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# Identification of Fusarium proliferatum chrysovirus 1 and its biocontrol activity of phytopathogenic *Fusarium* species

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## Abstract

*Fusarium proliferatum*, a pathogenic fungus that causes root rot, poses a significant threat to the yield and quality of *Salvia miltiorrhiza*. This study introduces Fusarium proliferatum Chrysovirus 1 (FpCV1), a novel mycovirus isolated from strain RZJJ28. FpCV1 consists four double-stranded RNA segments (FpCV1-1 to FpCV1-4), each encoding an open reading frame (ORF). Phylogenetic analysis, corroborated by BLASTp comparison and multiple sequence alignment, identified FpCV1 as a novel member of the genus *Betachrysovirus* within the family *Chrysoviridae*. Pathogenicity assays revealed that FpCV1 attenuates the virulence of *F. proliferatum*. Notably, FpCV1 can be horizontally transmitted to other *Fusarium* pathogens of *S. miltiorrhiza* root rot, reducing their virulence. Furthermore, FpCV1-carrying strain RZJJ28 has good potential as a biocontrol agent for the control of *S. miltiorrhiza* root rot. Additionally, FpCV1 can be also transferred to the pathogen *F. oxysporum*, which causes Fusarium wilt, thereby inducing hypovirulent effect. This report provides new insights and resources for the ecofriendly management of root rot disease in *S. miltiorrhiza* and potentially other Chinese medicinal plants. It also opens up the possibility of using one mycovirus (such as FpCV1) to control a variety of plant diseases caused by *Fusarium* species.

**Keywords** FpCV1, *Salvia miltiorrhiza* root rot, *Hypovirulence*, *Chrysoviridae*

## Background

Mycoviruses, also known as fungal viruses, are widely distributed across almost all fungal taxa, including yeasts, mushrooms, oomycetes, and filamentous fungi (Xie and Jiang 2014; Ghabrial et al. 2015; Kondo et al. 2022; Myers and James 2022). According to the International Committee on Taxonomy of Viruses (ICTV; https://ictv.global/), the genome types of mycoviruses are classified as single-stranded DNA (ssDNA), negative single-stranded RNA (-ssRNA), positive single-stranded RNA (+ssRNA), and double-stranded RNA (dsRNA) (Xie and Jiang 2014; Myers and James 2022; Hough et al. 2023). The dsRNAs included eleven families (*Amalgaviridae*, *Chrysoviridae*, *Curvulaviridae*, *Endornaviridae*, *Megabirnaviridae*, *Partitiviridae*, *Picobirnaviridae*, *Polymycoviridae*, *Quadriviridae*, *Spinareoviridae*, and *Totiviridae*) and one unspecified genus (*Botybirnavirus*) (Jiang et al. 2022; Song et al. 2024).

The family *Chrysoviridae* contains the genera *Alphachrysovirus* and *Betachrysovirus*, which can be distinguished through phylogenetic analysis of their RNA-dependent RNA poly-merase (RdRp) sequences (Shahi et al. 2021; Zheng et al. 2023). The genome of

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*Chrysoviridae* contains 3 to 7 segments (typically 4), with sizes ranging from 0.8 kb to 3.7 kb. The diameter of the virus particles is approximately 40 nm. Generally, each viral segment contains a single-stranded open reading frame (ORF), with the largest dsRNA1 encoding RdRp (Zheng et al. 2023). More than 40 chrysoviruses have been reported in the ICTV, covering almost all major phytopathogenic fungi (Jiang et al. 2022; Wu et al. 2023; Zou et al. 2024). Some chrysoviruses can induce low virulence in their hosts, such as Magnaporthe oryzae chrysovirus 1-A (MoCV1-A), Magnaporthe oryzae chrysovirus 1-B (MoCV1-B), and Magnaporthe oryzae chrysovirus 1-D (MoCV1-D) (Urayama et al. 2010, 2014; Higashiura et al. 2019). Infections with these viruses cause growth defects, changes in colony morphology, and inhibition of conidial germination in *Magnaporthe oryzae*, thereby leading to a hypovirulent phenotype. Notably, this reduction in virulence may be associated with the viral structural proteins. For example, the heterologous expression of MoCV1-A-ORF4 suppresses the growth of *Saccharomyces cerevisiae*, causing vacuolar enlargement and nuclear fragmentation (Urayama et al. 2012). The heterologous expression of the MoCV1-A-ORF4 protein can also inhibit conidial germination in *M. oryzae* (Urayama et al. 2016).

*Fusarium* species are important plant pathogenic fungi in agricultural production. Some *Fusarium* species infect medicinal crops, food crops, and cash crops, causing significant diseases such as root rot, stem rot, and base rot (Yang et al. 2021). Dozens of mycoviruses have been reported in *Fusarium* species, several of which can reduce host fungal virulence (Zhang et al. 2023; Zou et al. 2024). For example, Fusarium graminearum virus 1 (FgV1) can reduce the hyphal growth rate, increase pigmentation, and decrease the virulence of *F. graminearum*, making it a potential biocontrol agent against Fusarium head blight (Chu et al. 2002). Additionally, Fusarium graminearum virus China 9 (FgV-ch9) reduces the growth rate, lowers sporulation ability, disrupts cellular structures, and decreases the virulence of *F. graminearum* (Darissa et al. 2012; Bormann et al. 2018). Fusarium oxysporum alternavirus 1 (FoAV1) also reduces the virulence of *F. oxysporum*, providing a biocontrol option against bitter gourd wilt (Wen et al. 2021).

Five known Chrysoviruses have been documented in *Fusarium* species: Fusarium oxysporum chrysovirus 1 (FoV1), Fusarium oxysporum f. sp. dianthi mycovirus 1 (FodV1), Fusarium sacchari chrysovirus 1 (FsCV1), Fusarium graminearum dsRNA mycovirus 2 (FgV2), and Fusarium graminearum mycovirus China 9 (FgV-ch9) (Darissa et al. 2011; Yu et al. 2011; Lemus-Minor et al. 2015; Yao et al. 2020). Previous studies have shown that FodV1, FgV2, and FgV-ch9 affect the biological traits of their host fungi and reduce their virulence (Zou et al. 2024). However, there have been no reports of mycoviruses in *F. proliferatum*.

*Salvia miltiorrhiza bge* is a traditional and significant medicine in China (Jia et al. 2019). In recent years, as the cultivated area has gradually increased, various diseases, including root rot of *S. miltiorrhiza*, have a significant impact on both yield and quality, and their severity has been increasing (Han et al. 2023). Pathogens of *S. miltiorrhiza* root rot includes *F. solani*, *F. proliferatum*, and *F. oxysporum* (Yang et al. 2021). Current control measures for *S. miltiorrhiza* root rot primarily involve agricultural practices, chemical treatments, and biological control methods (Sa et al. 2022). Although chemical control is convenient and effective, it may give raise to concerns such as antifungal resistance and pesticide residues (Bamisi et al. 2024). Consequently, biological control, as an environmentally friendly measure, has shown significant scientific potential and application value in controlling *S. miltiorrhiza* root rot (Sa et al. 2022; Song et al. 2024). In recent years, the use of hypovirulent mycoviruses for plant disease control has received growing attention. For instance, Cryphonectria hypovirus 1 (CHV1) has been successfully employed to control chestnut blight (Anagnostakis 1982; Milgroom and Cortesi 2004). Similarly, Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1) has been used to treat Sclerotinia stem rot, with the SsHADV-1-infected strain DT-8 not only controlling the pathogen but also endophytically colonizing rapeseed, promoting plant growth and enhancing immunity (Yu et al. 2013; Zhang et al. 2020; Qu et al. 2021). However, the use of mycoviruses to control diseases in medicinal plants remains rare.

