 2019; Hallaj Salahipour et al. 2019; Ma et al. 2021). DON plays a significant role in the virulence of the fungus, being involved in various pathogenic processes of *F. graminearum* and contributing to fungal colonization, spread, and survival during the plant's infection (Voigt et al. 2005; Stephens et al. 2008). DON is a virulence factor known to be important for spread through rachis tissues (Bai et al. 2002; Bönnighausen et al. 2019). Currently, using the pesticide is the primary strategy for preventing and controlling FHB during the wheat flowering period. However, many fungicides have been observed to enhance DON production when used for disease control (Duan et al. 2020; Zhou et al. 2020). The biosynthesis of DON in *F. graminearum* involves a cluster of genes known as the *Tri* genes, which are responsible for the synthesis and regulation of DON, as well as other related trichothecene mycotoxins. The *Tri* gene cluster of *F. graminearum* consists of 12 genes, *Tri1* to *Tri16* (Kimura et al. 2007; Menke et al. 2013). Each gene within the cluster performs a specific function in the biosynthetic pathway of DON (Zhu et al. 2020). Understanding the relationship between DON synthesis

and fungal virulence is essential for managing *F. graminearum* infections.

To date, a few mycoviruses have been identified as potential biological control agents for *F. graminearum*. For instance, Fusarium graminearum virus 1 (FgV1), a double-stranded RNA (dsRNA) mycovirus with hypovirulence-associated features, infects *F. graminearum*, reducing mycelial growth and pathogenicity (Chu et al. 2002). Fusarium graminearum gemytripvirus 1 (FgGMTV1), the first multi-component single-stranded DNA virus identified from fungi, can also cause *F. graminearum* to exhibit typical low-toxicity phenomena. Additionally, it has been developed as a virus-induced gene silencing (VIGS) vector suitable for *F. graminearum* (Li et al. 2020; Zhang et al. 2023).

We previously demonstrated the infection of *F. graminearum* by CHV1. In this study, we further investigate the effects of CHV1 infection on pathogenicity, the production of DON and lipid droplet formation of *F. graminearum*. We observed hypovirulence-inducing characteristic of CHV1 and an intricate relationship between CHV1 replication and DON biosynthesis in *F. graminearum*.

Results

CHV1 infection reduces the pathogenicity and sporulation of *F. graminearum*

In our previous study, we successfully introduced CHV1 into *F. graminearum* (PH-1 strain). CHV1 infection caused changes in morphology and a reduction in hyphal growth when compared to virus-free *F. graminearum* in Potato dextrose agar (PDA) medium (Bian et al. 2020). In this study, to investigate the impact of CHV1 infection on fungal pathogenicity, sporulation, and germination, we conducted the following experiments. To assess fungal pathogenicity, conidia obtained from CHV1-infected and virus-free *F. graminearum* were inoculated onto tomato fruits, wheat coleoptiles or wheat heads, following which the fungal lesion and disease index were measured. CHV1-infected *F. graminearum* produced significantly smaller lesions on tomato fruits and wheat coleoptiles than the virus-free *F. graminearum* strain (Fig. 1a–d). CHV1-infected *F. graminearum* produced milder head blight symptoms on inoculated wheat heads than the virus-free *F. graminearum* strain (Fig. 1e, f). Additionally, CHV1 infection also caused a significant reduction in asexual sporulation (Fig. 1g) but not in the morphology or germination of asexual spores (Fig. 1h). CHV1 infection did not affect the formation of perithecia (ascocarps) or the development of ascospores (Fig. 1i). The number of perithecia per cm^2 was similar between CHV1-infected and virus-free *F. graminearum* (Fig. 1j). Therefore, in addition to inhibiting vegetative growth, CHV1 infection also reduces the pathogenicity and conidiation of *F. graminearum*.

Vertical transmission reduces CHV1 accumulation

We also assessed the efficiency of both horizontal transmission through hyphal fusion and vertical transmission through conidia or ascospores of CHV1 in *F. graminearum*. To examine horizontal transmission, the virus-free strains were co-cultured with CHV1-infected strains (Fig. 2a). The resulting fusant isolates obtained from the virus-free side were then tested for viral infection through the extraction of viral dsRNA and subsequent RT-PCR analysis. All fusant isolates were found to contain the virus (Fig. 2b). Individual germlings generated from conidia or ascospores of CHV1-infected *F. graminearum* were examined for viral infection using an RT-PCR assay. The results revealed that the transmission efficiency of CHV1 was 94.7% and 80% for conidia and ascospores, respectively (Fig. 2c, d). Intriguingly, the inhibition of fungal growth caused by CHV1 infection in the fungal progenies derived from hyphal fusion was as severe as in the original infected strain, but CHV1 symptoms were significantly less severe in fungal progenies derived from conidia and even less in those derived from ascospores (Fig. 2e). qRT-PCR showed that CHV1 genomic RNA accumulation was, from lowest to highest levels, in the fungal progenies derived from ascospores, conidia, and fusants (Fig. 2f). These results showed that vertical transmission negatively affects CHV1 accumulation in the fungal progenies.

CHV1 infection elevates DON production and toxisome formation in *F. graminearum*

As CHV1 infection reduced the pathogenicity of *F. graminearum*, we further investigated whether CHV1 infection affects the production and secretion of DON. The amount of DON produced by the virus-free and CHV1-infected *F. graminearum* strains cultured in DON-inducing medium was quantified. Interestingly, CHV1-infected strain showed a significant increase in DON production compared to the virus-free *F. graminearum* strain, regardless of whether they were cultured in Trichothecene biosynthesis inducing (TBI) (7 days), rice (28 days), or wheat head (15 days) medium (Fig. 3a). To further examine whether CHV1 infection enhances toxisome formation, CHV1 was introduced to a transgenic *F. graminearum* strain expressing a key DON synthetase protein, FgTri1, fused to GFP (FgTri1-GFP) driven by an endogenous FgTri1 promoter, which served as a reporter protein for toxisome formation (Tang et al. 2018), through hyphal fusion with a CHV1-infected fungal strain (Additional file 1: Figure S1). The virus-free and CHV1-infected FgTri1-GFP strains were grown in

