Effects of a Dark Septate Fungal Endophyte on the Growth and Physiological Response of Seedlings to Drought in an Epiphytic Orchid

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Dark septate endophytes (DSE) are a group of facultative biotrophic root-colonizing fungi that live within a plant for a part of their life cycle without causing any apparent, overt negative effects. These fungi have been found in > 600 different plant species, including orchids. Although the precise ecological functions of dark septate fungal endophytes are not yet well understood, there is increasing evidence that they enhance host growth and nutrient acquisition, and improve the plant's ability to tolerate biotic and abiotic stresses. In this research, we tested the effects of a DSE isolated from the roots of the epiphytic orchid Coelogyne viscosa on the growth and drought tolerance of orchid seedlings. Our results showed that addition of DSE inoculum significantly enhanced biomass of seedlings and increased the activities of drought resistance related enzymes and the accumulation of osmoregulatory substances. These results suggest that DSE can fulfill important ecological functions in stressful environments and potentially play an important role in the life cycle of epiphytic orchids.

Keywords: Coelogyne viscosa, orchids, root endophytes, dark septate endophytes (DSE), drought

INTRODUCTION

Endophytic fungi are fungi that reside mostly within plant tissues and grow within roots, stems, or leaves, without causing symptoms of disease (Rodriguez et al., 2009; Hardoim et al., 2015). Although endophytic fungi are known as important components of plant micro-ecosystems (Cordovez et al., 2019), their ecological function is not fully clear. There is increasing evidence that endophytes and plants often engage in a mutualistic relationship, with the endophyte enhancing host growth and nutrient acquisition, improving the plant's ability to tolerate abiotic stresses, such as drought or salinity, and decreasing biotic stresses by enhancing plant resistance to insects, pathogens, and herbivores (Hardoim et al., 2015; Bamisile et al., 2018; Moraga, 2020). In exchange plants provide habitats and nutrients for the endophytic fungi (Latz et al., 2018).

One important group of fungal endophytes are dark septate endophytes (DSE), a group of anamorphic and root-inhabiting fungi, with dark septate or hyaline hyphae colonizing plant roots (Jumpponen and Trappe, 1998; Addy et al., 2005). DSE are widely distributed and are often the
dominant fungal groups in plants growing in harsh habitats, such as Arctic, Antarctic, and alpine habitats, heavily polluted environments, arid ecosystems, and saline fields (Barrow, 2003; Mandyam and Jumpponen, 2005). In these harsh habitats, it has been demonstrated that DSE play a non-negligible role in promoting plant growth and development and mitigating biotic and abiotic stresses (Knapp et al., 2015; Qin et al., 2017; Chen et al., 2018). For example, the DSE *Phialophora mustea* has been shown to enhance metal tolerance and promote plant growth in tomato (Zhu et al., 2018). *Phialophora* sp. and *Leptosphaeria* sp. were shown to enhance antioxidase activity and drought tolerance in *Hedysarum scoparium* (Li et al., 2019).

In recent years, DSE have also been occasionally detected in the roots of orchids (Yuan et al., 2009; Ma et al., 2015; Novotná et al., 2017; Wang et al., 2021; Selosse et al., 2022). Orchids are known for their characteristic mycorrhizal symbiosis. Because an individual orchid produces thousands of tiny dust-like seeds, they have become fully reliant on typical orchid mycorrhizal fungi (OMF) to induce germination and subsequent establishment of seedlings. Besides, most adult orchids retain mycorrhizal associations as well (Rasmussen and Rasmussen, 2010) and these fungi have been recognized as an important factor influencing the distribution and abundance of orchid populations (McCormick and Jacquemyn, 2014; Pecoraro et al., 2018) and ecological adaptation of orchids (Herrera et al., 2018; Li et al., 2021). However, there is mounting evidence that fungi other than typical OMF reside in the roots of orchids, including DSE (Selosse et al., 2022), but to what extent DSE contribute to the ecology of orchids remains largely unclear.

Previous research has shown that several endophytic fungi can fulfill similar functions as typical OMF and contribute to seed germination and nutrient adsorption (Zhao et al., 2014), promote plant growth (Zhang et al., 2013), and induce pathogen resistance (Ma et al., 2015). In addition, it has been reported that endophytic fungi are able to enhance drought resistance (Kulda and Bacon, 2008). As such, these fungi may fulfill an important role in the life cycle of epiphytic orchids, which by nature grow on the surface of tree barks and have limited access to water, especially during prolonged periods of drought in the dry season. However, whether DSE colonizing orchid roots are able to induce physiological changes that enhance drought resistance in orchids is still unclear.