The horizontal transmission ability of mycoviruses limits their potential as biocontrol agents. In Europe, CHV1 effectively controls chestnut blight, but it failed in North America. This could be due to the presence of multiple vegetative compatibility groups (VCGs) in the chestnut blight pathogen in North America that restrict virus transmission (Nuss 2005). *Fusarium* diseases are often caused by complex *Fusarium* species, such as *F. asiaticum*, *F. graminearum*, *F. tricinctum*, and *F. pseudograminearum*, which cause Fusarium head blight in wheat (Kettle et al. 2015; Song et al. 2022), or *F. solani*, *F. proliferatum*, and *F. oxysporum*, which cause root rot in *S. miltiorrhiza* (Yang et al. 2021). Therefore, studying the cross-species transmission capabilities of mycoviruses within the *Fusarium* genus is crucial for the application of mycoviruses in disease control. Previous studies have shown that Fusarium graminearum virus-DK21 (FgV1-DK21), Fusarium poae virus 1-Fa (FpV1-Fa), and Fusarium avenaceum alternavirus 1 (FaAV1) (Lee et al.

2011; Song et al. 2022; Zhang et al. 2023) can successfully cross-transmit to other *Fusarium* species, demonstrating the promising application prospects of mycoviruses in controlling *Fusarium*-related diseases.

In this study, we identified a mycovirus, Fusarium proliferatum chrysovirus 1 (FpCV1), from the *F. proliferatum* strain RZJJ28, which causes root rot in *S. miltiorrhiza*. We analyzed the viral structure and phylogeny of FpCV1 and tested its hypovirulent effects on the fungal host. Furthermore, we successfully achieved the horizontal transmission of FpCV1 to other *Fusarium* pathogens, thereby reducing their virulence. This study aims to provide new strategies and insights for using mycoviruses in the control of plant diseases.

## Results

### DsRNA segments in strain RZJJ28

The *F. proliferatum* strain RZJJ28 was isolated from the roots of *S. miltiorrhiza* plants that exhibited root rot symptoms in Ruzhou city, Henan Province, China (Fig. 1a). This strain harbors four double-stranded RNA (dsRNA) segments, designated dsRNA1, dsRNA2, dsRNA3, and dsRNA4. Following treatment with DNase I and S1 nuclease, the sizes of these dsRNA segments were determined to be approximately 3.6 kb, 2.8 kb, 2.8 kb, and 2.5 kb, respectively (Fig. 1b).

### Molecular characterization of FpCV1 in the *F. proliferatum* strain RZJJ28

The complete sequence lengths of the four dsRNA segments from the *F. proliferatum* strain RZJJ28 have been deposited in the NCBI GenBank database with accession numbers OR765976, OR765977, OR765978, and OR765979. These segments have lengths of 3570, 2810, 2779, and 2583 bp, respectively, and their GC contents are 48%, 52%, 52%, and 50%, respectively. The 5' untranslated regions (UTRs) of the plus strands of the four dsRNA segments were 81, 84, 96, and 39 bp long, whereas the 3'-UTRs were 72, 86, 124, and 51 bp long, respectively. Sequence analysis revealed that each segment contains an open reading frames (ORFs) encoding putative proteins (Fig. 1c), specifically.

DsRNA1 contains an ORF (ORF1) that encodes a polyprotein of 1138 amino acids (aa), with a molecular mass of approximately 128.2 kDa. This protein includes a conserved RdRp domain spanning amino acids 543 to 846. A BLASTp search revealed that this protein shares significant similarity with the RdRp encoded by Fusarium

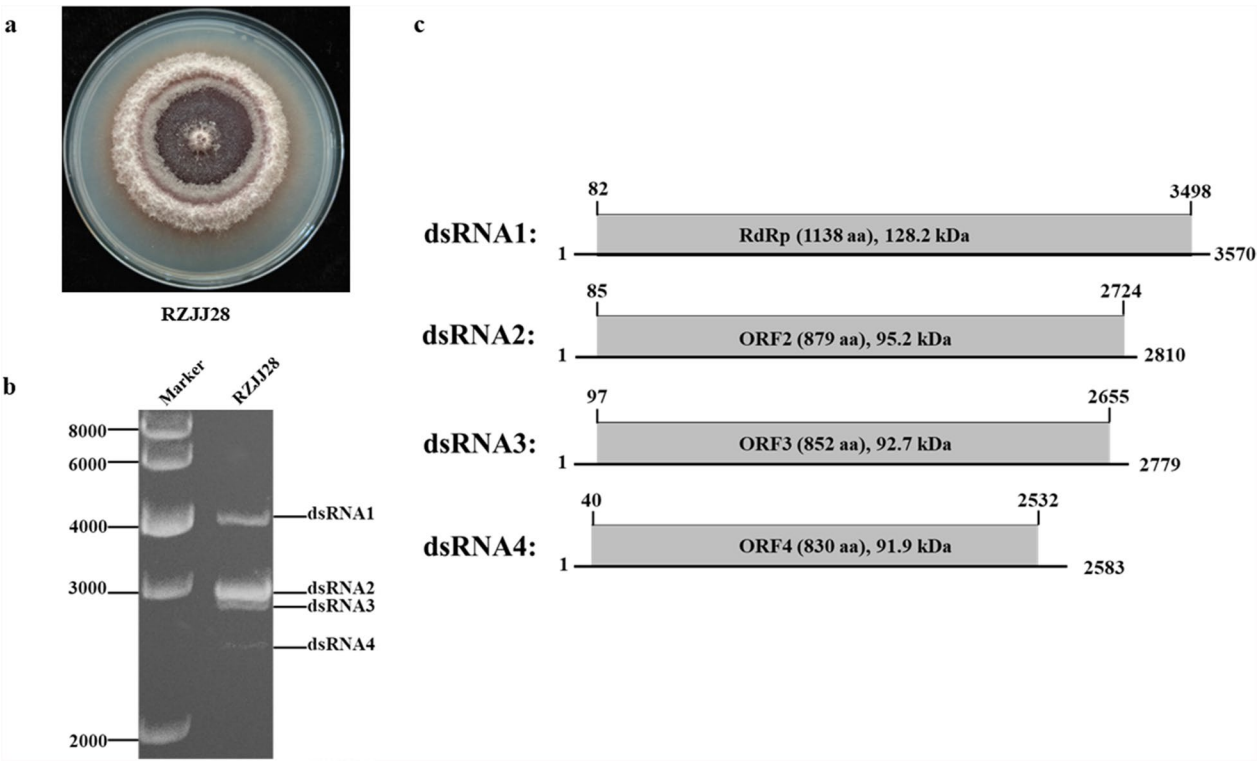



**Fig. 1** The colony morphology of strain RZJJ28, electrophoresis of FpCV1 and its genome structure diagram. **a** Colony morphology of the *F. proliferatum* strain RZJJ28 after seven days of growth. **b** Agarose gel electrophoresis of dsRNA treated with DNase I and S1 nuclease. **c** Genome diagram of FpCV1

sacchari chrysovirus 1 (QIQ28417.1), with 87.45% identity and an E-value of 0.0 (Table 1).

DsRNA2 contains an ORF (ORF2) encoding a putative 879-aa protein with a molecular mass of approximately 95.2 kDa. BLASTp searches revealed that this protein is most similar to the putative protein encoded by Fusarium oxysporum f. sp. dianthi mycovirus 1 (YP_009158914.1), with 87.14% identity and an E-value of 0.0 (Table 1).