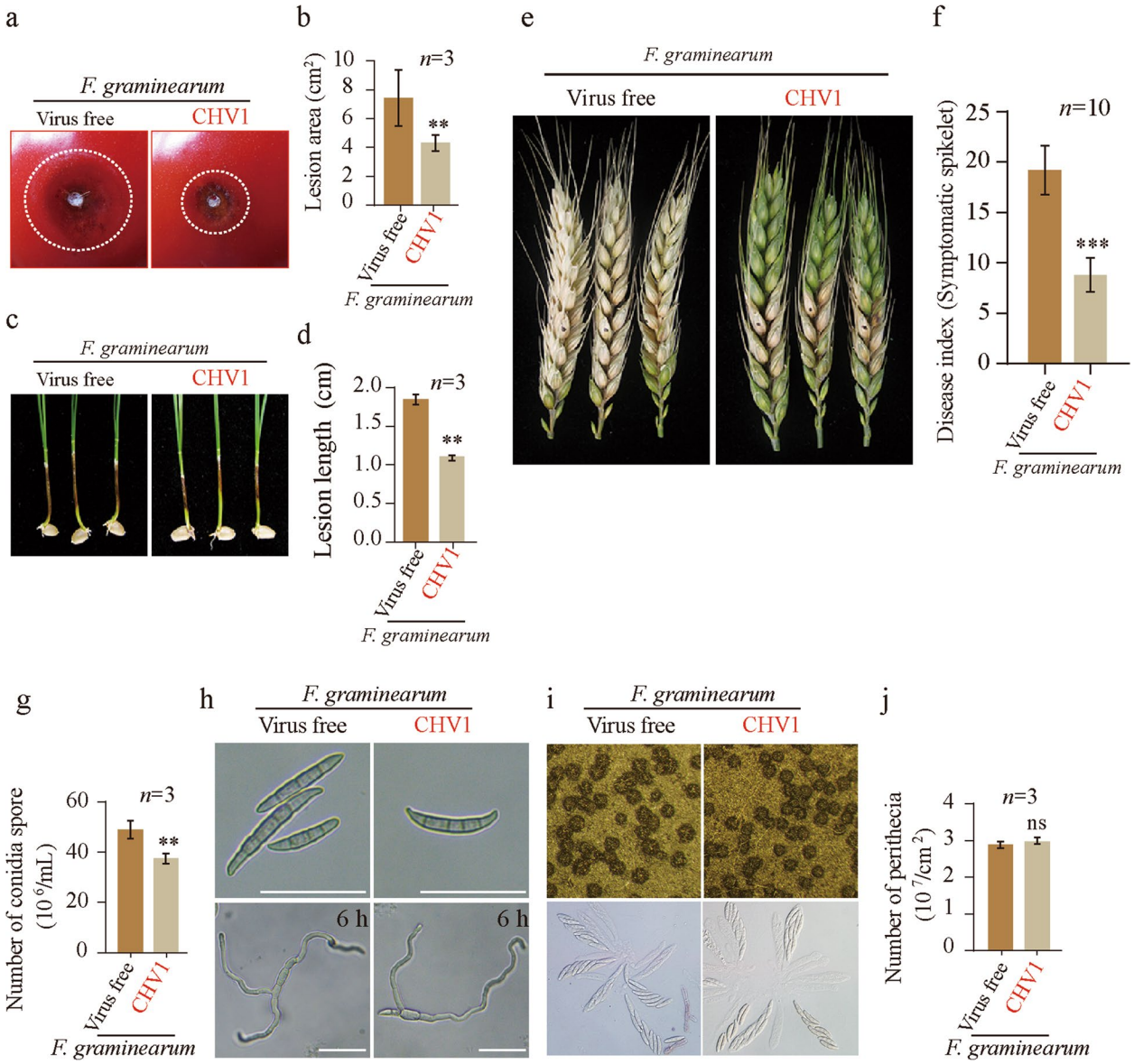

Fig. 1 Effects of CHV1 infection on the development and pathogenicity of *F. graminearum*. **a**, **c**, and **e** Infection assays were conducted on tomato fruits, coleoptiles, and wheat heads, using virus-free and CHV1-infected *F. graminearum* PH-1 (PH-1). Disease symptoms were photographed at 7 days and 14 days post inoculation (dpi). **b**, **d**, and **f** The lesion area of tomato fruits, lesion length of coleoptiles, and FHB disease progression were measured. **g** The impact of CHV1 infection on conidia was assessed. **h** CHV1 infection reduced the number of conidia but did not affect conidia morphology and germination after 6 hours of incubation. **i** and **j** The formation of perithecia and asci/ascospores was observed in virus-free and CHV1-infected strains after 15 days of culture in carrot medium at 25 and the number of perithecia was calculated. The data are expressed as mean ± SD (n=3 or n=10). ** indicates a significant difference at p<0.01, and *** indicates a significant difference at p<0.001 (Student's t-test). Scale bars: 20 μm

24-well plates supplemented with TBI medium, and toxisome formation was observed using a confocal laser. After 48 h of culture, a higher number of GFP-labeled toxisomes was observed in the CHV1-infected strain than in the virus-free strain (Fig. 3b), indicating that CHV1 infection promotes toxisome formation. Consistent with the microscopic observation, the accumulation level of the Tri1-GFP transcript and protein detected by qRT-PCR and western blot assays was significantly higher in the CHV1-infected strain than in the CHV-free strain (Fig. 3c, d).

Next, the effect of CHV1 infection on the transcript expression of *Tri* genes (*FgTri1*, *FgTri4*, *FgTri5*, and *FgTri6)*, which are involved in the biosynthesis of DON

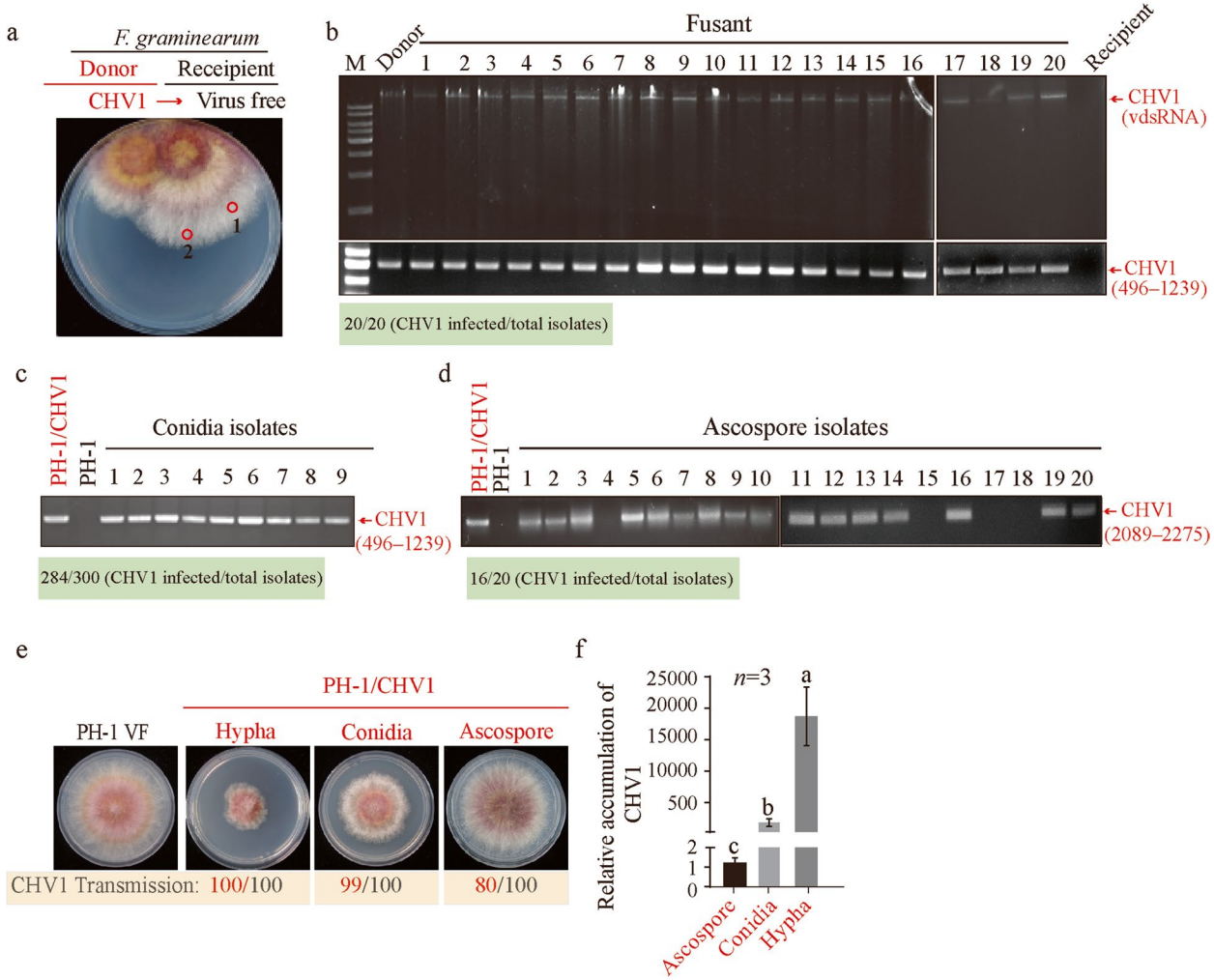

Fig. 2 Efficiency of CHV1 transmission in *F. graminearum*. **a** Virus-free and CHV1-infected *F. graminearum* strains were co-cultured in PDA medium. After 5 days of hyphal contact, mycelia were collected from two specific locations on the recipient side (indicated with a red circle). **b** The resulting fusant mycelia were then grown in fresh PDA medium for RNA preparation. The extracted dsRNA, including samples from the donor, recipient, and fusant were electrophoresed on a 1.0% agarose gel in a 1xTAE buffer system and stained with ethidium bromide (top row). RT-PCR was used to detect CHV1 accumulation in fungal hosts by amplifying the CHV1 genomic RNA from 496 to 1239 nt or 2089 to 2275 nt using specific primers (bottom row). The number of CHV1-infected colonies versus the total number of tested fungal colonies is indicated below the gel. **c**, **d** CHV1-infected mycelia were cultured in CMC liquid medium for 3 days to induce conidiation, while carrot medium was used for 14 days to induce ascospore production. The resulting single conidia/ascospore spores were germinated and grown on a fresh PDA plate for RNA preparation. RT-PCR was used to detect CHV1 accumulation in these spores' germlings using specific primers as described above. The resulting RT-PCR products were electrophoresed on a 1.0% agarose gel in a 1xTAE buffer system and stained with ethidium bromide. The number of CHV1-infected samples versus the total number of tested samples is indicated below the gel. **e** The phenotypes of fungal colonies with CHV1 through horizontal hyphal anastomosis and vertical conidial and ascospore transmission were observed. The efficiency of CHV1 transmission was calculated and is presented below the fungal phenotype images. **f** Quantification of CHV1 RNA accumulation levels in fungal strains was performed using qRT-PCR. The data are shown as mean ± SD (n=3). Different letters indicate a significant difference at $p<0.05$ (one-way ANOVA)

in *F. graminearum*, was analyzed by qRT-PCR. When *F. graminearum* was grown in PDA medium (non-DON-inducing medium), the expression levels of *Tri1*, *Tri4*, and *Tri6* (Xu et al. 2021), but not *Tri5*, were upregulated by CHV1 infection (Fig. 3e), while in TBI medium, CHV1 infection enhanced the transcript expression of all detected *Tri* genes, including the *Tri5* gene, which showed a drastic increase (Fig. 3f). Tri5 (trichodiene synthase) catalyzes the first step in DON synthesis, facilitating the cyclization of farnesyl diphosphate (FPP) to produce the sesquiterpene trichodiene (Tijerino et al. 2011; Graziani et al. 2019). Taken together, these results show that CHV1 infection promotes DON biosynthesis in *F. graminearum*.