In this study, we have isolated a DSE fungal strain from the roots of the epiphytic orchid *Coelogyne viscosa* growing on tree barks in tropic forest in Xishuangbanna, Southwest China. Previous research has shown that this orchid commonly associates with OMF of the Tulasnellaceae, Ceratobasidiaceae, and Sebacinaeae, and few other Basidiomycota members, such as Marasmiaceae, Thelephoraceae, Ganodermataceae, and unknown Basidiomycota (Xing et al., 2015), but presence of DSE in this epiphytic orchid was hitherto unknown. In order to understand the potential ecological functions of the isolated DSE fungus, we aimed at answering the following questions: (1) Does inoculation of orchid seedlings with this DSE affect growth of seedlings? (2) Does inoculation of the DSE induce physiological changes that enhance drought resistance of this epiphytic orchid? To answer these questions, sterile seedlings of *C. viscosa* were inoculated with DSE inoculum and subsequent seedling growth, enzyme activities and concentrations of osmoregulatory substances were compared between inoculated and non-inoculated control plants.

**MATERIALS AND METHODS**

**Orchid Species and Sampling**

*Coelogyne viscosa* is an epiphytic orchid that grows in clusters on tree barks or rock surfaces. The species is widely distributed in northeastern India, Burma, southern China, Laos, Vietnam, Thailand, and peninsular Malaysia, where it grows in the evergreen lowland and montane forests at elevations between 700 and 1000 m a.s.l. The flowering period of *C. viscosa* ranges from September to November, and individual plants produce 2–4 flowers (Chen and Dudley, 2009).

This study was conducted in the Menglun sub-reserve (21°41’ N–101°25’ E), one of the five sub-reserves of the national nature reserve established in Xishuangbanna, Yunnan province, China. Ten individual plants were randomly selected and four root fragments (3–5 cm) were collected from each individual plant. Slight yellowish or opaque roots of *C. viscosa* were selected with surface being cleaned three times with sterile water to minimize the detection of substrate fungi. Roots were stored at 4°C for isolation of mycorrhizal associates. In addition, three mature capsules of *C. viscosa* were collected, and the capsules were surface sterilized and kept in plastic tubes at 4°C for sowing.

**Isolation and Identification of Fungal Associates**

Root treatment and fungal isolation were performed according to Zhu et al. (2008). The collected roots were cut into 3-cm-long fragments, surface sterilized for 30 s in 75% ethanol, 3 min in 1% sodium hypochlorite solution, and 30 s in 75% ethanol, and washed six times in sterile water. Root hairs, epidermis, velamen, and other attachments were peeled off with a needle and forceps. Segments were scraped and teased apart. This released individual pelotons from the cortex cells. Pelotons were collected in a 60 mm sterile Petri dish containing 10 mL sterile distilled water. Then Petri dishes with pelotons were placed under a microscope at low power to observe the pelotons suspended in water. A 2.5 μL solution containing individual pelotons was absorbed onto 1 cm² potato dextrose agar (PDA) with Streptomyces sulfite (100 μg/mL) and potassium Penicillin G (100 μg/mL) and cultured in a 25°C dark growth chamber. Visible hyphae emerged on the media within 1–2 weeks. The tips of the hyphae growing from the pelotons were cut and transferred to PDA several times for purification.

Genomic DNA was extracted from fungal isolates using the CTAB Plant Genome DNA Rapid Extraction kit provided by Beijing Aidlab Biotech Co., Ltd., Beijing, China according to the manufacturer’s instructions. For fungal identification, sequence amplification was performed using the universal primers ITS-1 and ITS-4 (White et al., 1990). All of the PCR reactions were carried out in a thermal cycler (BIO-RAD, America). The 25 μL PCR reaction system contained 12.5 μL Taq PCR Master (2×),...
µL of each primer, 2 µL DNA template, and the
remaining system was replenished to 25 µL with ddH2O. The
PCR program was as follows: 95°C for 5 min, 35 cycles at 95°C
for 45 s, 50°C for 30 s, and 72°C for 1 min with a final extension
of 72°C for 10 min. The quality of extracted DNA was checked by
1% agarose gel electrophoresis and spectrophotometry (optical
density at 260/280 nm ratio). Then the PCR solutions with
single and bright bands were sent to the Taihe Biotechnology
Co. Ltd. Beijing, China. for bi-directional DNA sequencing.
Taxonomic identification, sequencing results were compared
with known sequences from GenBank available on the National
Center for Biotechnology Information (NCBI) website for a
BLAST alignment.

