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Biocontrol action of *Trichothecium roseum* against the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici*

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Trichothecium roseum is known to be a mycoparasite and inhibit phytopathogenic fungi. However, so far, only scarce information is available on the impacts of *T. roseum* on powdery mildews. Based on the morphological and molecular analysis, we identified *T. roseum* as a mycoparasite on colonies of the wheat powdery mildew fungus (*Blumeria graminis* f. sp. *tritici*, Bgt, recently clarified as *B. graminis* s. str.) and then showed that *T. roseum* was capable of efficiently impairing colony formation and conidial distribution of Bgt. After inoculation of *T. roseum* conidia on Bgt colonies, the biomasses of Bgt significantly decreased 1.46, 1.64, 7.55, and 10.49 times at 2, 4, 6, and 8 dpi, respectively. Thus, *T. roseum*, acting as a potential biological agent, impeded the developments of Bgt, making it a viable alternative for wheat powdery mildew control. Utilizing the *Agrobacterium tumefaciens*-mediated transformation (ATMT) system, a *T. roseum* strain that constitutively expressed green fluorescent protein was produced to improve the visualization of the *T. roseum*-Bgt interaction and showed direct hyphae interaction of *T. roseum* with Bgt structures during parasitic processes. These findings indicate that ATMT is a potent and efficient method for transforming *T. roseum*. Nevertheless, our results suggest that *T. roseum* is an antagonistic parasite of the wheat powdery mildew fungus, and hence, can be considered for phytopathogen management.

KEYWORDS

biological control, *Trichothecium roseum*, fungal identification, wheat, powdery mildew fungus, *Blumeria graminis*, *Agrobacterium tumefaciens*-mediated transformation, fluorescent protein

Background

Blumeria graminis f. sp. *tritici* (Bgt), recently clarified as *B. graminis* s. str., infests wheat and causes a destructive foliar disease (Liu et al., 2021). It inflicts severe economic losses and is regarded as the world's sixth most crucial fungal phytopathogen (Dean et al., 2012). Currently, chemical fungicides, which potentially harm human health and environment, have been widely used to control the wheat powdery mildew disease. However, chemical fungicide treatments cause powdery mildews to develop resistance (Safaei et al., 2022). Thus, environmentally friendly and human-friendly alternatives for controlling Bgt were in the focus of recent research (Kiss, 2003; Zhu et al., 2022a). Previously, *Ampelomyces quisqualis, Pseudozyma flocculosa, Verticillium lecanii* and *Tilletiopsis spp*. were the main biocontrol agents (BCAs) for powdery mildew diseases (Hajlaoui and Bélanger, 1993; Falk et al., 1995; Dik et al., 1998; Köhl et al., 2019). So far, commercial biological control products for efficiently controlling cereal powdery mildew are still in demand and thus the identification of novel BCAs is needed.

The *Trichothecium* genus consists of at least 77 species, which mostly are plant pathogenic fungi (http://www.mycobank.org/). *T. roseum* is reported as a primarily fungus causing postharvest disease that infest a variety of vegetables, fruits, and crops (Barnett and Hunter, 1972), including tomato, cucumber, orange, apple, mango, strawberry, Hami melon, peppers and maize (Yang et al., 2003; Kasuyama and Tanina, 2007; Dal Bello, 2008; Kwon et al., 2010; Inácio et al., 2011; Hamid et al., 2014; Lin et al., 2016; Xue et al., 2016; Li et al., 2022). *T. roseum* promotes fruit degradation after infection and then disrupts the long-term preservation of fruits and vegetables (Wei et al., 2018). While it is a pathogen on plants, *T. roseum* is also a BCA against multiple plant diseases, including fungi and insects (Zhang et al., 2010). It was reported that *T. roseum*, acting as a BCA, infected the psyllid insect *Pauropsylla buxtoni* and suppressed the gemination of *Phakopsora pachyrhizi* uredospore (Kumar and Jha, 2002; Batta, 2020). Moreover, *T. roseum* was shown to be a BCA against plant phytopathogens, such as *Sclerotinia sclerotiorum, Rhizoctonia solani* and *Penicillium digitatum* (Freeman and Morrison, 1949; Huang and Kokko, 1993; Tesfagiorgis and Laing, 2010). However, little is known about the capability of *T. roseum* on efficiently parasitizing and/or suppressing powdery mildew fungi, including Bgt.