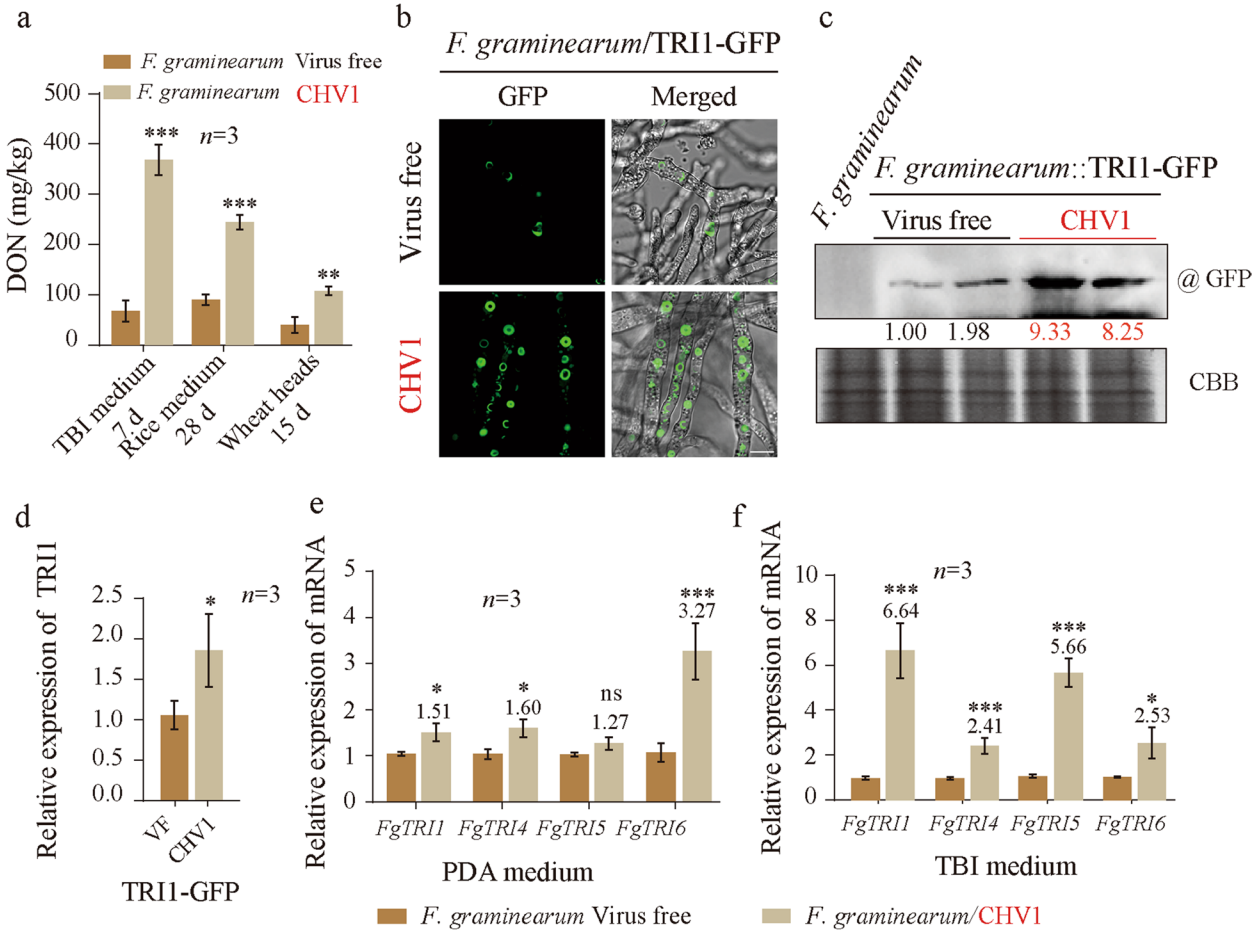

Fig. 3 CHV1 infection increases the biosynthesis of DON. **a** DON production was determined in virus-free and CHV1-infected strains grown in TBI, rice medium, and wheat spikes. **b** The formation of toxisomes in the mycelia of virus-free and CHV1-infected Tri1-GFP strains grown in TBI for 48 hours was observed. The Tri1-GFP strain carries a reporter construct that expresses Tri1 fused with green fluorescence protein (GFP) under its native promoter. GFP fluorescence was visualized using confocal laser scanning microscopy (CLSM). **c** The total proteins were extracted from the mycelia and subjected to western blotting analysis using GFP-specific antibodies. **d** The total RNA was extracted from the mycelia and the transcript expression of Tri1-GFP was analyzed by qRT-PCR. **e**, **f** The transcript expression of key genes (*Tri1*, *Tri4, Tri5*, and *Tri6*) involved in the biosynthesis of DON was analyzed. The total ssRNA was isolated from the mycelia of virus-free and CHV1-infected strains grown in PDA or TBI, and qRT-PCR analysis was carried out. The data are presented as mean ± SD (n=3). ns indicates no differences, * indicates significant differences at p<0.05, ** indicates a significant difference at p<0.01, *** indicates significant differences at p<0.001 (Student's t-test)

Induction of DON production enhances CHV1 accumulation in *F. graminearum*

As we observed that CHV1 infection promotes DON production in *F. graminearum*, we then questioned whether the presence of a higher amount of DON benefits CHV1 infection in *F. graminearum*. Therefore, we examined the effects of supplying exogenous DON on CHV1 symptoms and accumulation in *F. graminearum.* The virus-free and CHV1-infected strains were grown in PDA/PDB medium containing 50 μg/mL commercial DON for phenotypic observation and RNA preparation (Wang et al. 2018). Interestingly, the CHV1-infected strain exhibited significantly slower growth in DON-containing medium than in DON-free medium, whereas the addition of DON to the medium did not affect the growth of virus-free *F. graminearum* strains (Fig. 4a, b), showing that CHV1 induces more severe symptoms in the presence of exogenous DON. The CHV1 accumulation level was then analyzed using qRT-PCR. The results showed that exogenous application of DON did not significantly affect CHV1 RNA accumulation levels in *F. graminearum* cultured in Potato Dextrose Broth (PDB) medium, but CHV1 RNA accumulation was highly elevated in *F. graminearum* cultured in TBI medium (Fig. 4c). These results suggest that DON does not directly affect CHV1 replication but enhances CHV1 symptom induction in *F. graminearum*.

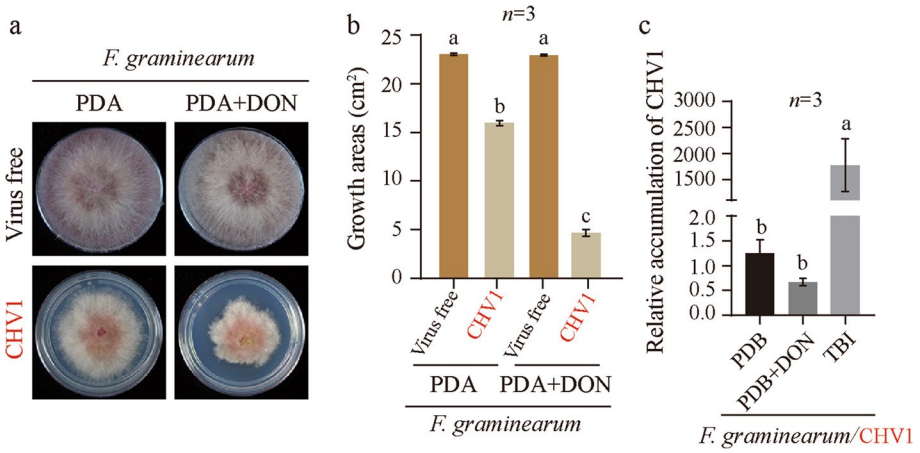

Fig. 4 The effect of the exogenous application of DON on CHV1 infection. **a** Phenotypic characteristics of virus-free and CHV1-infected strains were observed after 5 days of growth in PDA with or without the exogenous application of DON. **b** The growth areas of fungal strains were measured. **c** CHV1 accumulation was analyzed using qRT-PCR. Total ssRNAs were isolated from fungal mycelia cultured in PDB, PDB containing 40 μg/mL DON, or TBI medium and subjected to qRT-PCR. The data are presented as mean ± SD (n=3). The different letters indicate a significant difference at $p<0.05$ (one-way ANOVA)

Moreover, CHV1 infection enhances the process of DON biosynthesis, which, in turn, may promote virus replication.

CHV1 infection interferes with the biosynthesis of lipid droplets (LDs) in *F. graminearum*

The regulation of DON biosynthesis is a complex process that involves various regulatory mechanisms, including pathway-specific and global regulators, signal transduction pathways, and epigenetic modifications (Chen et al. 2019). A previous study has reported that the deletion of *FgVEA*, encoding the velvet protein in *F. graminearum* can induce the aggregation of LDs and decrease DON production (Jiang et al. 2011). Both LDs and toxin-producing bodies originate from the endoplasmic reticulum (Chen et al. 2019; Chen et al. 2021), suggesting a close relationship between the production of DON and the aggregation of LDs in *F. graminearum* (Liu et al. 2019). To investigate whether CHV1 infection also affects LDs biosynthesis in *F. graminearum*, mycelia of CHV1-infected *F. graminearum* strains grown in PDB were then stained with BODIPY to monitor LDs (green dots) using Confocal Laser Scanning Microscope (CLSM) (Fig. 5a). As controls, virus-free strains were grown in PDB or PDB containing 40 μg/mL Triacsin C, a compound that reduces LDs formation (Dechandt et al. 2017). As expected, Triacsin C treatment markedly decreased LDs formation in the mycelial cells as compared to that in mycelial cells of non-treated virus-free strain (Fig. 5a, b), showing Triacsin C activities in reducing LDs biosynthesis in *F. graminearum*. Interestingly, CHV1 infection, similar to Triacsin C treatment, decreased LDs formation in *F. graminearum* compared to virus-free *F. graminearum* cultured in PDB (Fig. 5a, b). These results show that CHV1 infection not only increase DON production but also suppress LDs formation. Therefore, it is possible that CHV1 affects both biosynthesis of LDs and DON. Moreover, in contrast to the application of DON, the addition of Triacsin C increased CHV1 accumulation (Figs. 4c and 5c), suggesting that suppression of LDs biosynthesis is necessary to promote CHV1 infection in *F. graminearum*.