DsRNA3 contains an ORF (ORF3) encoding a putative 852-aa protein with a molecular mass of approximately 92.7 kDa. BLASTp searches revealed that this protein has significant similarity to the putative coat protein encoded by Fusarium sacchari chrysovirus 1 (QIQ28419.1), with 88.01% identity and an E-value of 0.0 (Table 1).

DsRNA4 contains an ORF (ORF4) encoding a putative 830-aa protein with a molecular mass of approximately 91.9 kDa. BLASTp searches revealed that this protein is most similar to another protein (QIQ28420.1), with 81.28% identity and an E-value of 0.0 (Table 1).

These findings suggest that the four dsRNA segments in the *F. proliferatum* strain RZJJ28 likely play important roles in the virus life cycle, potentially involving in replication, transcription, and possibly structural functions, given the presence of a coat protein-like sequence.

The RdRp sequences of this virus have been compared with those of other members of the *Chrysoviridae* family through conserved domain database retrieval and multiprotein alignment analysis. The results revealed that there are highly conserved amino acid sequences within the RdRp of FpCV1 and across other *Chrysoviridae* families (Fig. 2).

These findings suggest that the mycovirus isolated from the *F. proliferatum* strain RZJJ28, which possesses four double-stranded RNA (dsRNA) segments, represent a novel member of the *Chrysoviridae* family. Consequently, this virus has been named 'Fusarium proliferatum chrysovirus 1' (FpCV1). This represents the first reported instance of a chrysovirus associated with *F. proliferatum*, highlighting its potential importance in the context of viral diversity and the biology of *Fusarium* species.

The four dsRNA segments of FpCV1 each contain UTRs at both the 5' and 3' ends. Multiple sequence alignment of these UTRs sequences revealed that both the 5'-UTRs and 3'-UTRs of FpCV1 contain highly conserved nucleotide sequences (Fig. 3). The presence of conserved sequences in UTRs is a common feature among viruses, indicating their functional importance in the viral life cycle.

The classification and phylogenetic relationships of FpCV1 were investigated by comparing the RdRP amino acid sequence of FpCV1 with those of known members of the *Chrysoviridae* family, with a specific focus on the *Alphachrysovirus* and *Betachrysovirus* genera. Using the *Totiviridae* family as an outgroup, a phylogenetic tree was constructed via the maximum likelihood (ML) method with 1000 bootstrap repeats to ensure the robustness of the tree. These results indicate that FpCV1 clusters within the *Betachrysovirus* genus of the *Chrysoviridae* family (Fig. 4). This finding suggests that FpCV1 is a novel member of this genus that shares common ancestry and potentially functional similarities with other betachrysoviruses.

### Biological effects of FpCV1 on *F. proliferatum* strain RZJJ28

To investigate the potential impact of FpCV1 on the virulence of its host, *F. proliferatum* strain RZJJ28, we conducted a pot experiment using *S. miltiorrhiza* as the host plant. The experimental design involved inoculating *S. miltiorrhiza* seedlings with *F. proliferatum* strains of either RZJJ28 alone or DS20-2 (virus-free) and comparing their growth and development to those of uninoculated control (CK) seedlings.

At 40 days post-inoculation, the plants in the DS20-2 treatment group exhibited significant stunting and yellowing of the above-ground portions, with some plants wilting. In contrast, the plants in the RZJJ28 treatment group showed normal growth, with no obvious signs of

**Table 1** Identity between FpCV1-encoded proteins and equivalent proteins of other chrysoviruses

| Virus | ORF1 | | ORF2 | | ORF3 | | ORF4 | |
|---|---|---|---|---|---|---|---|---|
| | E-value | Identity | E-value | Identity | E-value | Identity | E-value | Identity |
| Fusarium sacchari chrysovirus 1 | 0 | 87.45 | 0 | 86.8 | 0 | 88.01 | 0 | 81.28 |
| Fusarium oxysporum f. sp. dianthi mycovirus 1 | 0 | 87.09 | 0 | 87.14 | 0 | 87.32 | 0 | 80.36 |
| Fusarium graminearum dsRNA mycovirus 2 | 0 | 62.93 | 0 | 48.52 | 0 | 51.18 | 0 | 41.77 |
| Fusarium graminearum mycovirus China 9 | 0 | 62.15 | 0 | 47.52 | 0 | 51.06 | 0 | 41.04 |
| Coniothyrium diplodiella chrysovirus 1 | 0 | 35.63 | 2.00E-46 | 28.8 | 1.00E-35 | 25.32 | 3.00E-09 | 25.56 |
| Colletotrichum fructicola chrysovirus 1 | 0 | 35.09 | 3.00E-45 | 29.43 | 1.00E-39 | 28.59 | N | N |
| Erysiphe necator associated chrysovirus 1 | 1.00E-127 | 35.6 | 7.00E-37 | 29.64 | 9.00E-41 | 26.05 | 3.00E-06 | 27.13 |

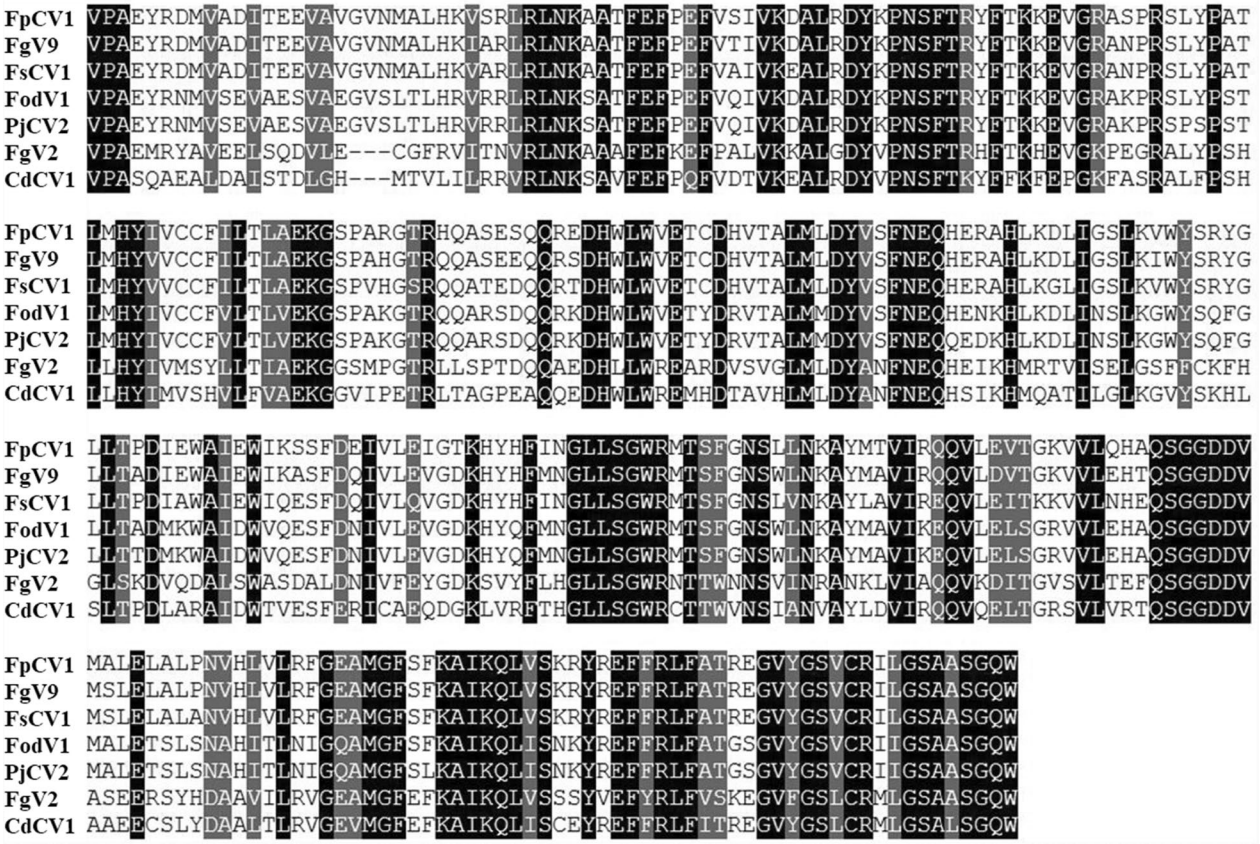



**Fig. 2** Multiple alignment of the conserved RdRp amino acid sequences encoded by FpCV1 and other *Chrysoviridae* family members