Identification of parasites that are capable of inhibiting cereal phytopathogens is crucial for developing BCAs against these diseases. In this study, we reported the discovery and identification of a *T. roseum* strain isolated from Bgt-infested wheat leaves under natural environmental conditions. Since there is little information available on the mycoparasitism and/or biocontrol effects of *T. roseum* on powdery mildews, we examined the disease-suppressive properties of *T. roseum* on Bgt developments. To enhance the visualization of the interactions between *T. roseum* and Bgt, a green fluorescent protein (GFP)-transformed strain of *T. roseum* was constructed using the *Agrobacterium tumefaciens*-mediated transformation (ATMT) system.

Materials and methods

Fungal and plant materials

Wheat seeds (*Triticum aestivum* L. cv. Aikang 58) were sown in plastic pots (Ø 9 cm) with sterilized nutrient soil (including peat soil, vermiculite and perlite) and pots were placed in growth chambers (temperature, 22°C; humidity, 70%; light/dark, 16 h/8 h) (Zhu et al., 2022b). *Blumeria graminis* f. sp. *tritici* (Bgt) (isolate Bgtzm2022) was propagated on its host wheat leaves under the same conditions. One day before inoculation, the powdery mildew-infected leaves were shaken to obtain fresh spores.

The mycoparasite was collected from mildew-infected wheat grown at Henan Normal University in 2021. And the single spore of the mycoparasite was isolated. To obtain a pure strain, individual spores of the mycoparasite were isolated and transferred to PDA and incubated at 25°C in the dark (Zhu et al., 2020a).

Microscopic observation

In order to morphologically identify the isolated mycoparasite, the fungus was firstly observed by light microscopy (Sunny Optical, EX30, Zhejiang, China), then the spores and mycelial structure were observed by scanning electron microscopy (SEM) (Hitachi TM3030Plus, Japan) according to a previously described method (Zhu et al., 2020a).

DNA extraction and amplification

The total genomic DNA of the mycoparasite was extracted according to the cetyltrimethylammonium bromide (CTAB) method (Rogers and Bendich, 1994). The mycoparasite ribosomal ITS sequence was amplified by polymerase chain reaction (PCR) with universal primers, ITS1 and ITS4 (White et al., 1990). Reaction mixture composition of PCR include 2X M5 HiPer plus Taq HiFi PCR mix (25 μL), Forward primer: ITS1 (5′-TCCGTAGGTGAACCT0GCGG-3′) (2.5 μL), Reverse primer: ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (2.5 μL), templete (1 μL), ddH_2O (19 μL). PCR was conducted on a C1000 TouchTM Thermal Cycler (Bio-Rad, Hercules, California, United States), applying following program: 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, 72°C for 1 min, and final elongation at 72°C for 5 min. 1.5% agarose gel was used for electrophoresis detection of PCR

products. The amplicon was sequenced (Invitrogen, Shanghai, China) and the obtained sequence was deposited into GenBank with Accession No. MZ292093.

Phylogenetic analysis

In order to further identify *T. roseum*, a phylogenetic tree of *Trichothecium* spp. ITS sequences was built. Sequences were retrieved and analyzed in MEGA software. The phylogenetic tree is constructed according to Maximum likelihood method with the options as 1000 bootstrap method, Tamura-Nei model, 50% site coverage cut off. The ITS sequences of *Arthrociadiella mougeotii* and *Blumeria graminis* were applied as outgroups (Zhu et al., 2020b, 2021a).

Hyperparasitism assays

To determine whether *T. roseum* was a mycoparasite, conidia of Bgt were inoculated onto healthy wheat leaves (cv. Aikang 58). Six days post inoculation (dpi), mildew-infected plants were inoculated with spore suspension (concentration 1×10^6 spores mL^{-1}) of the identified *T. roseum* strain and kept in a growth chamber. Bgt-inoculated plants treated with water were served as controls. At 2, 4, 6, and 8 dpi, the leaves were collected for microscopic analysis. Then the leaves were cut to 2 cm in size for discoloring and the fungal structures were stained with trypan blue according to a previous study and observed under a light microscopy (Sunny Optical, EX30, Zhejiang, China) to morphologically identify the fungal characteristics (Zhu et al., 2017). Meanwhile the leaves were air-dried for observation by the SEM.