It had been demonstrated that target of rapamycin (TOR) pathway regulates LD synthesis in *F. graminearum*. The genes involved in TOR signaling pathway, such as FgNem1/FgCak1/FgSpo7 or FgPah1 play roles in the TOR-mediated regulation of LD biogenesis. Deletion of FgNem1/FgCak1/FgSpo7 or FgPah1 impairs the response to TOR signaling, and associates with decreased LD synthesis. Notably, absence of FgNem1/FgCak1/FgSpo7 or FgPah1 also leads to reduced levels of DON synthesis, hypha growth and pathogenicity in *F. graminearum* (Liu et al. 2019). To investigate whether CHV1 infection affects TOR pathway and LD synthesis, we examined the transcriptional changes of these genes with or without virus infection. CHV1 infection leads to reduced transcription levels of FgNem1/FgCak1/FgSpo7 or FgPah1 (Fig. 6a), accompanied with decreased LD formation (Fig. 6b, c) in *F. graminearum* growing on TBI medium. This observation suggests that CHV1 infection modulate the TOR signaling pathway and negatively affects the LD formation.

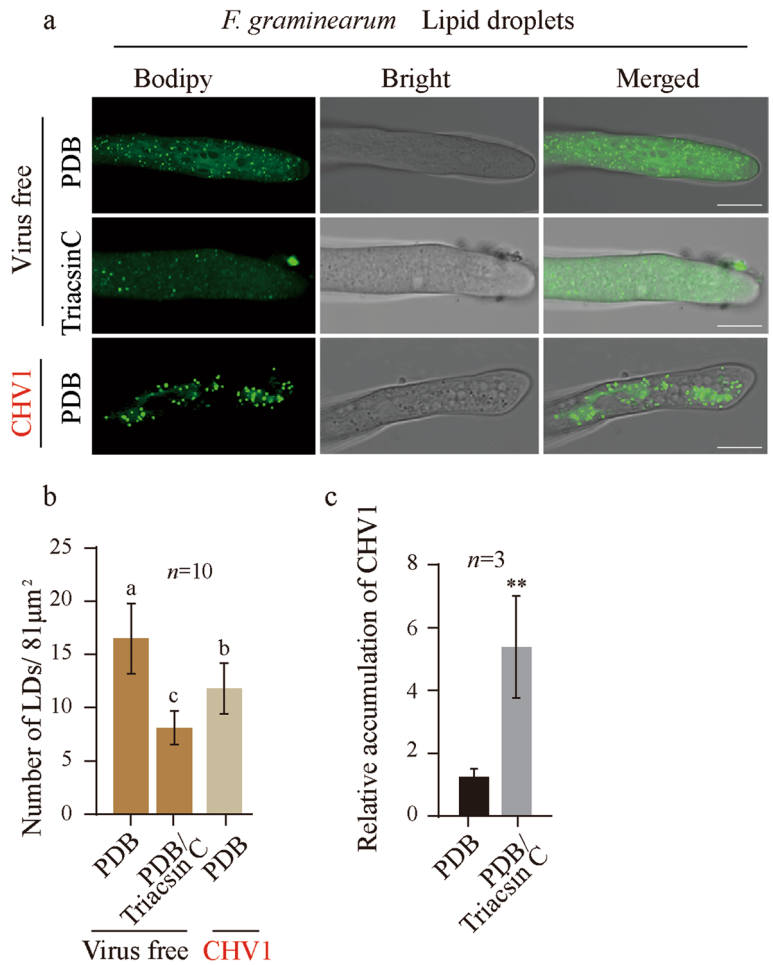

Fig. 5 CHV1 reduces the quantity of lipid droplet (LD) and inhibiting LD biosynthesis enhances CHV1 replication. **a** The subcellular distribution of LDs in the hyphal cells of *F. graminearum* was examined using BODIPY staining (green fluorescence). The mycelia were cultured in PDB, PDB containing 5 μM/mL Triacsin C, and mycelia of the CHV1-infected strain were cultured in PDB medium. Confocal laser scanning microscopy was used to observe LDs. **b** The number of LDs in hyphal cells was measured in an area of 81 μm^2. Data are presented as mean ± SD (n=10). **c** The accumulation of CHV1 in fungal strains was analyzed by qRT-PCR. The total ssRNA was extracted from the mycelia of CHV1-infected strains cultured in PDB and PDB Triacsin C (5 μM/mL) and subjected to qRT-PCR analysis. The data are presented as mean ± SD (n=3). The different letters indicate a significant difference at p<0.05 (one-way ANOVA). ** indicates a significant difference at p<0.01 (Student's t-test)

Discussion

Mycoviruses have attracted significant attention as potential biological control agents against pathogenic fungi. Researchers have investigated how mycoviruses affect the virulence and development of their fungal hosts, leading to a deeper understanding of the complex interactions between mycoviruses and fungi (Xie et al. 2014; Myers et al. 2022). The study of hypovirulence mechanisms, in particular, has unveiled the molecular and cellular processes involved in fungal pathogenesis. CHV1 is a well-known mycovirus that causes hypovirulence in its natural host, *C. parasitica* (Sun et al. 2008), as well as in a heterologous host, *V. mali* (Yang et al. 2021). CHV1 infection leads to milder or attenuated disease symptoms in both hosts. We previously introduced CHV1 into the phytopathogenic fungus *F. graminearum*, the causal agent of FHB worldwide (Bian et al. 2020). By utilizing the CHV1-*F. graminearum* virus-fungal host system, we investigated the effects of the virus on the fungal host's biology and fitness. This knowledge can be useful in developing strategies to control fungal diseases.

We discovered that CHV1 infection significantly reduced the pathogenicity and conidiation of *F. graminearum* (Fig. 1), which is a typical feature of viral control agents of phytopathogenic fungi. The efficient transmission of a virus from one fungal host to another is crucial for the application of hypovirulence in

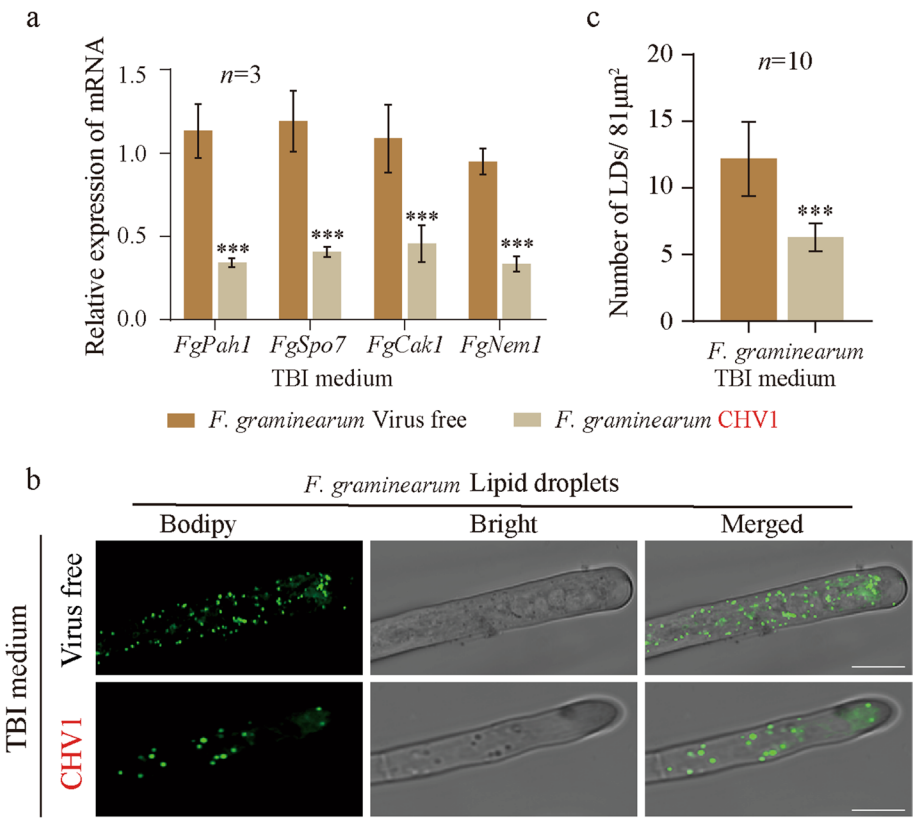

Fig. 6 CHV1 reduced the transcription of FgNem1/FgCak1/FgSpo7 or FgPah1 and decreased LD formation. **a** Both virus-free and CHV1-infected strains of *F. graminearum* cultured in TBI medium. The total ssRNA was isolated from the mycelia and analyzed using qRT-PCR. The results are displayed as mean ± SD (*n*=3). **b**, **c** BODIPY staining (green fluorescence) was employed to investigate the LD formation in the hyphal cells of *F. graminearum*. LD observation was performed using confocal laser scanning microscopy, and the quantification of LDs in hyphal cells within an 81μm^2 area is presented, as mean ± SD (*n*=10). *** indicates a significant difference at $p < 0.001$ (Student's *t*-test)