Germination and Growth of Seedlings

Three mature capsules of *C. viscosa* were sterilized following
methods outlined in Dutra et al. (2009). Uncracked capsules were
transferred, and a total of 60 bottles was used. Meanwhile, five
cultivation bottles. For each bottle, a cluster of five seedlings was
and subsequently seedlings were transferred to the substrate in

Substrates used for seedling cultivation were prepared as follows:
pine bark was crushed into small pieces (0.5–1 cm in diameter),
soaked in water for 2 days for full water absorption and mixed
with crumpled dry sawdust and chestnut leaves at a volume ratio
of 1:1:1. Water was added to the substrate to obtain a relative
water content of 80%. Then the mixed substrate was put into

Preparation of Dark Septate Endophytes-Inoculated Seedlings

Substrates used for seedling cultivation were prepared as follows:
the fungi were visible in the substrate a week later. All seedlings were cultured for 100 days to establish symbiosis.
To investigate how inoculation with DSE affected growth of
seedlings, fresh weight of seedlings was determined for inoculated
and non-inoculated groups (n = 10 clusters of seedlings per
treatment). The seedlings were carefully removed from the
culture substrate and subsequently washed with running water,
drained with filter paper and finally weighed to the nearest mg.

Physiological Response to Artificial Drought

To assess how inoculation with DSE affected the physiological
response of seedlings that were exposed to drought, the gas-
permeable sealing film was removed from the cultivation bottles
to mimic a natural drought. Twenty bottles with inoculated
seedlings and 20 bottles with non-inoculated seedlings (control)
were exposed to air at 25°C, 60% relative air humidity and
3000 lux light intensity (light/dark = 12/12 h). The experiment
lasted for 12 days. Leaves were sampled at day 0, 3, 6, 9, and 12
for the measurement of physiological indexes. At each sampling
timepoint, four bottles of seedlings were randomly selected from
inoculated and non-inoculated groups, and leaves collected from
each group were pooled. The sampled leaves were immediately
wrapped in tinfoil and placed in liquid nitrogen, and then
transferred to the –80°C refrigerator for storage. The culture
bottles were weighed while sampling to record changes in relative
water content of the substrate.

Physiological Indexes Measurement

Crude enzyme extract was obtained by adding 0.2M PBS
buffer (pH = 7.0) pre-cooled at 4°C at a ratio of 1 mL
buffer per 0.1 g leaves according to the net weight of leaves.
Subsequently, the mixture was grinded at a low temperature. After
centrifugation at 4°C and 4000 rpm for 10 min, the
supernatant was collected and stored at 4°C for 1–2 days for the
measurement of peroxidase (POD), superoxide dismutase (SOD),
and catalase (CAT) activities, and the concentrations of soluble
proteins, sugars, and proline (PRO). For each determination,
three technical replicates were used.

The POD and CAT activities were determined by a POD Assay
Kit (colorimetric method) and a CAT Assay Kit provided by
Nanjing Jiancheng Bioengineering Institute. The SOD activity
and soluble proteins were measured by a Total SOD Assay
Kit with NBT and a Bradford Protein Assay Kit provided by
Shanghai Beyotime Biotechnology Co., Ltd. Shanghai, China.
The assays were performed in general accordance to the
instructions provided by the manufacturer. Before the SOD
determination, the crude enzyme extract needed to be diluted
eightfold to fortyfold.

The soluble sugars and PRO in the control and fungus-treated
groups were determined by the Plant Soluble Sugar Content
Assay Kit and PRO Content Assay Kit provided by Beijing Box
Biotechnology Technology Co., Ltd. Beijing, China, respectively.
For the determination of soluble sugars, the tested sample solution was configured according to a ratio of 0.05 g tissue to 0.5 mL distilled water. For the determination of PRO, the tested sample solution was configured according to a ratio of 0.05 g tissue to 0.5 mL extract from the kit. The rest of the steps were performed with reference to the manufacturer’s instructions.

**Data Analysis**

To assess the taxonomic position of the isolated DSE, a Maximum likelihood (ML) tree was constructed. The ITS sequence obtained in this study and reference sequences (closely similar sequences and published DSE sequences retrieved from GenBank) were aligned in Clustal X version 2.0 (Larkin et al., 2007). The best-fit model for the dataset was identified using the Akaike Information Criterion implemented in jModel Test2 (Darriba et al., 2012). Evolutionary model K2 + G was identified as the best model. The ML phylogeny was constructed with MEGA 7.0 and clade support was estimated using a non-parametric bootstrap analysis with 1000 pseudo-replicated datasets.