Fungal biomass determination

To determine whether Bgt biomasses were affected by *T. roseum*, mildew-infested leaves were treated with water or *T. roseum* spore suspension. At 2, 4, and 6 dpi, leaves were collected and total DNA was isolated using a previously described method (Zhu et al., 2019). The elongation factor 1 alpha of wheat, *T. roseum* and Bgt was amplified with primers TaEF1 (wheat elongation factor 1, TaEF1-F: TGGTGTCATCAAGCCTGGTATGGT and TaEF1-R: ACTCATGGTGCATCTCAACGGACT), TrEF2 (*T. roseum* elongation factor 2, TrEF2-F: CGTCGCTTCTGACTCCAAGA and TrEF2-R: AGCCTCAACAGCCTTACCAG) and BgtEF1 (Bgt elongation factor 1, BgtEF1-F: AAGCTAAAGGCCGAACGTGA and BgtEF1-R: GCACAGTCAGCTTGAGAGGT), respectively (Coram et al., 2008; Hu et al., 2018). Each of the sequences was separately fused into the pMD^{TM}19-T vector (Takara, Japan). Real time PCR mixture [2x AceQ qPCR SYBR Green Master Mix (7.5 μL, Vazyme), forward primer (0.3 μL), reverse primer (0.3 μL), DNA Templete (1.5 μL), ddH_2O (5.4 μL)] was used for anlyzing. RT-qPCR experiments were conducted on a LightCycler 96 real-time PCR instrument (Roche, Switzerland) as followings processes: initiated at 95°C for 15 min, followed by 45 cycles at 95°C for 10 s and 60°C for 30 s. The fungal BgtEF1, TrEF1, and TaEF1 plasmids were diluted into a serial concentration for generation of the standard curves. Ct means were determined using qPCR. The Bgt, *T. roseum* and wheat DNA concentrations were calculated according to the corresponding standard curves. Then *T. roseum* and Bgt biomass changes were calculated based on the DNA concentration ratios of *T. roseum*/wheat or Bgt/wheat according to a previously reported method (Zhu et al., 2022a). The data was obtained from three ($n = 3$) independent biological replicates.

Green fluorescent protein transformation and observation of *T. roseum*

To transform *T. roseum*, *Agrobacterium tumefaciens* strain GV3101 was transferred with the binary vector pPK2Tgfp (Martínez-Cruz et al., 2017) and then grown for 24 h at 28°C in 2 ml of YEP medium (Yeast Extract Peptone Broth Medium) containing 50 μg ml^{-1} kanamycin and 50 μg ml^{-1} gentamicin in an orbital shaker at 140 rpm. The suspension of transferred *Agrobacterium tumefaciens* was directly pipetted onto *T. roseum* (strain ZM-Tr2021) grown on PDA and incubated for 4 days at room temperature (Zhu et al., 2022c). Then the spores of *T. roseum* were inoculated onto PDA medium containing 50 μg ml^{-1} carbendazim and 100 μg ml^{-1} cefotaxime and incubated for 10 days at room temperature. The newly formed colonies were selected as transformed *T. roseum* and used for further experiments.

To observe the interaction of *T. roseum* and Bgt, mildew-infested wheat leaves were treated with GFP-transformed *T. roseum* and incubated for 6 days under the same condition described above. The structures of *T. roseum* and Bgt were observed with a fluorescence microscope (BX63, Olympus, Japan).

Results

Identification of *T. roseum* as a mycoparasite in Bgt colony

Initially a mycoparasite on the wheat powdery mildew fungus *Blumeria graminis* f. sp. *tritici* (Bgt) was found. To identify the mycoparasite, the morphological characteristics and genetical analysis were conducted. The colonies of the fungus

on PDA were granular pink and white, with dark pink on the reverse side (Figures 1A,B). Conidia were hyaline, two-celled, and single-septate, ellipsoid to pyriform (15–25 × 8 to 12 μm), with truncation in the middle, and produced in clusters. Mycelia displayed hyaline hyphae and slender, 2–4 μm in diameter, and conidiophores were branched (Figure 1C). According to the morphological characteristics, the pathogen was initially identified as *Trichothecium roseum*.