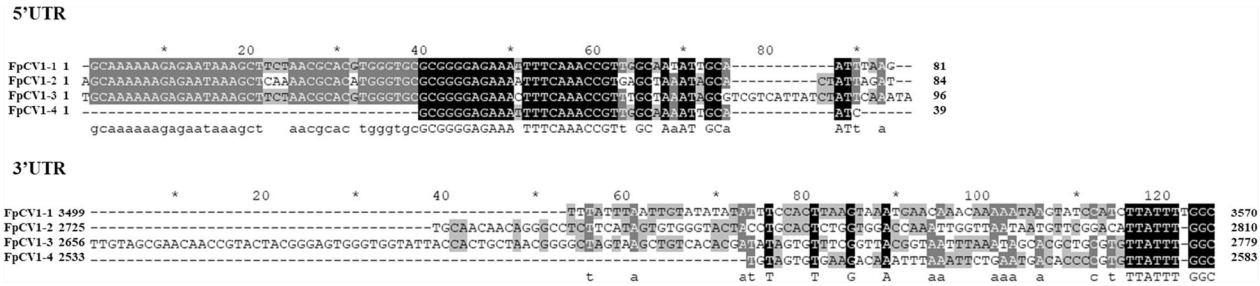



**Fig. 3** Multiple sequence alignment of FpCV1 dsRNA segments in the 5′ and 3′UTRs

wilting or yellowing (Fig. 5a, b). Additionally, there was no significant difference in root length or root weight between the RZJJ28 and CK treatment groups (Fig. 5c, d). These results suggest that FpCV1 may reduce the virulence of its host *F. proliferatum* strain RZJJ28 toward *S. miltiorrhiza*.

To further clarify the hypovirulent effect of FpCV1 on its host, we conducted additional experiments to remove FpCV1 from strain RZJJ28 via protoplast regeneration. A total of 10 regenerated strains were obtained during this process, and subsequent dsRNA extraction revealed that 5 of these strains were free of FpCV1, indicating a 50% virus-free rate in the protoplast progeny of RZJJ28. (Additional file 1: Figure S1a). Further validation via RT–PCR confirmed that strains RZJJ28-VF-4 and RZJJ28-VF-5 were virus-free (Additional file 1: Figure S1b).

We then examined the colony morphology, colony diameter, and conidia production of both the strains RZJJ28 and RZJJ28-VF. Compared with the




**Fig. 4** Phylogenetic analysis of the RdRp of FpCV1 and other mycoviruses via the maximum likelihood (ML) method in MEGA 11 with 1000 bootstrap replicates




**Fig. 5** Detection of the biological effects of living *S. miltiorrhiza*. **a**, **b** Growth status of *S. miltiorrhiza* plants 40 days after inoculation with a 10 mL spore suspension ($10^7$ $mL^{-1}$) of *F. proliferatum* DS20-2 (virus-free) or RZJJ28. **c, d** Analysis of the significance of differences in the length and weight of *S. miltiorrhiza* roots treated with *F. proliferatum* DS20-2 (virus-free) or RZJJ28. ($P < 0.05$)

wild-type strain RZJJ28, the virus-free strain RZJJ28-VF presented reduced colony pigmentation (Fig. 6a), faster growth (Fig. 6b), and no significant change in conidia production (Fig. 6c). These findings suggest that FpCV1 infection may influence the growth characteristics of the host fungus.

To determine whether FpCV1 infection could reduce the virulence of *F. proliferatum* in *S. miltiorrhiza* infection, we inoculated *S. miltiorrhiza* seedlings with strains RZJJ28 and RZJJ28-VF; untreated plants were used as controls. The results of the pot experiment revealed that plants inoculated with RZJJ28-VF exhibited

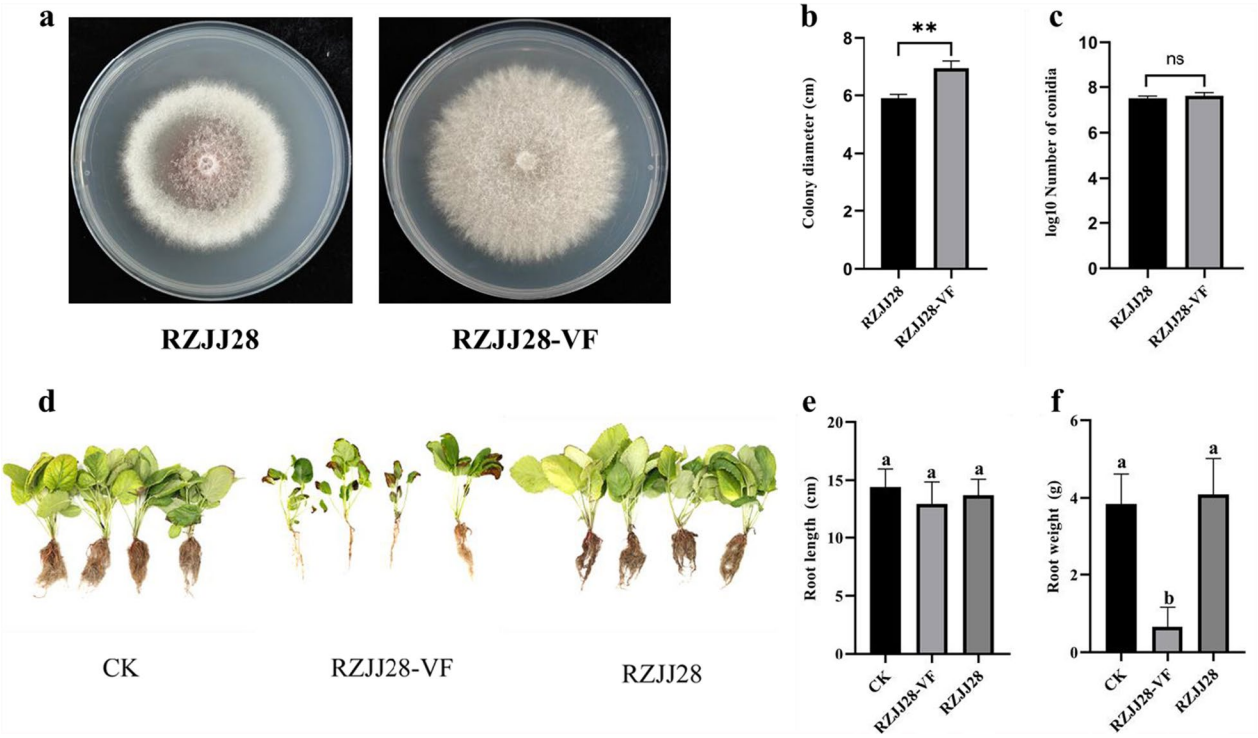



**Fig. 6** Characterization of FpCV1-infected and cured *F. proliferatum* strains RZJJ28 and RZJJ28-VF. **a** Colony morphology of RZJJ28 and RZJJ28-VF after five days of growth. **b** Colony diameters of RZJJ28 and RZJJ28-VF. **c** Conidial production of RZJJ28 and RZJJ28-VF. **d** Growth status of *S. miltiorrhiza* plants 40 days after inoculation with a 10 mL spore suspension ($10^7$ $mL^{-1}$) of *F. proliferatum* strains RZJJ28 and RZJJ28-VF. **e, f** Difference significance analysis of the root length and weight of *S. miltiorrhiza* plants ($P < 0.05$)