To investigate whether physiological indexes differed between seedlings inoculated with HB3-1 and controls, a two-way ANOVA was used with inoculation treatment, culture time and their interaction included as fixed factors and physiological indexes as dependent variables. In addition, to assess how fungal inoculation affected the fresh weight of seedlings and the physiological indicators measured at the given days, paired and unpaired t-tests were used. Fresh weight, CAT, SOD, and POD activities, the concentration of soluble proteins, sugars, and PRO were used as dependent variables, whereas fungal treatment (inoculated versus non-inoculated plants) as independent variable. All analyses were performed using the statistical software GraphPad Prism version 7.0 for Windows (GraphPad Software, San Diego, CA, United States).

**RESULTS**

**Isolation and Identification of Dark Septate Endophytes**

In total, 35 fungal isolates were retrieved by picking out pelotons directly from the cortex cells of root segments of *C. viscosa*. Combining morphological characteristics with ITS sequences, the 35 fungal isolates were identified as 7 fungal strains, four of them belonging to Ascomycota and three to Basidiomycota, respectively (Table 1). One fungal strain (HB3-1) had dark septate hyphae (Figure 1) and showed a 95% sequence similarity with *Exophiala* species [E. equina (MT4573276), Uncultured *Exophiala* (LC440267), etc.]. The sequence of HB3-1 was submitted to GenBank under the accession number OM327422. Based on the ITS sequences of HB3-1 and reference sequences of known DSE isolated from other plant species, we constructed a ML tree, and the result showed that HB3-1 clustered well with sequences of other *Exophiala* fungi from the same clade (Figure 2).

Colonies of strain HB3-1 grown on PDA were flat and fluffy, had an even edge and varied in color from dark to black-brown (Figure 1). There were few aerial hyphae around the inoculum. Colony thickness decreased gradually from the center to the edge, and colonies produced dark brown pigmentation on PDA medium. Colonies grew slowly, only reaching 6 cm in diameter after 4 months of culturing under 22 ± 2°C in darkness. The mycelium had branched, septate, light brown hyphae (2–6 μm in diameter). No sexual or asexual spores were observed.

**Inoculation and Verification of Dark Septate Endophytes Colonization**

Microscopic observation of the roots of seedlings 100 days after they were inoculated with strain HB3-1 showed substantial fungal colonization. The colonized hyphae had a dark-brown moniliform structure. HB3-1 formed atypical pelotons in the velamen and exodermis cells (Figures 3A,B), and further extended inward and penetrated the cortical cells (Figure 3C). Furthermore, microsclerotia, a typical structure of DSE, were observed in the cortical intercellular space (Figure 3D). From these morphological observations, it was concluded that the observed strain showed all features characteristic of typical DSE and therefore had successfully colonized seedlings.

**Effect of Dark Septate Endophytes Inoculation on Fresh Weight of Seedlings**

HB3-1 had a significant effect on growth of *C. viscosa* seedlings. When seedlings of *C. viscosa* were transplanted from the

| Fungal isolate | Closest matches in GenBank (accession numbers) | BLAST match sequence Max ident (%) | Proposed identification | Number of pelotons* |
|----------------|-----------------------------------------------|-----------------------------------|-------------------------|---------------------|
| hy-1           | Uncultured Tulasnellaeeae (JF691394)           | 97.10                             | Tulasnellaeeae sp.       | 4                   |
| hy-111         | Tulasnella sp. (MK651838)                      | 98.58                             | Tulasnella sp.           | 5                   |
| HBS1           | Flammulina velutipes (MH469688)               | 96.97                             | Flammulina sp.           | 8                   |
| HB3-1          | Exophiala equina (MT4573276)                  | 95.20                             | Exophiala sp.            | 4                   |
| C-11           | Diaporthaceae phaseolorum (KT964565)          | 97.35                             | Diaporthaceae sp.        | 7                   |
| C-22           | Acremonium charticolae (MK312365)             | 99.79                             | Acremonium sp.           | 4                   |
| cvl-1          | Gliomastix polychrome (AB540566)              | 97.45                             | Gliomastix sp.           | 3                   |

*Number of pelotons from which each fungal strain was recovered.
FIGURE 1 | Morphology of fungal strain HB3-1. (A) Colony of HB3-1 on potato dextrose agar (PDA). (B) Mycelial morphology of HB3-1. The septate hyphae are visible. Scale bar, Panel (A) = 1 cm, Panel (B) = 20 µm.