The ITS region of the mycoparasite rDNA was PCR-amplified. The sequence of the amplicon (accession No. MZ292093) was 613 bp and showed 100% identity to a reported *T. roseum* (EU552162) strain on *Leucadendron xanthoconus*. To conduct phylogenetic analyse, the ITS sequences of *Trichothecium* sp. were retrieved and applied for phylogenetic analysis. The phylogenetic tree clearly showed that *T. roseum* (EU552162) and this identified fungus were clustered in the same branch (Figure 2). Therefore, the isolated mycoparasite was identified and confirmed as *T. roseum* based on the morphological characteristics and molecular analysis.

The mycoparasitical action of *T. roseum* on Bgt colonies

To test whether *T. roseum* was capable of parasiting on Bgt colonies, spore suspension of *T. roseum* or water was separately sprayed on Bgt-infected of wheat leaves and incubated for 8 days (Figure 3). Compared to the groups (water treatment), colonies of Bgt turned from white to dark and conidiophores were collapsed. By development on Bgt colonies, structures of *T. roseum* covered on Bgt colonies and interacted with Bgt conidia (Figures 3A–C,E–G). The SEM analysis showed the same results: conidiophores and conidia of Bgt were twined and destroyed by *T. roseum* structures (Figures 3D,H). In addition, *T. roseum* did not infect healthy wheat (Supplementary Figure 1). Therefore, the mycoparasitical action of *T. roseum* was confirmed.

The developments of *T. roseum* on Bgt

To further determine the developmental processes of *T. roseum* on Bgt, a mycoparasitism experiment was performed. Comparing to the control groups (Bgt treated with water) (Figures 4A–C), *T. roseum* started to form mycelia at 2 dpi, produced conidia at 4 dpi and extensively formed fungal strutures at 6 dpi. At the meantime, Bgt conidiophores and conidia were collapsed by *T. roseum* (Figures 4D–F).

T. roseum directly decreases biomasses of Bgt on wheat leaves

To quantitatively measure the biomass changes during the interactions, mildew-infested wheat leaves were inoculated with *T. roseum* and the biomasses of both fungi were quantified using RT-qPCR (Figures 5A–C). During the growth of *T. roseum,* the biomasses of *T. roseum* notably increased 1.74 times at 2 dpi, 366.36 times at 4 dpi, 1,458 at 6 dpi and 6,964 at 8 dpi when compared to that at 0 dpi (Figure 5D). In contrast, the biomasses of Bgt significantly decreased 1.46 times at 2 dpi, 1.64 time at 4 dpi, 7.55 times at 6 dpi and 10.49 times at 8 dpi when compared to that at 0 dpi (Figure 5E).

Green fluorescent protein transformation of *T. roseum*

To enhance the visualization of the mycoparasitism interaction between *T. roseum* and Bgt, spores of GFP transformed and wild type (untransformed) *T. roseum* were separately inoculated onto mildew-infested wheat leaves and examined under a fluorescence microscope. The GFP-transformed *T. roseum* strain showed direct interaction with Bgt structures (Figures 6A–C). In some cases, Bgt conidiophores and conidia were observed with fluorescent signals (Figures 6D–F). In addition, the mycoparasitical activities of the GFP-transformed *T. roseum* strain was not affected when compared to the wild-type strain.

Discussion

Identification of a hyperparasite on *Blumeria graminis* f. sp. *tritici* colony

T. roseum has been repeatedly reported as a fungus causing postharvest disease on a variety of fruits and vegetables (Yang et al., 2003; Kasuyama and Tanina, 2007; Dal Bello, 2008; Kwon et al., 2010; Inácio et al., 2011; Hamid et al., 2014; Lin et al., 2016; Tang et al., 2016; Xue et al., 2016; Li et al., 2022). In addition, *T. roseum* was shown to be a potential agent for biological control of plant fungal diseases (including soybean rust) and *Pauropsylla buxtoni,* a disease-causing insect (Freeman and Morrison, 1949; Huang and Kokko, 1993; Kumar and Jha, 2002; Tesfagiorgis and Laing, 2010; Batta, 2020). However, to date, there is no information available concerning the antagonistic effects of *T. roseum* on the developments of the economically and agriculturally important powdery mildew pathogens, including Bgt.