stunted growth and appeared relatively smaller, whereas those inoculated with RZJJ28 displayed only mild disease symptoms and were overall healthier with relatively mild symptoms (Fig. 6d). Additionally, we measured the root length and weight of the *S. miltiorrhiza* plants. The results revealed no significant difference in root length among the three treatments (Fig. 6e). However, the root weight was significantly reduced in the RZJJ28-VF group (Fig. 6f), whereas no significant difference was detected between the CK and RZJJ28 groups. These results indicate that FpCV1 infection can significantly reduce the virulence of *F. proliferatum*, which in turn alleviates the damage caused by root rot in *S. miltiorrhiza*. This finding highlights the potential role of FpCV1 in regulating the virulence of its host and suggests that this mycovirus could be explored as a biological control agent for managing *Fusarium* diseases in agricultural settings.

### Horizontal transmission capacity and hypovirulence of FpCV1

To investigate the horizontal transmission capability of FpCV1 to the three pathogens of *S. miltiorrhiza* root rot. We co-cultured RZJJ28 with three *S. miltiorrhiza* root rot pathogens-*F. proliferatum* strain DS20-2, *F. solani* strain F21, and *F. oxysporum* strain TD-1. The results showed that FpCV1 was successfully horizontally transmitted from RZJJ28 to strains DS20-2, F21 and TD-1 confirmed by dsRNA extraction (Fig. 7b) and RT-PCR (Fig. 7c), resulting in strains DS20-2 + V, F21 + V, and TD-1 + V (Fig. 7a).

To examine the impact of FpCV1 on the virulence of the *S. miltiorrhiza* root rot pathogen, we inoculated wild-type strains DS20-2, F21, TD-1, and FpCV1-carrying strains DS20-2 + V, F21 + V, TD-1 + V onto *S. miltiorrhiza* seedlings, using untreated plants as controls. After 30 days, the seedlings inoculated with wild-type strains (DS20-2, F21, and TD-1, respectively) exhibited significant growth inhibition, whereas those inoculated with FpCV1-carrying strains (DS20-2 + V, F21 + V, and TD-1 + V, respectively) displayed normal growth (Fig. 7d). Additionally, there were no significant differences in root length among three treatments (Fig. 7e). However, compared to the control, the root weight of plants treated with wild-type strains was significantly reduced, while no significant difference on those treated with FpCV1-carrying strains (Fig. 7f). These results indicate that FpCV1 infection can also effectively reduce the virulence of *F. proliferatum*,

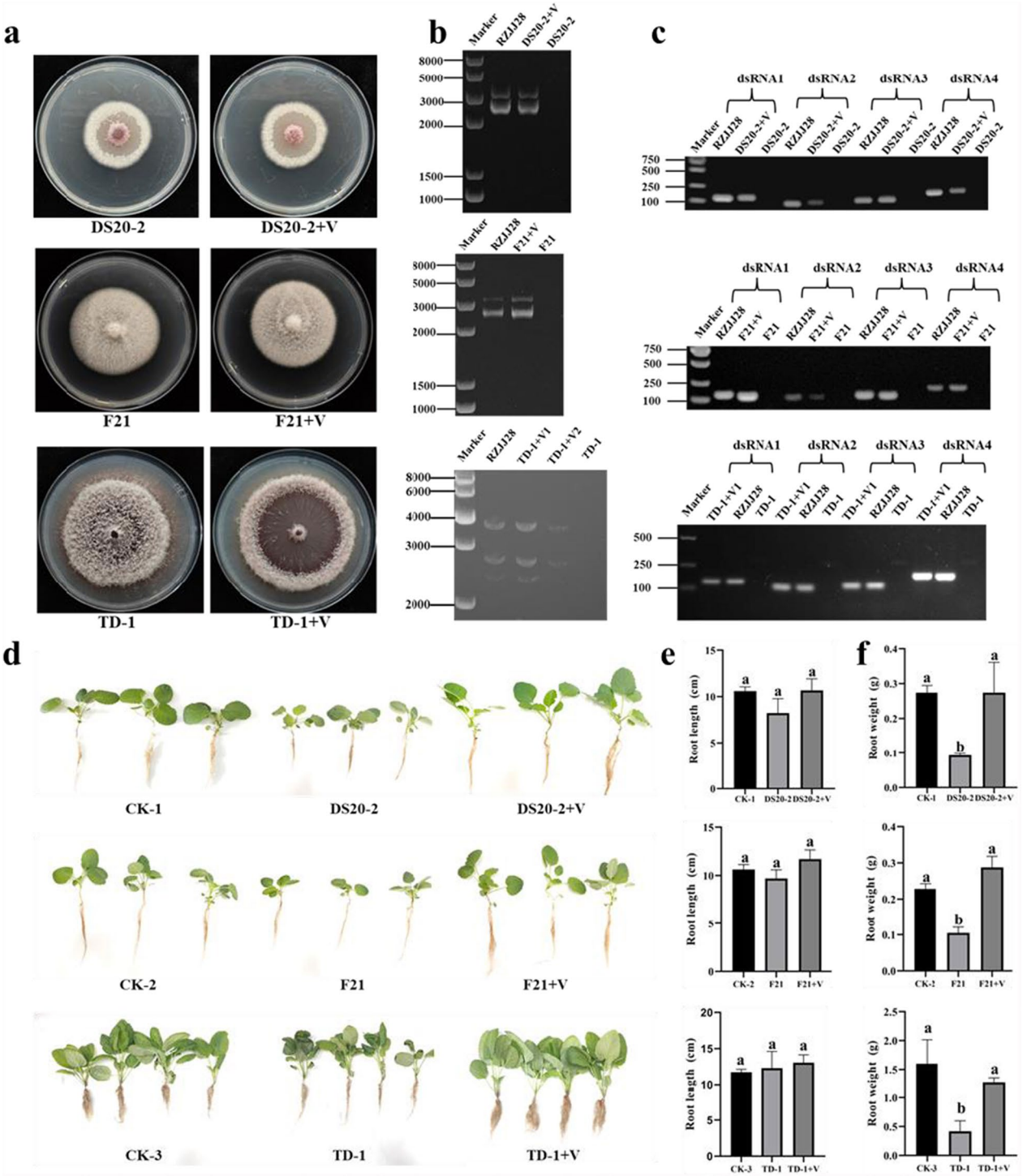



**Fig. 7** FpCV1 can transmit horizontally and reduce pathogen virulence. **a** The colony morphology of virus-free strains and virus-infected strains on PDA medium. **b** Horizontal transmission of FpCV1 detected via dsRNA extraction. **c** Horizontal transmission of FpCV1 detected by RT-PCR. **d** Growth status of *S. miltiorrhiza* plants 30 or 40 days after inoculation with a 10 mL spore suspension ($10^7$ $mL^{-1}$) of *F. proliferatum* strain DS20-2 and DS20-2 + V, *F. solani* strain F21 and F21 + V, *F. oxysporum* strain TD-1 and TD-1 + V, respectively. **e** Difference significance analysis of root length in *S. miltiorrhiza* plants. **f** Difference significance analysis of root weight in *S. miltiorrhiza* plants ($P < 0.05$)

*F. solani*, and *F. oxysporum*, the main pathogens of *S. miltiorrhiza* root rot, by horizontal transmission.