biological control. Effective transmission ensures that the mycovirus can spread through the population of the target pathogen, exerting a substantial impact on its virulence. Therefore, we examined the transmissibility of CHV1 through hyphal fusion or spores (Fig. 2). Similar to CHV1 transmission efficacy in its fungal hosts, *C. parasitica* and *V. mali*, CHV1 exhibited a relatively high spread efficiency in *F. graminearum*. However, the virus load varied depending on the transmission pathway. The highest virus load was observed through hyphal fusion, while the lowest occurred via ascospores (Fig. 2f). This explains why CHV1 had less effect on the morphology of strains originating from ascospore germination (Fig. 2e). Indeed, it is commonly considered that ascospores are rarely involved in mycovirus transmission, and limited studies have been conducted on this subject (McFadden et al. 1983; Tuomivirta et al. 2009; Xie et al. 2014). One possible reason for the limited transmission of mycoviruses through ascospores is the potential physical and biochemical barriers that exist within the ascus, hindering the transfer and infection of mycoviruses (Xie et al. 2006; Son et al. 2013). Additionally, ascospore production in certain fungi might not coincide with active mycoviral infection or replication, further limiting the possibility of mycovirus transmission through this spore type (Gross et al. 2012; Khalifa et al. 2013). It is crucial to fully understand the mechanisms of mycovirus transmission, including the potential involvement of ascospores, to explore approaches that enhance the spread of mycoviruses in fungal populations for biological control applications.

By infecting and replicating within fungal cells, viruses can disrupt or modify the expression of key genes involved in virulence and secondary metabolite biosynthesis. This interference can result in the attenuation or enhancement of virulence traits and the modulation of secondary metabolite production (Hurwitz et al. 2013). Fungal toxins, such as DON, are secondary metabolites produced by fungi under specific conditions (Chen et al. 2019). DON belongs to the B group of monotelomeric compounds and is widely distributed and influential as a

fungal toxin worldwide. It is commonly referred to as a vomiting toxin and is primarily involved in fungal virulence (Desjardins et al. 1993). While viral infection can attenuate fungal virulence, the regulation of DON production can vary depending on the specific mycovirus-fungus interaction. For instance, a hypovirus, Fusarium graminearum hypovirus 2 (FgHV2), which also induces hypovirulence in *F. graminearum,* can reduce the accumulation of DON (Li et al. 2015). However, in our study, we noted that CHV1 reduced the phytopathogenicity of *F. graminearum* whereas increased DON production (Figs. 1 and 3). Moreover, genes related to DON biosynthesis were also upregulated upon CHV1 infection, particularly *Tri5,* which was significantly upregulated in TBI medium compared to PDA medium. Additionally, CHV1 infection induced a high accumulation of Tri-GFP-labeled toxisomes. While the wild-type strain required 72 h for toxisome induction, CHV1 infection achieved the same in just 48 h (Fig. 3). These results indicate that CHV1 upregulated the biosynthesis of DON production. In our study, we also observed that either DON production or the replication of CHV1 in TBI medium was significantly increased compared to that in PDB medium (Fig. 4). However, the addition of exogenous DON toxin did not actually increase the accumulation of CHV1 (Fig. 4). This indicates that the production process of DON is beneficial for the replication of CHV1, rather than the DON itself.

Previous studies have suggested that stressors such as H_2O_2 can stimulate DON synthesis (Ponts et al. 2006). Furthermore, CHV1 infection was shown to change stress enzyme activity in *C. parasitica*, and associated with the accumulation of H_2O_2 (Nuskern et al. 2017). This implies that CHV1 infection induces a stress condition during fungal growth, leading to increased DON accumulation. Given that CHV1 has a broad impact on host fungal metabolism (Brusini et al. 2017), it is likely that CHV1 affect diverse factors involved in *F. graminearum* pathogenicity beside DON biosynthesis. Further studies are needed to investigate the mechanism underlying CHV1's contrasting effects in increasing DON production whereas diminishing the pathogenicity of *F. graminearum*.

LDs are widely present dynamic organelles that store and supply lipids in all eukaryotic cells and some prokaryotic cells for energy metabolism and lipid membrane synthesis (Olzmann et al. 2019), An increasing number of studies indicate that LDs are related to viral replication; for example, hepatitis C virus (HCV) relies on the host's lipid metabolism pathway for replication, and its particle assembly occurs on LDs and endoplasmic reticulum membranes (Miyanari et al. 2007). LDs occur within the leaflets of the phospholipid bilayer in the endoplasmic reticulum (Kassan et al. 2013; Choudhary et al. 2015). A previous study by Liu et al. (2019) demonstrated the significant involvement of the TOR pathway in regulating LDs, DON biosynthesis, and phytopathogenicity in *F. graminearum*. FgNem1/FgCak1/FgSpo7 or FgPah1 are key players in TOR-mediated regulation of LD biogenesis. Deletion of FgNem1/FgCak1/FgSpo7 or FgPah1 disrupts the response to TOR signaling, resulting in decreased LD synthesis. The absence of these genes also reduced DON synthesis, as well as the pathogenicity in *F. graminearum* (Liu et al. 2019). Notably, in our observation, CHV1-infection downregulated the transcription of FgNem1/FgCak1/FgSpo7 or FgPah1 (Fig. 6a) and inhibited LD formation (Fig. 6b, c) during growth on TBI medium, suggesting the possibility that CHV1 diminishes LD formation through suppression of TOR pathway. Conversely, CHV1 infection upregulates the transcription levels of *Tri* genes (Fig. 3f) and enhances DON biosynthesis, thereby increasing DON production (Fig. 3). Further investigations are necessary to determine if CHV1 also influences other factors related to LD formation and DON synthesis.

Conclusion

In this study, we investigated the impact of CHV1 infection on *F. graminearum*. Our analysis revealed that CHV1 reduced the pathogenicity and conidiation of *F. graminearum* while enhancing the production of DON, a crucial factor in *F. graminearum*'s virulence. We observed that CHV1 triggered the biosynthesis and DON production, which in turn promoted virus replication. Additionally, our findings indicated that CHV1 infection disrupts the formation of lipid droplets while also leading to an increase in DON production.

Methods

Fungal strains and culturing

The wild-type fungal strain *F. graminearum* PH-1 (PH-1 strain) was kindly provided by Prof. Huiquan Liu (Northwest A&F University, China) (Xin et al. 2023). The Tri1-GFP-expressing *F. graminearum* strain (FgTri1-GFP) (Tang et al. 2020) was kindly provided by Prof. Cong Jiang (Northwest A&F University, China). Fungal protoplast isolation and CHV1 transfection were performed following the methods described by Bian et al. 2020. All fungal strains used in this study were cultivated in PDA at 25℃ for 3–5 days for morphological observation and RNA preparation. All experiments were independently repeated at least three times.

Virus transmission assay

The horizontal transmission of CHV1 through hyphal fusion was carried out as previously described (Chiba

et al. 2015). In brief, recipient strains of *F. graminearum* were co-cultured with CHV1-infected *F. graminearum* strains in PDA plates. After 5–7 days of co-culturing, mycelial plugs were collected from two different positions (1 and 2) on the recipient side. These mycelial plugs were then cultured on a new PDA plate layered with cellophane to extract RNAs for viral dsRNA analysis or perform reverse transcription-polymerase chain reaction (RT-PCR) detection. The vertical transmission of CHV1 was evaluated separately for asexual spores (conidia) and sexual spores (ascospores) in this study. Asexual spores were prepared in the liquid medium Carboxymethylcellulose (CMC) as described (Zhou et al. 2010). On the other hand, sexual spores were assayed in carrot solid medium (Kong et al. 2018). Both conidia and ascospores were collected and suspended in water before being placed on a PDA plate. Individual strains derived from a single conidium and ascospore were isolated and examined for CHV1 infection.

Pathogenesis assay

The pathogenesis of fungal colonies was assessed on coleoptiles, tomatoes and wheat heads following the methods as previously described (Zhang et al. 2012; Jiang et al. 2020; Ren et al. 2022). For each experiment, conidia were obtained from the tested fungal colonies grown in CMC medium for 5 days. These conidia were then resuspended in sterile water to a concentration of 10^6 spores/mL.