Initially, we isolated a fungus with suspected mycoparasitic potential from mildew-infected wheat leaves. Due to the fact that the morphological and molecular characterization of this

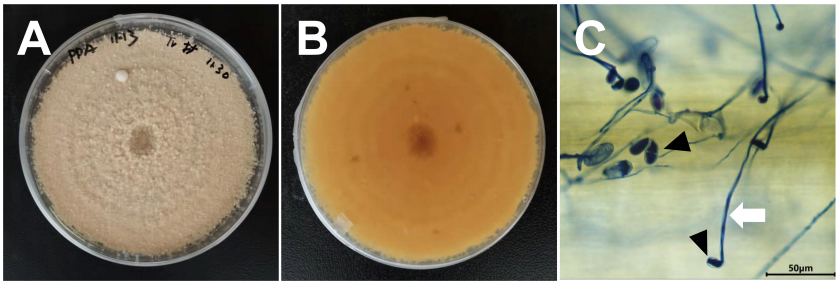

FIGURE 1
Morphological characteristics of *T. roseum*. **(A,B)** *T. roseum* colonies on PDA 12 dpi. **(C)** Mycelia and conidia of *T. roseum* on Bgt colonies. White arrow indicated mycelia of *T. roseum*. Black arrow indicated conidia of *T. roseum*.

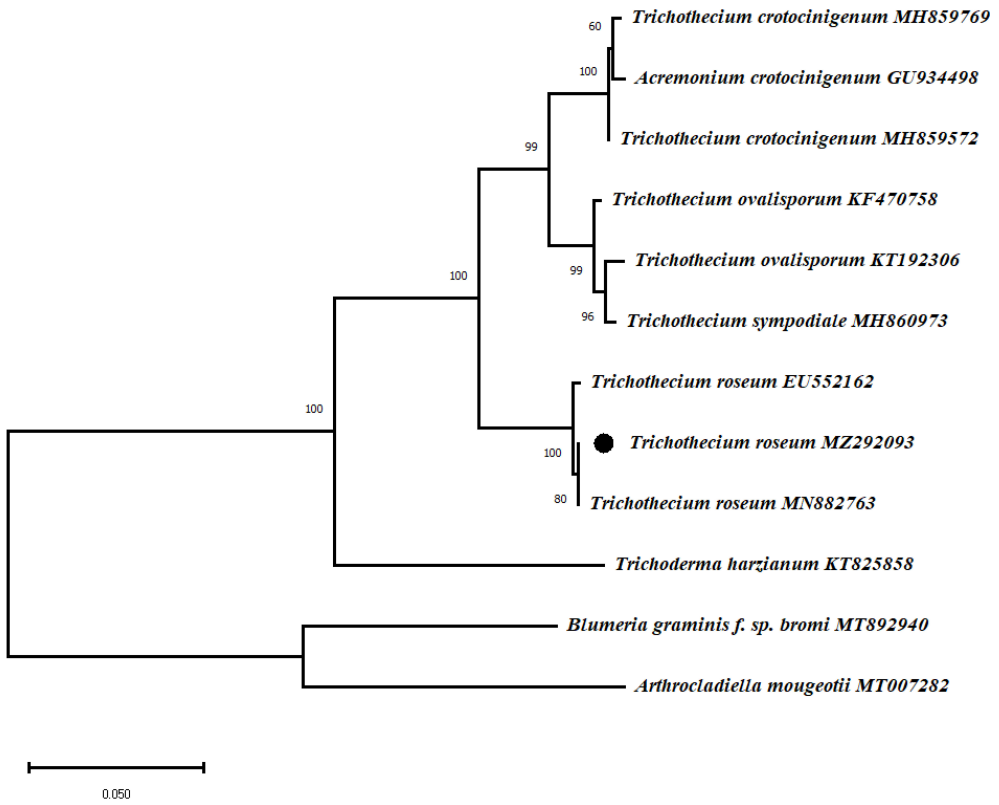

FIGURE 2
Phylogenetical analysis of *T. roseum* and *Trichothecium* sp. The maximum likelihood tree was constructed in MEGA software with 1000 bootstrap replicates and Tamura-Nei model. The bar indicates a distance of 0.050. Bold words highlighted *T. roseum*. *Arthrocladiella mougeotii* and *Blumeria graminis* f. sp. *bromi* were included as outgroups.

mycoparasite was consist with previously reported *T. roseum* (Marincowitz et al., 2008; Tang et al., 2015), we identified and confirmed that the mycoparasite was *T. roseum* (Figures 1, 2).