*Fusarium* species are important plant pathogens, which can cause many serious crop diseases, especially in soil-borne diseases. To investigate if the FpCV1 could spread to other fusarium pathogens, we successfully horizontally transmitted FpCV1 from strain RZJJ28 to the *F. oxysporum* strain KG-MC which is a soil-borne pathogen causing Fusarium wilt, and detected FpCV1 through dsRNA extraction and RT-PCR (Additional file 1: Figure S2b, c). Compared to wild-type strain KG-MC, the FpCV1-carrying strain KG-MC + V exhibited increased pigmentation, reduced growth rate, and no significant change in sporulation (Additional file 1: Figure S2a, d, e). Pot experiments demonstrated that the virulence of KG-MC + V was significantly lower than that of KG-MC (Additional file 1: Figure S2f). These findings suggest that FpCV1 can be horizontally transmitted to other soil-borne *Fusarium* pathogen and also reduce its virulence.

### Biological control effects of FpCV1 against *S. miltiorrhiza* root rot

To investigate the biocontrol potential of FpCV1-carrying strain when directly applied for disease control of *S. miltiorrhiza* root rot infected with multiple pathogens. We inoculated *S. miltiorrhiza* seedlings in the biocontrol treatment group with 10 mL of a spore suspension ($10^7$ $mL^{-1}$) of the FpCV1-carrying strain RZJJ28. Two days later, both the pathogen treatment group and the biocontrol treatment group were inoculated with 10 mL ($10^7$ $mL^{-1}$) of a mixed spore suspension of the pathogens *F. proliferatum* strain DS20-2, *F. solani* strain F21 and *F. oxysporum* strain TD-1, using untreated plants as control (CK). After 30 days, compared to the control, the pathogens mixture groups exhibited stunted growth, whereas the pathogens mixture + RZJJ28 strain groups plants displayed normal growth (Fig. 8a). There were no significant differences in root length between the three treatment groups (Fig. 8b). However, the root weight of pathogens mixture groups plants was significantly lower than that of both control and pathogens mixture + RZJJ28 strain groups plants (Fig. 8c). These results indicate the

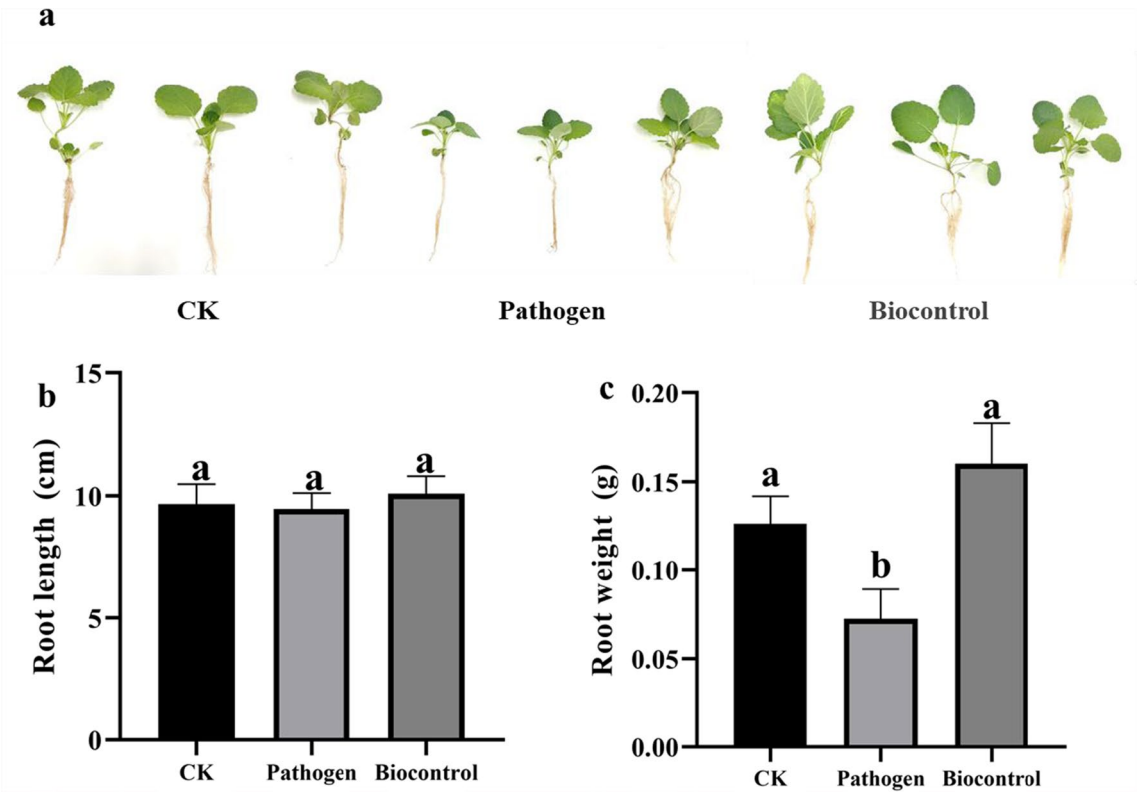



**Fig. 8** The virus-infected *F. proliferatum* strain RZJJ28 protects plants against simultaneous infection by three pathogens. **a** Using untreated plants as controls, the growth status of *S. miltiorrhiza* plants in both the pathogens mixture groups (Pathogen) and pathogens mixture + RZJJ28 strain groups (Biocontrol) was assessed after 30 days. **b** Difference significance analysis of root length in *S. miltiorrhiza* plants. **c** Difference significance analysis of root weight in *S. miltiorrhiza* plants ($P < 0.05$)

good potential of FpCV1 as a biocontrol agent against *S. miltiorrhiza* root rot.

## Discussion

In recent years, numerous novel mycoviruses have been identified in various phytopathogenic fungi (Xie and Jiang 2024). However, no mycoviruses have been identified in *F. proliferatum*, a phytopathogen that causes root rot in *S. miltiorrhiza*. Our study introduces FpCV1, a novel mycovirus isolated from the *F. proliferatum* strain RZJJ28, which belongs to the *Betachrysovirus* genus within the family *Chrysoviridae*. This represents the first documentary discovery of a Chrysovirus in *F. proliferatum*.

*Fusarium* contains many phytopathogenic species and can cause several devastating diseases, such as Fusarium head blight (FHB), Fusarium wilt, and root rot in various important crops. To date, mycoviruses have been reported in at least 19 *Fusarium* species (Zhou et al. 2024), and some of them have been reported to have a profound effect on their hosts. For example, FodV1, FgV2, and FgV-ch9 can decrease the growth rate of the host mycelium and reduce conidial production while also exhibiting reduced virulence (Li et al. 2019). Additionally, FodV1 may affect the colonization rate of the host fungus in plants (Torres-Trenas et al. 2019). However, there have been no reports of mycoviruses attenuating the virulence of *F. proliferatum*. In this study, we present the first evidence of such an occurrence with FpCV1. Compared with the strain RZJJ28-VF (virus-free), the strain RZJJ28 infected with FpCV1 presented increased colony pigmentation, slower mycelial growth, and no significant difference in spore production, but a significant reduction in the virulence of *F. proliferatum* was detected. This finding is significant, as it opens new possibilities for biological control strategies against diseases caused by *F. proliferatum*.