In the pathogenicity assay using spikelet, flowering wheat heads of the cultivar Xiaoyan 22 were inoculated with 10 μL conidia suspension (10^6 spores/mL) on the third or fourth spikelet from the base of the inflorescence. The wheat heads were enclosed in plastic bags for 48 h to maintain moisture. Scab symptoms were observed 14 days post-inoculation (dpi) (Ren et al. 2022). For the pathogenicity assay using coleoptiles, the top of the wheat coleoptile was excised, and 2 μL conidia suspension (10^6 spores/mL) was inoculated into the wounds for 7 days (Zhang et al. 2012). Pathogenicity assays on tomatoes were conducted as previously described (Jiang et al. 2020). The tomato fruits were wounded after surface disinfection and then inoculated with 10 μL conidia suspension (10^6 spores/mL). The inoculated tomatoes were incubated at 25℃ and 100% humidity for 7 days.

dsRNA and total RNA extraction, RT-PCR, quantitative RT-PCR (qRT-PCR)

dsRNA extraction from mycelia was conducted as described previously (Li et al. 2019). Total RNA was extracted from the mycelia as previously described (Suzuki et al. 2002). To eliminate fungal chromosomal DNA, the extracted total RNA was treated with RQ1 DNase I (Promega, USA, M6101) at 37℃ for 30 min, as previously described (Xiong et al. 2019). The first-strand cDNAs were synthesized using EasyScript Reverse Transcriptase (TransGen, China, AE101-02) and utilized for virus RT-PCR detection and qRT-PCR. The qRT-PCR reactions were performed with Green qPCR SuperMix (TransGen, China, AQ601-01-V2) on a CFX96TM Real-Time PCR Detection System. The *18S* gene served as an internal control for normalization (Wei et al. 2019). All primers used in this study are listed in Additional file 2: Table S1. The relative expression levels of each gene were calculated with the $2^{-\triangle\triangle Ct}$ method. Biological triplicates were included for each gene analyzed.

Analysis of DON production

Conidia were obtained from 3–5-day cultures of *F. graminearum* in liquid CMC. The conidia were then suspended and cultured in trichothecene biosynthesis induction (TBI) medium, and DON production was assessed as previously described (Ren et al. 2022) with some modifications. For TBI culturing, the conidia (20 μL, 10^6 spores/mL) were placed in 2 mL TBI medium in a 24-well plate, wrapped in tin foil, and incubated at 25℃ for 7 days. The resulting mycelia from the TBI medium were collected for RNA preparation. For wheat head culturing, a previously described method was followed (Jiang et al. 2020). The conidia (10 μL, 10^6 spores/mL) were inoculated onto wheat spikelets, as described above. In the case of rice medium, 6 g of rice was soaked in deionized water for 1 h and then sterilized at 121℃ for 1 h. The cooked rice was placed on a PDA plate and grown with fungal mycelia at 25℃ for 28 days (Dong et al. 2020). Afterward, the fungal mycelia in these culture media were collected and freeze-dried using a freeze dryer (LABCONCO, USA, Freezone PI J 2.5). The collected samples were ground into a homogeneous flour and subjected to mycotoxin extraction, as described previously (Ren et al. 2022). DON was quantified using gas chromatography-mass spectrometry (the GC-MS-QP2010 system with AOC-20i autoinjector (Jiang et al. 2020).

Fluorescent imaging and lipid droplets (LDs) staining

An FgTri1-GFP strain infected with CHV1 was obtained through hyphal fusion. Virus-free FgTri1-GFP and FgTri1-GFP with CHV1 were cultured in TBI medium at 25℃ for 5 days. To observe the expression of the FgTril-GFP fusion protein in the mycelium, confocal laser scanning microscopy was employed using an Olympus FV3000 instrument with a laser excitation/emission wavelength of 488/510–550 nm, as described previously (Niu et al. 2022). To monitor the lipid droplets (LDs) in the mycelium, the LD staining dyes BODIPY 493/503 (Sigma-Aldrich, USA, 790389) was used (Liu et al. 2019).

Staining was performed following the manufacturers' instructions.

Western blotting analysis

The western blotting analysis was carried out as previously described (Sun et al. 2006). The FgTril-GFP protein was detected by anti-GFP (1:5000; GFP Mouse Monoclonal Antibody, Beyotime, China, AF0159). For secondary antibody detection, a goat anti-rabbit immunoglobulin G-horseradish peroxidase (IgG-HRP, 1:5000; pRoteintech) was used.

DON and Triacsin C treatment

Fungus was cultured in a PDA medium containing 40 μg/mL DON (MedChemExpress, USA, HY-N6684) at 25℃ for 5 days (Wang et al. 2018). The conidia were harvested from CHV1-infected *F. graminearum* strains that were cultured in CMC medium at 25℃ for 5 days. The resulting conidia were suspended in 100 μL (10^6 spores/mL) and cultured in 20 mL medium, including PDB, PDB containing 40 μg/mL DON or 5 μM/mL Triacsin C (MedChemExpress, USA, HY-N6707) (Dechandt et al. 2017), and liquid TBI medium, at 25℃ for 3 days. The mycelia were collected for qRT-PCR assay and LD staining. All experiments were repeated at least three times.

Abbreviations:

cDNAs	Complementary DNAs
CHV1	Cryphonectria hypovirus 1
CLSM	Confocal Laser Scanning Microscope
CMC	Carboxymethylcellulose
C. parasitica	Cryphonectria parasitica
DON	Deoxynivalenol
dpi	days post-inoculation
dsRNA	Double-stranded RNA
FgGMTV1	Fusarium graminearum gemytripvirus 1
FgHV2	Fusarium graminearum hypovirus 2
F. graminearum	Fusarium graminearum
FgV1	Fusarium graminearum virus 1
FHB	Fusarium head blight
HCV	Hepatitis C virus
H_2O_2	Hydrogen peroxide
LDs	Lipid droplets
ORFs	Open reading frames
PDA	Potato dextrose agar
PDB	Potato dextrose broth
qRT-PCR	Quantitative real-time PCR
RT-PCR	Reverse transcription-polymerase chain reaction
TBI	Trichothecene biosynthesis induction
TOR	Target of rapamycin
Tri	Trichothecene
VIGS	Virus-induced gene silencing
V. mali	Valsa mali

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00272-7.

Additional file 1: Figure S1. Transfection of CHV1 into the FgTri1-GFP strain. **a** The FgTri1-GFP strain was co-cultured with CHV1-infected *F. graminearum* in PDA. CHV1 was transfected through hyphal anastomosis. **b** Detection of CHV1 infection in the FgTri1-GFP strain was achieved using dsRNA and RT-PCR analysis. **c** The FgTri1-GFP strains, with or without CHV1 infection, were cultured on PDA for 5 days and then photographed. Both virus-free and CHV1-infected *F. graminearum* PH-1 strains were cultured in parallelfor comparison.

Additional file 2. Table S1. A list of primers used in this study.

Acknowledgments

We thank the High-Performance Computing Center of Northwest A&F University for providing computing resources.

Authors' contributions

LS and RB designed research; RB, HR, MJ, ZZ, SZ, and TP performed research; RB, HR, and IBA analyzed data; RB and LS wrote the manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (32170163) and the Natural Science Foundation of Xinjiang Uygur Autonomous Region (2021D01D12) to LS.

Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the paper, its supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors of the manuscript have reviewed and consented to the publication of identifiable details, including photographs and any information within the text in the journal of *Phytopathology Research*.

Competing interests

Authors declare no conflict of interests.

Received: 27 March 2024 Accepted: 5 July 2024
Published online: 27 September 2024

References

Allen TD, Nuss DL. Specific and common alterations in host gene transcript accumulation following infection of the chestnut blight fungus by mild and severe hypoviruses. J Virol. 2004;78:4145–55.

Andika IB, Kondo H, Suzuki N. Dicer functions transcriptionally and posttranscriptionally in a multilayer antiviral defense. Proc Natl Acad Sci U S A. 2019;116:2274–81.

Bai GH, Desjardins AE, Plattner RD. Deoxynivalenol-nonproducing fusarium graminearum causes initial infection, but does not cause disease spread in wheat spikes. Mycopathologia. 2002;153:91–8.

Bian RL, Andika IB, Pang TX, Lian ZQ, Wei S, Niu EB, et al. Facilitative and synergistic interactions between fungal and plant viruses. Proc Natl Acad Sci U S A. 2020;117:3779–88.

Boenisch MJ, Schäfer W. Fusarium graminearum forms mycotoxin producing infection structures on wheat. BMC Plant Biol. 2011;11:110.

Bönnighausen J, Schauer N, Schäfer W, Bormann J. Metabolic profiling of wheat rachis node infection by Fusarium graminearum - decoding deoxynivalenol-dependent susceptibility. New Phytol. 2019;221:459–69.

Brusini J, Wayne ML, Franc A, Robin C. The impact of parasitism on resource allocation in a fungal host: the case of *Cryphonectria parasitica* and its mycovirus, Cryphonectria Hypovirus 1. Ecol Evol. 2017;7:5967–76.

Chen B, Nuss DL. Infectious cDNA clone of hypovirus CHV1-Euro7: a comparative virology approach to investigate virus-mediated hypovirulence of the chestnut blight fungus *Cryphonectria parasitica*. J Virol. 1999;73:985–92.