To test the mycoparasitic actions of *T. roseum* on Bgt, *T. roseum* spore suspension was then sprayed on the mildew-infested wheat leaves. Our results showed distinct interactions between *T. roseum* and Bgt (Figure 3), suggesting *T. roseum* was capable of parasitizing on powdery mildews. Therefore, we, for the first time, illustrated that *T. roseum* was a mycoparasite of Bgt and that, in agreements with previous studies, *T. roseum* was a natural biocontrol agent against phytopathogens under field and laboratory conditions.

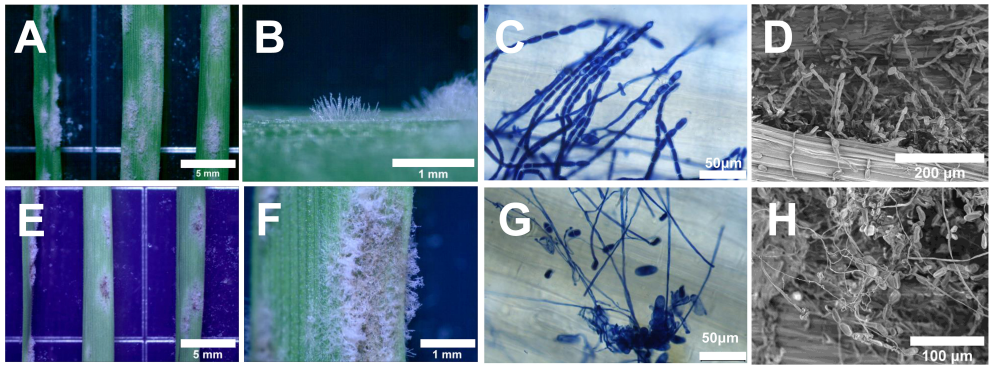

FIGURE 3
Morphological characteristics of Bgt and *T. roseum* colonies during interaction. **(A–D)** Water-treated Bgt colonies. **(E–H)** Bgt colonies treated with *T. roseum*. The colonies were monitored under stereo microscopy **(A,B,E,F)**, light microscopy **(C,G)** and scanning microscopy **(D,H)**. The scale bars were 5 mm in **(A,E)**, 1 mm in **(B,F)**, 50 μm in **(C,G)**, 200 μm in **(D)** and 100 μm in **(H)**.

T. roseum mediated suppression of Bgt conidial formation and distribution

Bumeria graminis is an obligate biotrophic ascomycete that causes powdery mildews on various plant species (Liu et al., 2021; Zhu et al., 2021a,b). To determine the inhibitory effect of *T. roseum* on Bgt, we observed the growth of Bgt at 2, 4, and 6 d after spraying *T. roseum* spore suspension, and found that Bgt spores collapsed significantly and the mycelium of *T. roseum* entangled on Bgt (Figure 4). In previous studies on Bgt biocontrol, *Sporothrix flocculosa* was shown to be able to grow rapidly and produce large numbers of spores on wheat powdery mildew spore clusters within 12 h, and to cause collapse of the powdery mildew conidial chain and inhibit conidial production within 24 h (Hajlaoui and Bélanger, 1993). Our experimental results also showed that the parasitization of *T. roseum* caused the collapse of the conidia chains of Bgt. *T. roseum* directly wrapped around mildew conidiophores at 2 dpi, erupted hyphae from mildew colonies at 4 dpi and disrupted conidia distribution of Bgt from 4 dpi (Figure 4). In addition, the biomasses of *T. roseum* notably increased during mycoparasitic processes (Figure 5D). However, the biomasses of Bgt significantly reduced by inoculation of *T. roseum* (Figure 5E). These results suggested that *T. roseum* was able to efficiently control Bgt. Previously, *T. roseum* was reported to inhibit uredospore germination of the soybean rust fungus *Phakopsora pachyrhizi* and to entomopathogenically infect *Pauropsylla buxtoni* on fig (*Ficus carica*) leaves (Kumar and Jha, 2002; Batta, 2020). Secondary metabolites of *T. roseum* were found to be active in suppressing the disease-causing fungus (Jayaprakashvel et al., 2010). However, whether the secondary metabolites of *T. roseum* have the same inhibitory effects on Bgt is unclear. Further studies on secondary metabolites of *T. roseum* will provide more insights into the suppression effects of this mycoparasite on phytopathogens.