*S. miltiorrhiza* is an important Chinese medicinal plant. With the rapid expansion of planting areas, monoculture and repeated cropping, diseases, including root rot of *S. miltiorrhiza* caused by *Fusarium* spp., are becoming increasingly common and serious (Jia et al. 2019; Yang et al. 2021). Current control measures for *S. miltiorrhiza* root rot primarily involve agricultural practices, chemical treatments, and biological control methods (Sa et al. 2022). In recent years, mycoviruses have been successfully used to control plant diseases. For example, CHV1 has been utilized to manage chestnut blight in Europe, and SsHADV1 has been employed to control the Sclerotinia stem rot (Anagnostakis 1982; Yu et al. 2013). However, reports on the use of mycoviruses to control *S. miltiorrhiza* root rot are still limited. In this study, the ability of FpCV1 to reduce the virulence of *F. proliferatum* had been confirmed, as observed in *S. miltiorrhiza* seedling assays. Interestingly, the FpCV1 can successfully transmit and cause a hypovirulent effect not only in other *F. proliferatum* strain, but also in *F. solani* and *F. oxysporum* strains, which are the main pathogens responsible for *S. miltiorrhiza* root rot in the field. In addition, the apply directly with the FpCV1-carrying strain RZJJ28 has a good control effect to *S. miltiorrhiza* root rot infected with multiple pathogens, suggesting that this mycovirus has the potential to be used in managing root rot disease in medicinal plants such as *S. miltiorrhiza*. This is also particularly relevant given the limited reports on the use of one mycovirus for controlling diseases caused by variety of pathogens.

Mycoviruses generally lack an active transmission ability and are primarily horizontally transmitted through fungal hyphal fusion. Some studies have shown that mycoviruses can be horizontally transmitted from low-virulence fungi to high-virulence fungi in the field, reducing the virulence of high-virulence strains and thereby controlling plant diseases (Liu et al. 2016; Deng et al. 2022; Song et al. 2022; Hai et al. 2024). Research has shown that proline produced by plants can promote the spread of mycoviruses (Hai et al. 2024). The horizontal transmission capability of mycoviruses is crucial for their use in controlling agricultural diseases. Leptosphaeria biglobosa botybirnavirus 1 (LbBV1) can be successfully transmitted to *Botrytis cinerea* (Deng et al. 2022). The interspecies transmission of mycoviruses between *Fusarium* species has been documented more than once. FgV1-DK21 can be transmitted from *F. boothii* to *F. asiaticum*, *F. graminearum*, and *F. oxysporum* (Lee et al. 2011). FpV1-Fa can be successfully transferred from *F. asiaticum* to *F. poae* and *F. tricinctum* (Song et al. 2022). FaAV1 is transmitted from *F. avenaceum* to other *Fusarium* species (Zhang et al. 2023). In this study, we successfully horizontally transmitted FpCV1 from the *F. proliferatum* strain to other *Fusarium* species, like *F. proliferatum*, *F. solani* and *F. oxysporum*. And the FpCV1-obtained strains were also showed reduced virulence against *S. miltiorrhiza*. Besides, FpCV1 is successfully horizontally transmitted to the *F. oxysporum* f. sp. *momordicae* strain, an important pathogenic pathogen of Fusarium wilt, resulting in reduced virulence. Unfortunately, we tried other horizontal transmission experiments with FpCV1 from *F. proliferatum* to other *Fusarium* species, such as *F. graminearum*, *F. pseudograminis*, while these attempts were unsuccessful. Our findings have indicated that FpCV1 is a hypovirulence-associated mycovirus with a better horizontal transmission capability. It provides novel strategies and insights for using a mycovirus (such as FpCV1) to prevent multiple diseases (like root rot and

Fusarium wilt). This provides a new direction for the application of mycovirus to control plant diseases.

## Conclusions

In this study, we identified a novel chrysovirus, designated as, 'Fusarium proliferatum chrysovirus 1' (FpCV1), from the *F. proliferatum* strain RZJJ28. BLASTp analysis, multiple sequence alignment and phylogenetic analysis revealed that FpCV1 is a new member of the *Betachrysovirus* genus, the *Chrysoviridae* family. This represents the first discovery of a Chrysovirus in *F. proliferatum*. Our findings indicate that FpCV1 can reduce the virulence of *F. proliferatum* and can also be horizontally transferred to *F. solani* and *F. oxysporum*, the additional pathogens causing *S. miltiorrhiza* root rot, thereby reducing their virulence. Based on this, the FpCV1-carrying strain RZJJ28 can be used as a biocontrol agent to control *S. miltiorrhiza* root rot infecting by multiple Fusarium pathogens. Additionally, the FpCV1 can also be transferred to *F. oxysporum* causing Fusarium wilt, resulting in a hypovirulent effect. Our study highlights the potential of FpCV1 as a biological control agent for controlling root rot of *S. miltiorrhiza* and underscores the importance of further research to explore its practical applications of using a mycovirus to prevent multiple diseases in agricultural settings.

## Methods

### Strains and culture conditions

Ten fungal strains were used in this study. Strain RZJJ28 was isolated from the roots of a *S. miltiorrhiza* plant infected with root rot in Ruzhou city, Henan Province, China. *F. proliferatum* strain DS20-2 and *F. solani* strain F21 were kindly provided by Professor Wang Fei of Henan Academy of Agricultural Sciences. The strain TD-1 is a pathogenic strain causing root rot in *S. miltiorrhiza*, while the strain KG-MC is a highly virulent *F. oxysporum* f. sp. *momordicae* strain responsible for bitter gourd wilt, preserved by the Biological Control Laboratory of the College of Plant Protection at Henan Agricultural University. The virus-infected strains DS20-2 + V, F21 + V, TD-1 + V, and KG-MC + V were obtained through a co-culturing process involving RZJJ28 and DS20-2, F21, TD-1, and KG-MC, respectively, on PDA plates. All strains were cultured on PDA media under dark conditions at 28℃ for 5 days, after which they were preserved in 20% glycerol at −80℃.

### DsRNA extraction and purification

DsRNA extraction and purification were performed as previously described (Zhao et al. 2020). Fresh mycelium was cultured on PDA media covered with glass paper for 3–5 days. The fresh mycelium was rapidly frozen with liquid nitrogen, ground, and the dsRNA selectively absorbed using cellulose powder CF11 (Sigma-Aldrich, China). The extracted dsRNA was treated with RNase-free DNase I and S1 nuclease (Takara, Dalian, China) to remove genomic DNA and single-stranded RNA, respectively. The processed dsRNA was electrophoresed on a 1% agarose gel at 120 V for 50 min. The nucleic acids were stained with SuperRed (Seven, China) and visualized via a gel documentation and image analysis system (InGenius LhR, Syngene, UK).

### cDNA synthesis, cloning, and sequencing

cDNA synthesis was performed using the purified dsRNA as the template, as described previously (Zhao et al. 2020). As described previously (Zhao et al. 2020), cDNA synthesis was performed using the purified dsRNA as the template. Briefly, the purified dsRNA was reverse transcribed into the first cDNA strand via the random primers RACE3RT and HiScript III Reverse Transcriptase (Vazyme, Nanjing, Jiangsu, China). PCR amplification was subsequently carried out via the RACE3 primer. The PCR products were sequenced, and the obtained sequences were used to design specific primers for RT–PCR amplification. The sequences were then assembled via SnapGene 7.0. To obtain the 5' and 3' terminal sequences, PC3-T7 loop primers were ligated to the ends of the dsRNA via T4 RNA ligase, followed by RT–PCR amplification with terminal sequence primers and PC2. All PCR products were subsequently cloned and inserted into the pMD19-T vector, which was subsequently transformed into *E. coli* DH5α for sequencing. Sequencing was performed by Shanghai Bioengineering Company. To obtain high-quality consensus sequences, each nucleotide of the full-length cDNA was sequenced at least three times. All primers used for cDNA cloning and sequencing are listed in (Additional file 2: Table S1).