Chen F, Yan B, Ren J, Lyu R, Wu YF, Guo YT, et al. FIT2 organizes lipid droplet biogenesis with ER tubule-forming proteins and septins. J Cell Biol. 2021;220(5):e201907183.
Chen Y, Kistler HC, Ma ZH. *Fusarium graminearum* Trichothecene Mycotoxins: Biosynthesis, Regulation, and Management. Annu Rev Phytopathol. 2019;57:15–39.
Chiba S, Suzuki N. Highly activated RNA silencing via strong induction of dicer by one virus can interfere with the replication of an unrelated virus. Proc Natl Acad Sci U S A. 2015;112:E4911–4918.
Chiba S, Velasco L, Ayllón MA, Suzuki N, Lee-Marzano SY, Sun LY, et al. ICTV Virus Taxonomy Profile: Hypoviridae 2023. J Gen Virol. 2023;104. https://doi.org/10.1099/jgv.0.001848.
Choi GH, Nuss DL. Hypovirulence of chestnut blight fungus conferred by an infectious viral cDNA. Science. 1992;257:800–3.
Choudhary V, Ojha N, Golden A, Prinz WA. A conserved family of proteins facilitates nascent lipid droplet budding from the ER. J Cell Biol. 2015;211:261–71.
Chu YM, Jeon JJ, Yea SJ, Kim YH, Yun SH, Lee YW, et al. Double-stranded RNA mycovirus from *Fusarium graminearum*. Appl Environ Microbiol. 2002;68:2529–34.
Chun J, Ko YH, Kim DH. Transcriptome Analysis of Cryphonectria parasitica Infected With Cryphonectria hypovirus 1 (CHV1) Reveals Distinct Genes Related to Fungal Metabolites, Virulence, Antiviral RNA-Silencing, and Their Regulation. Front Microbiol. 2020;11:1711.
Chun J, Ko YH, So KK, Cho SH, Kim DH. A fungal GPI-anchored protein gene functions as a virulence and antiviral factor. Cell Rep. 2022;41: 111481.
Craven MG, Pawlyk DM, Choi GH, Nuss DL. Papain-like protease p29 as a symptom determinant encoded by a hypovirulence-associated virus of the chestnut blight fungus. J Virol. 1993;67:6513–21.
Dechandt CRP, Zuccolotto-Dos-Reis FH, Teodoro BG, Fernandes A, Eberlin MN, Kettelhut IC, et al. Triacsin C reduces lipid droplet formation and induces mitochondrial biogenesis in primary rat hepatocytes. J Bioenerg Biomembr. 2017;49:399–411.
Deng FY, Allen TD, Hillman BI, Nuss DL. Comparative analysis of alterations in host phenotype and transcript accumulation following hypovirus and mycoreovirus infections of the chestnut blight fungus *Cryphonectria parasitica*. Eukaryot Cell. 2007;6:1286–98.
Desjardins AE, Hohn TM, Mccormick SP. Trichothecene biosynthesis in Fusarium species: chemistry, genetics, and significance. Microbiol Rev. 1993;57:595–604.
Dodds JA. Association of type 1 viral-like dsRNA with club-shaped particles in hypovirulent strains of *Endothia parasitica*. Virology. 1980;107:1–12.
Dong F, Zhang X, Xu JH, Shi JR, Lee YW, Chen XY, et al. Analysis of *Fusarium graminearum* Species Complex from Freshly Harvested Rice in Jiangsu Province (China). Plant Dis. 2020;104:2138–43.
Duan YB, Lu F, Zhou ZH, Zhao HH, Zhang J, Mao YS, et al. Quinone outside inhibitors affect DON biosynthesis, mitochondrial structure and toxisome formation in *Fusarium graminearum*. J Hazard Mater. 2020;398:122908.
Ghabrial SA, Castón JR, Jiang D, Nibert ML, Suzuki N. 50-plus years of fungal viruses. Virology. 2015;479–480:356–68.
Giraldo MC, Valent B. Filamentous plant pathogen effectors in action. Nat Rev Microbiol. 2013;11:800–14.
Graziani F, Pinton P, Olleik H, Pujol A, Nicoletti C, Sicre M, et al. Deoxynivalenol inhibits the expression of trefoil factors (TFF) by intestinal human and porcine goblet cells. Arch Toxicol. 2019;93:1039–49.
Gross A, Zaffarano PL, Duo A, Grünig CR. Reproductive mode and life cycle of the ash dieback pathogen *Hymenoscyphus pseudoalbidus*. Fungal Genet Biol. 2012;49:977–86.
Hallaj Salahipour M, Hasanzadeh S, Malekinejad H, Razi M, Farrokhi-Ardebili F. Deoxynivalenol reduces quality parameters and increases DNA damage in mice spermatozoa. Andrologia. 2019;51:e13238.
Hillman BI, Halpern BT, Brown MP. A viral dsRNA element of the chestnut blight fungus with a distinct genetic organization. Virology. 1994;201:241–50.
Hurwitz BL, Hallam SJ, Sullivan MB. Metabolic reprogramming by viruses in the sunlit and dark ocean. Genome Biol. 2013;14:R123.
Jiang C, Hei RN, Yang Y, Zhang SJ, Wang QH, Wang W, et al. An orphan protein of *Fusarium graminearum* modulates host immunity by mediating proteasomal degradation of TaSnRK1α. Nature Communications. 2020;11:4382.
Jiang JH, Liu X, Yin YN, Ma ZH. Involvement of a velvet protein FgVeA in the regulation of asexual development, lipid and secondary metabolisms and virulence in *Fusarium graminearum*. PLoS One. 2011;6:e28291.
Kassan A, Herms A, Fernández-Vidal A, Bosch M, Schieber NL, Reddy BJ, et al. Acyl-CoA synthetase 3 promotes lipid droplet biogenesis in ER microdomains. J Cell Biol. 2013;203:985–1001.
Khalifa ME, Pearson MN. Molecular characterization of three mitoviruses coinfecting a hypovirulent isolate of *Sclerotinia sclerotiorum* fungus. Virology. 2013;441:22–30.
Kimura M, Tokai T, Takahashi-Ando N, Ohsato S, Fujimura M. Molecular and genetic studies of fusarium trichothecene biosynthesis: pathways, genes, and evolution. Biosci Biotechnol Biochem. 2007;71:2105–23.
Kong XJ, Van Diepeningen AD, Van Der Lee TaJ, Waalwijk C, Xu JS, Xu J, et al. The *Fusarium graminearum* Histone Acetyltransferases Are Important for Morphogenesis, DON Biosynthesis, and Pathogenicity. Front Microbiol. 2018;9:654.
Kotta-Loizou I. Mycoviruses and their role in fungal pathogenesis. Curr Opin Microbiol. 2021;63:10–8.
Li H, Bian RL, Liu Q, Yang L, Pang TX, Salaipeth L, et al. Identification of a Novel Hypovirulence-Inducing Hypovirus From Alternaria alternata. Front Microbiol. 2019;10:1076.
Li PF, Tedersoo L, Crowther TW, Wang BZ, Shi Y, Kuang L, et al. Global diversity and biogeography of potential phytopathogenic fungi in a changing world. Nat Comm. 2023;14:6482.
Li PF, Wang SC, Zhang LH, Qiu DW, Zhou XP, Guo LH. A tripartite ssDNA mycovirus from a plant pathogenic fungus is infectious as cloned DNA and purified virions. Sci Adv. 2020;6:eaay9634.
Li PF, Zhang HL, Chen XG, Qiu DW, Guo LH. Molecular characterization of a novel hypovirus from the plant pathogenic fungus *Fusarium graminearum*. Virology. 2015;481:151–60.
Liu H, Wang H, Liao XL, Gao B, Lu X, Sun D, et al. Mycoviral gene integration converts a plant pathogenic fungus into a biocontrol agent. Proc Natl Acad Sci U S A. 2022;119:e2214096119.
Liu N, Yun YZ, Yin YN, Hahn M, Ma ZH, Chen Y. Lipid droplet biogenesis regulated by the FgNem1/Spo7-FgPah1 phosphatase cascade plays critical roles in fungal development and virulence in *Fusarium graminearum*. New Phytol. 2019;223:412–29.
Ma TL, Zhang LX, Wang MH, Li YQ, Jian YQ, Wu L, et al. Plant defense compound triggers mycotoxin synthesis by regulating H2B ub1 and H3K4 me2/3 deposition. New Phytol. 2021;232:2106–23.
Machado AK, Brown NA, Urban M, Kanyuka K, Hammond-Kosack KE. RNAi as an emerging approach to control Fusarium head blight disease and mycotoxin contamination in cereals. Pest Manag Sci. 2018;74:790–9.
Mccabe PM, Pfeiffer P, Van Alfen NK. The influence of dsRNA viruses on the biology of plant pathogenic fungi. Trends Microbiol. 1999;7:377–81.
Mcfadden JJP, Buck KW, Rawlinson CJ. Infrequent Transmission of Double-stranded RNA Virus Particles but Absence of DNA Proviruses in Single Ascospore Cultures of Gaeumannomyces graminis. J Gen Virol. 1983;64:927–37.
Menke J, Weber J, Broz K, Kistler HC. Cellular development associated with induced mycotoxin synthesis in the filamentous fungus *Fusarium graminearum*. PLoS One. 2013;8:e63077.
Miyanari Y, Atsuzawa K, Usuda N, Watashi K, Hishiki T, Zayas M, et al. The lipid droplet is an important organelle for hepatitis C virus production. Nat Cell Biol. 2007;9:1089–97.
Myers JM, James TY. Mycoviruses. Curr Biol. 2022;32:R150–r155.
Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L. Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. Front Public Health. 2016;4:148.
Niu EB, Ye CZ, Zhao WY, Kondo H, Wu YF, Chen JP, et al. Coat protein of Chinese wheat mosaic virus upregulates and interacts with cytosolic glyceraldehyde-3-phosphate dehydrogenase, a negative regulator of plant autophagy, to promote virus infection. J Integr Plant Biol. 2022;64:1631–45.
Nuskern L, Tkalec M, Ježić M, Katanić Z, Krstin L, Ćurković-Perica M. Cryphonectria hypovirus 1-Induced Changes of Stress Enzyme Activity in Transfected Phytopathogenic Fungus *Cryphonectria parasitica*. Microbial ecology. 2017;74:302–11.
Olzmann JA, Carvalho P. Dynamics and functions of lipid droplets. Nat Rev Mol Cell Biol. 2019;20:137–55.