Green fluorescent protein transformation of *T. roseum* by the *Agrobacterium tumefaciens*-mediated transformation

Previously, the transformation of *T. roseum* with fluorescent proteins was based on the PEG-mediated protoplast transformation method (Dai et al., 2019, 2020; Wang et al., 2022). *Agrobacterium tumefaciens*-mediated transformation (ATMT) methods were repeatedly utilized for transfer GFP into biocontrol agents to reveal the interactions between parasites and powdery mildews (Németh et al., 2019). However, so far, to the best of our knowledge, the method of transferring GFP fluorescent protein in *T. roseum* and making it stably expressed using the ATMT method has not been reported. In our study, to enhance the visualization of the interaction between *T. roseum* and Bgt, we, for the first time, used the agrobacterium to transfer GFP into *T. roseum* and obtained a strain with stably expressed GFP fluorescent signal (Figure 6). This indicates that *T. roseum* can be transformed by the ATMT method. Our previous study has demonstrated the chromosome-scale genome sequence of *T. roseum* (Zhu et al., 2022c). Genetic overexpression and/or knockout is potent for analyzing the roles of specific genes. Therefore, functions of genes and proteins involved in pathogenicity, mycoparasitism and metabolism can be deeply mined combining the transformation method and genome sequence.

Conclusions

Screening and identification of mycoparasites on crop pathogenic fungi is crucial for exploring new biological control agents of crop diseases and for understanding the biodiversity of hyperparasites. Our study provides new

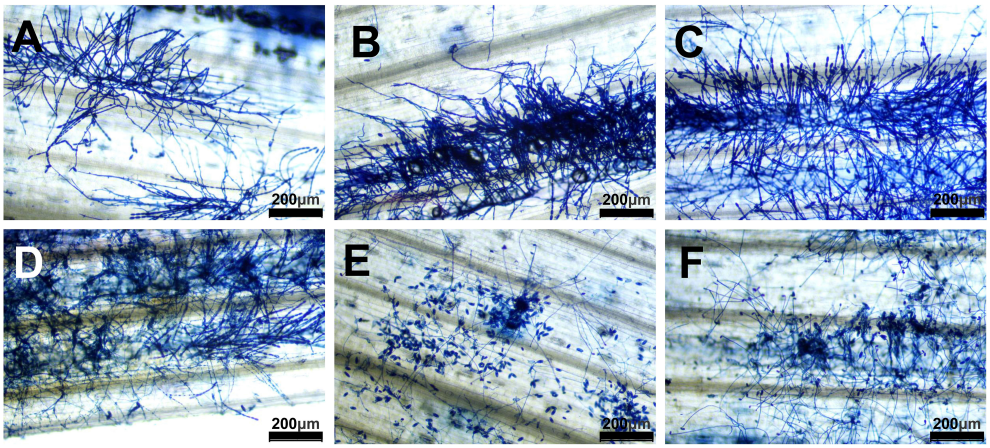

FIGURE 4
Colonies of Bgt with or without *T. roseum* interactions. **(A–C)** Bgt colonies treated with water at 2 dpi, 4 dpi and 6 dpi, respectively. **(D–F)** Bgt colonies treated with *T. roseum* suspension at 2, 4, and 6 dpi, respectively. Scale bars in **(A–F)** were 200 μm.