### Sequence and phylogenetic analyses

The ORFs and conserved domains of dsRNAs were predicted via ORF Finder and CD searches on the NCBI website (ncbi.nlm.nih.gov). Multiple sequence alignments with members of other partitiviruses were performed via the CLUSTALX (2.0) program (Thompson et al. 1997). The phylogenetic tree was constructed with MEGA11 software via the maximum likelihood (ML) method with 1000 bootstrap replicates (Tamura et al. 2021). The resulting phylogenetic tree was further refined and visualized via ITOL (Letunic and Bork 2021).

### Acquisition of RZJJ28-VF

Currently, the main methods for obtaining virus-free strains include the hyphal tip method, protoplast method,

ribavirin or cycloheximide treatment, and conidia progeny method (Liu et al. 2022; Urayama et al. 2010). To efficiently obtain virus-free strains, a combination of these methods can be used.

First, the mycelial tips of RZJJ28 were consecutively transplanted five times on PDA plates (ribavirin 100 μg/mL). Subsequently, five 1 $cm^2$ mycelial plugs were inoculated into 100 mL PDB medium and incubated at 28℃ with shaking at 180 rpm for 5 days. Subsequently, protoplasts were prepared following the previously described method (Nahalkova and Fatehi 2003). The RZJJ28 spore suspension obtained from shaking culture was filtered through a layer of Miracloth to collect spores. Transfer 5 mL of $1\times10^7$ spore suspension to 100 mL of fresh PDB medium and incubated on a shaker at 180 rpm and 28℃ for 12–14 h. The resulting mycelia were filtered through Miracloth and washed multiple times with 0.7 M NaCl solution to remove residual media. Clean mycelia (1 g fresh weight) were transferred into 20 mL of enzymatic digestion solution containing 0.1% snailase and 1% lysing enzymes dissolved in 0.7 M NaCl solution. The digestion was performed at 90 rpm and 28℃ for 3 h. The digestion mixture was then filtered through three layers of lens paper, washed thoroughly with 0.7 M NaCl solution, and centrifuged at 2246×*g* and 4℃ for 15 min. The resulting protoplasts were resuspended in an appropriate volume of STC buffer [20% sucrose, 50 mM $CaCl_2$, 50 mM Tris–HCl (pH = 8.0)]. After the protoplasts were diluted, they were cultured via the spread plate method. The hyphal tips were then selected to obtain protoplast progeny of RZJJ28. Successful mycovirus elimination was confirmed by extracting dsRNA and conducting RT-PCR.

#### Horizontal transmission of FpCV1

The horizontal transmission of FpCV1 was assessed according to previous methods (Liu et al. 2022; Zhang et al. 2023). The donor strain for the horizontal transmission experiment was RZJJ28, and the recipient strains were DS20-2 (Geneticin G418 resistance), F21 (Geneticin G418 resistance), TD-1 (hygromycin B resistant), and KG-MC (hygromycin B resistant). Mycelial discs from the donor and recipient strains were placed opposite each other on PDA plates, with a 2 cm distance between them. After allowing the mycelia of both strains to come into contact, the cultures were incubated for an additional 3 days. Five mycelial plugs were taken from the side of the plate near the recipient strain, for a total of 20 plugs. These mycelial plugs were then inoculated onto PDA plates containing Geneticin G418 or hygromycin B (50 μg/mL) and incubated in the dark at 28℃ for 3 days. The presence of FpCV1 in the recipient strains was confirmed through dsRNA extraction and RT–PCR.

#### RNA extraction and RT–PCR

To extract total RNA, strains were grown on the cellophane membrane overlying a PDA plate for 4–5 days, and fresh mycelia were harvested and ground to powder in liquid nitrogen. Total RNA was prepared using RNA reagent (TransGen Biotech, Beijing, China) according to the manufacturer's instructions.

For RT–PCR detection, first-strand cDNAs were synthesized via a HiScript III 1st Strand cDNA Synthesis Kit (+gDNA wiper) (Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer's instructions. PCR amplification was subsequently carried out via specific primers (Additional file 2: Table S1), which were designed on the basis of the sequences of FpCV1, to detect the virus-transmitted strains. The products amplified via RT–PCR were observed via electrophoresis in a 1.0% agarose gel.

#### Evaluation of the biocontrol potential of mycoviruses

To evaluate the impact of mycoviruses on fungi, the colony morphology, growth rate, spore production, and virulence of infected mycovirus strains were compared with those of virus-free strains. After 5 days of mycelial growth, observations were made, and photographs were taken. Mycelia were punched with a 0.5 cm diameter punch and placed in 15 mL of PDA medium for cultivation, with daily measurements of colony diameter. Pathogenicity testing was conducted through pot experiments in which uniformly growing *S. miltiorrhiza* plants were inoculated with 10 mL of spore suspension ($10^7$ $mL^{-1}$), whereas the control group received sterile water. Plants were grown in the greenhouse for several days, followed by photographic documentation and data collection. The virulence test of bitter gourd was performed using a method described previously (Wen et al. 2021). All the experiments were conducted in triplicate. The data were analyzed via Prism 8.0.2 software, and the treatment means were compared via the least significant difference test at the $P = 0.05$ level.

#### Abbreviations

| | |
|---|---|
| +ssRNA | Positive single-stranded RNA |
| -ssRNA | Negative single-stranded RNA |
| Aa | Amino acids |
| BLASTp | Basic local alignment search tools for proteins |
| CD | Conserved domain |
| cDNA | Complementary DNA |
| dpi | Days post-inoculation |
| dsRNA | Double-stranded RNA |
| FHB | Fusarium head blight |
| ICTV | International committee on classification of viruses |
| ITOL | Interactive tree of life |
| MEGA11 | Molecular evolutionary genetics analysis version 11 |
| ML | Maximum likelihood |
| NCBI | National Center for Biotechnology Information |
| ORFs | Open reading frames |

| | |
|---|---|
| PDA | Potato dextrose agar |
| PDB | Potato dextrose broth |
| RdRp | RNA-dependent RNA polymerase |
| Rpm | Revolutions per minute |
| RT-PCR | Reverse transcription-polymerase chain reaction |
| ssDNA | Single-stranded DNA |
| UTRs | Untranslated regions |
| VCGs | Vegetative compatibility groups |
| VF | Virus-free |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-025-00348-y.

**Additional file 1**: **Figure S1.** Detection of RZJJ28-virus-free (VF). **Figure S2.** Phenotypic effects of FpCV1 infection on KG-MC.

**Additional file 2**: **Table S1.** Primers used in the test.

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**Author contributions**
LS and YZ (Ying Zhao) designed the research. ZG, PS, MW, YZ (Yuanyuan Zhang), and MZ performed the experimental work. SL, YiZ (Ying Zhao), LS, YQ, CW, and FW analyzed the data and wrote the manuscript. YZ (Ying Zhao), ZG, CW, and FW modified and completed the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**
Not applicable.

## Declarations

**Ethics approval and consent to participate**
Not applicable.

**Consent for publication**
Not applicable.

**Competing interests**
The authors declare that they have no competing interests.

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