Pestka JJ. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol. 2010;84:663–79.
Ponts N, Pinson-Gadais L, Verdal-Bonnin MN, Barreau C, Richard-Forget F. Accumulation of deoxynivalenol and its 15-acetylated form is significantly modulated by oxidative stress in liquid cultures of *Fusarium graminearum*. FEMS Microbiol Lett. 2006;258:102–7.
Reid LM, Mather DE, Bolton AT, Hamilton RI. Evidence for a Gene for Silk Resistance to *Fusarium graminearum* Schw. Ear Rot of Maize J Heredity. 1994;85:118–21.
Ren JY, Zhang YH, Wang YH, Li CL, Bian ZY, Zhang X, et al. Deletion of all three MAP kinase genes results in severe defects in stress responses and pathogenesis in *Fusarium graminearum*. Stress Biol. 2022;2:6.
Shapira R, Choi GH, Hillman BI, Nuss DL. The contribution of defective RNAs to the complexity of viral-encoded double-stranded RNA populations present in hypovirulent strains of the chestnut blight fungus *Cryphonectria parasitica*. Embo j. 1991;10:741–6.
Son M, Lee KM, Yu J, Kang M, Park JM, Kwon SJ, et al. The HEX1 gene of *Fusarium graminearum* is required for fungal asexual reproduction and pathogenesis and for efficient viral RNA accumulation of Fusarium graminearum virus 1. J Virol. 2013;87:10356–67.
Stephens AE, Gardiner DM, White RG, Munn AL, Manners JM. Phases of infection and gene expression of *Fusarium graminearum* during crown rot disease of wheat. Mol Plant Microbe Interact. 2008;21:1571–81.
Sun LY, Nuss DL, Suzuki N. Synergism between a mycoreovirus and a hypovirus mediated by the papain-like protease p29 of the prototypic hypovirus CHV1-EP713. J Gen Virol. 2006;87:3703–14.
Sun LY, Suzuki N. Intragenic rearrangements of a mycoreovirus induced by the multifunctional protein p29 encoded by the prototypic hypovirus CHV1-EP713. Rna. 2008;14:2557–71.
Suzuki N, Maruyama K, Moriyama M, Nuss DL. Hypovirus papain-like protease p29 functions in trans to enhance viral double-stranded RNA accumulation and vertical transmission. J Virol. 2003;77:11697–707.
Suzuki N, Nuss DL. Contribution of protein p40 to hypovirus-mediated modulation of fungal host phenotype and viral RNA accumulation. J Virol. 2002;76:7747–59.
Tang GF, Chen AH, Dawood DH, Liang JT, Chen Y, Ma ZH. Capping proteins regulate fungal development, DON-toxisome formation and virulence in *Fusarium graminearum*. Mol Plant Pathol. 2020;21:173–87.
Tang GF, Chen Y, Xu JR, Kistler HC, Ma ZH. The fungal myosin I is essential for Fusarium toxisome formation. PLoS Pathog. 2018;14:e1006827.
Tang ZJ, Tang HY, Wang WQ, Xue YF, Chen DD, Tang WH, et al. Biosynthesis of a New Fusaoctaxin Virulence Factor in Fusarium graminearum Relies on a Distinct Path To Form a Guanidinoacetyl Starter Unit Priming Nonribosomal Octapeptidyl Assembly. J Am Chem Soc. 2021;143:19719–30.
Tijerino A, Cardoza RE, Moraga J, Malmierca MG, Vicente F, Aleu J, et al. Overexpression of the trichodiene synthase gene tri5 increases trichodermin production and antimicrobial activity in *Trichoderma brevicompactum*. Fungal Genet Biol. 2011;48:285–96.
Tuomivirta TT, Kaitera J, Hantula J. A novel putative virus of Gremmeniella abietina type B (Ascomycota: Helotiaceae) has a composite genome with endornavirus affinities. J Gen Virol. 2009;90:2299–305.
Voigt CA, Schäfer W, Salomon S. A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. Plant J. 2005;42:364–75.
Walter S, Nicholson P, Doohan FM. Action and reaction of host and pathogen during Fusarium head blight disease. New Phytol. 2010;185:54–66.
Wang QH, Chen DP, Wu MC, Zhu JD, Jiang C, Xu JR, et al. MFS Transporters and GABA Metabolism Are Involved in the Self-Defense Against DON in *Fusarium graminearum*. Front Plant Sci. 2018;9:438.
Wei S, Bian RL, Andika IB, Niu EB, Liu Q, Kondo H, et al. Symptomatic plant viroid infections in phytopathogenic fungi. Proc Natl Acad Sci U S A. 2019;116:13042–50.
Wu SS, Cheng JS, Fu YP, Chen T, Jiang DH, Ghabrial SA, et al. Virus-mediated suppression of host non-self recognition facilitates horizontal transmission of heterologous viruses. PLoS Pathog. 2017;13:e1006234.
Xie JT, Jiang DH. New insights into mycoviruses and exploration for the biological control of crop fungal diseases. Annu Rev Phytopathol. 2014;52:45–68.
Xie JT, Wei DM, Jiang DH, Fu YP, Li GQ, Ghabrial S, et al. Characterization of debilitation-associated mycovirus infecting the plant-pathogenic fungus Sclerotinia sclerotiorum. J Gen Virol. 2006;87:241–9.
Xin KY, Zhang Y, Fan LG, Qi ZM, Feng CJ, Wang QH, et al. Experimental evidence for the functional importance and adaptive advantage of A-to-I RNA editing in fungi. Proc Natl Acad Sci U S A. 2023;120:e2219029120.
Xiong Q, Zhang LQ, Waletich J, Zhang LL, Zhang C, Zheng XY, et al. Characterization of the Papain-Like Protease p29 of the Hypovirus CHV1-CN280 in Its Natural Host Fungus *Cryphonectria parasitica* and Nonhost Fungus *Magnaporthe oryzae*. Phytopathology. 2019;109:736–47.
Xu C, Chen HZ, Wu QY, Wu Y, Daly P, Chen J, et al. Trehalose-6-phosphate phosphatase inhibitor: N-(phenylthio) phthalimide, which can inhibit the DON biosynthesis of *Fusarium graminearum*. Pestic Biochem Physiol. 2021;178:104917.
Yang SA, Dai RY, Salaipeth L, Huang LL, Liu J, Andika IB, et al. Infection of Two Heterologous Mycoviruses Reduces the Virulence of Valsa mali, a Fungal Agent of Apple Valsa Canker Disease. Frontiers in Microbiology. 2021;12:659210.
Zhang HX, Xie JT, Fu YP, Cheng JS, Qu Z, Zhao ZZ, et al. A 2-kb Mycovirus Converts a Pathogenic Fungus into a Beneficial Endophyte for Brassica Protection and Yield Enhancement. Mol Plant. 2020;13:1420–33.
Zhang J, Chen J, Hu LF, Jia RM, Ma Q, Tang JJ, et al. Antagonistic action of Streptomyces pratensis S10 on *Fusarium graminearum* and its complete genome sequence. Environ Microbiol. 2021;23:1925–40.
Zhang LH, Wang SC, Ruan SJ, Nzabanita C, Wang YF, Guo LH. A Mycovirus VIGS Vector Confers Hypovirulence to a Plant Pathogenic Fungus to Control Wheat FHB. Adv Sci (Weinh). 2023;10:e2302606.
Zhang XW, Jia LJ, Zhang Y, Jiang G, Li X, Zhang D, et al. In planta stage-specific fungal gene profiling elucidates the molecular strategies of *Fusarium graminearum* growing inside wheat coleoptiles. Plant Cell. 2012;24:5159–76.
Zhou X, Heyer C, Choi YE, Mehrabi R, Xu JR. The CID1 cyclin C-like gene is important for plant infection in *Fusarium graminearum*. Fungal Genet Biol. 2010;47:143–51.
Zhou ZH, Duan YB, Zhou MG. 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. 2020;22:598–614.
Zhu MZ, Cen YF, Ye W, Li SN, Zhang WM. Recent Advances on Macrocyclic Trichothecenes, Their Bioactivities and Biosynthetic Pathway. Toxins (Basel). 2020;12(6):417.