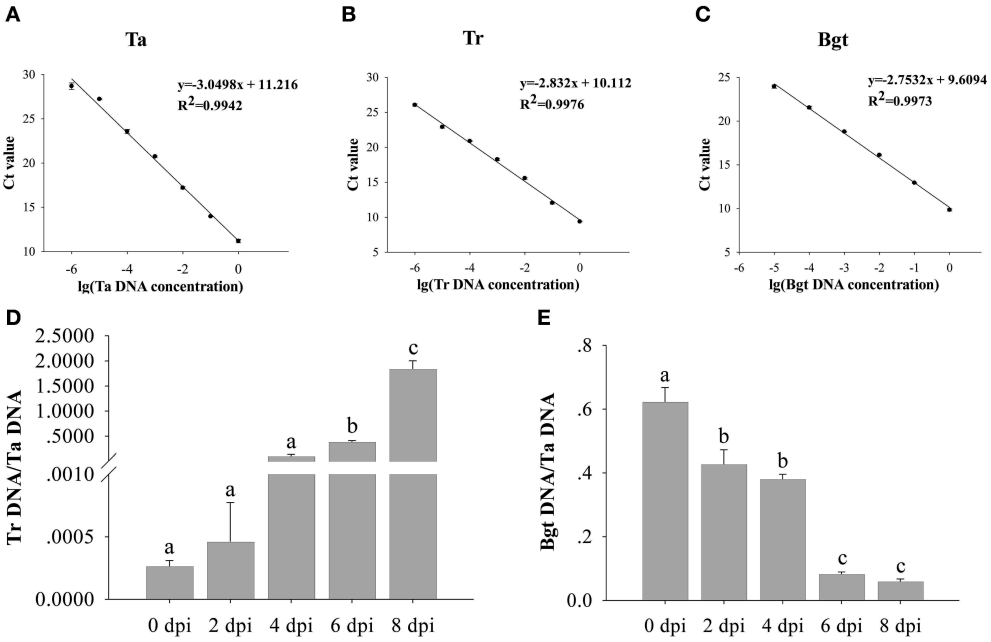

FIGURE 5
Biomass changes of *Blumeria graminis* f. sp. *tritici* (Bgt) and *T. roseum* during interactions. The DNA concentration standard curves of wheat (Ta) **(A)**, *T. roseum* (Tr) **(B)** and *B. graminis* f. sp. *tritici* (Bgt) **(C)** were used to calculate the biomasses of *T. roseum* **(D)** and Bgt **(E)**. The relative quantities of PCR product of TaEF1, TrEF1, and BgtEF1 in infected samples were calculated using the gene-specific standard curves to quantify wheat, *T. roseum* and Bgt gDNA. The standard curves derived from the serial dilutions were built utilizing the primers of elongation factors of Ta, Tr, and Bgt, respectively. Each value is given as mean ± SD of three independent biological experiments. Significant differences were determined in a One-way ANOVA test, with *post-hoc* Tukey test: different letters indicate significant differences ($P < 0.05$).

insights into the fungal host range and developmental processes of *T. roseum* and helps to fill knowledge gaps in the understanding of the interaction between *T. roseum* and Bgt. The major findings of this study are as follows: (1) *T. roseum* directly parasitizes on the wheat powdery mildew and suppresses conidiophore formation and conidial distribution of Bgt; (2) *T. roseum* decreases the biomasses of powdery mildew during mycoparasitic processes, suggesting its potential utilization in the control of wheat powdery mildew; (3) by using the ATMT method, the

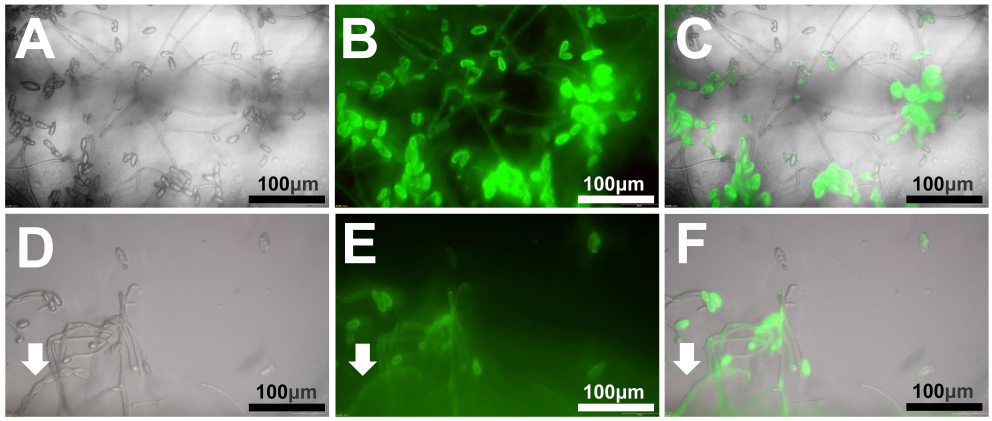

FIGURE 6
Visualization of interaction between GFP transformed *T. roseum* and Bgt. **(A,D)** Bright field. **(B,E)** Fluorescence field. **(C,F)** Merged images. The arrow heads indicate a conidiophore of Bgt. Scale bars = 50 μm.

visualization of *T. roseum* structures is improved with GFP transformation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/nuccore/MZ292093, MZ292093.

Author contributions

MZ designed and conceived of the study and wrote the initial manuscript. XD, PC, WZ, and YL participated in the experiments, performed the statistical analysis, and prepared the figures and tables. JC, ZL, and ZQ conceived of the study and critically edited the whole manuscript. All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2022.998830/full#supplementary-material

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