FIGURE 2 | Maximum-likelihood tree constructed using rDNA-ITS sequences of HB3-1 generated in this study and reference sequences downloaded from GenBank. Known dark septate endophytes (DSE) are marked in bold. DSE detected in orchids (Giryldia et al., 2002; Hou and Guo, 2009; Valdés et al., 2011; Sebastián et al., 2014; Zhao et al., 2014) were marked by the orchid names in brackets. The values of the bootstrap frequencies (BP > 70%) are shown beside the nodes.
nutrient-rich 1/2MS medium to the relatively infertile bark substrate, the growth rate of seedlings slowed down in both the treatment and control, due to their need to re-adapt to the new environment. One hundred days after inoculation, fresh weight of non-inoculated seedlings had reduced by 0.45 ± 0.07 g and was significantly lower \( t = 6.27, P < 0.01 \) than before transplantation. Interestingly, the fresh weight of seedlings inoculated with HB3-1 had increased by 0.39 ± 0.08 g and was significantly higher than before transplantation \( t = 5.04, P < 0.01 \) (Figure 4).

**Effect of Dark Septate Endophytes Inoculation on Physiological Indexes**

Inoculation with HB3-1 significantly affected the physiological variables measured in seedlings of *C. viscosa*. Two-way ANOVA revealed that the activities of all investigated physiological indexes were significantly influenced by inoculation, culture time and their interactions, except for POD and soluble protein, which were not influenced by inoculation, and CAT, which was not influenced by culture time (Table 2).

Superoxide dismutase activity in the seedlings inoculated with HB3-1 was significantly \( P < 0.01 \) higher than that in the control group during most of the experiment, and the difference in expression level increased as drought progressed, reaching a maximum value at day 12 (Figure 5A). Similarly, a significantly higher CAT activity \( P < 0.05 \) was detected in seedlings inoculated with HB3-1 than in control seedlings (day 0, 6, and 12) (Figure 5B). POD activity was slightly higher in control seedlings than in seedlings inoculated with HB3-1 in the beginning of the experiment (day 0 and 3) \( P < 0.05 \), but became significantly lower \( P < 0.01 \) at the end of the experiment (day 6 and 12) (Figure 5C).

The soluble protein content in control seedlings reached its peak at day 9 \( 0.751 ± 0.008 \text{ mg/mL} \), while in the HB3-1 treatment group this peak was already detected at day 6 \( 1.015 ± 0.036 \text{ mg/mL} \) and it was significantly higher \( P < 0.01 \) than in the control (Figure 5D). The soluble sugar content in both HB3-1-treated and control groups decreased during the experiment, but it was always significantly higher in the HB3-1 group than in the group control \( P < 0.01 \) (Figure 5E). PRO content did not differ significantly between the
**TABLE 2** | Effect of inoculation with HB3-1 (inoculation vs. control) and culture time on physiological indexes of Coelogyne viscosa seedlings.

| Extracellular enzyme | Inoculation | Culture time | Inoculation × Culture time |
|---------------------|-------------|--------------|----------------------------|
|                     | F           | p            | F                          | p        | F              | p            |
| SOD                 | 216.126     | <0.001       | 234.764                    | <0.001   | 148.273        | <0.001       |
| CAT                 | 53.430      | <0.001       | 1.845                      | 0.160    | 9.903          | <0.001       |
| POD                 | 0.789       | 0.835        | 11.936                     | <0.001   | 30.602         | <0.001       |
| Soluble protein     | 3.074       | 0.095        | 57.850                     | <0.001   | 51.708         | <0.001       |
| Soluble sugar       | 88.467      | <0.001       | 340.993                    | <0.001   | 8.571          | <0.001       |
| Proline             | 54.638      | <0.001       | 64.312                     | <0.001   | 43.873         | <0.001       |

Bold values are significant at $P < 0.05$.

**FIGURE 5** | Differences in physiological variables between control (CK) seedlings and seedlings inoculated with dark septate fungal endophyte strain HB3-1. (A) Superoxide dismutase (SOD) activity. (B) Catalase (CAT) activity. (C) Peroxidase (POD) activity. (D) Soluble protein content. (E) Soluble sugar content. (F) Proline (PRO) content. * $P < 0.05$, ** $0.001 \leq P < 0.01$, *** $0.0001 \leq P < 0.001$, **** $P < 0.0001$. 

HB3-1-treated seedlings and control seedlings in the beginning of the experiment, but it was significantly higher in the control group than in HB3-1-treated group at the end of the experiment (day 9 and 12, $P < 0.001$) (**Figure 5F**).

**DISCUSSION**

In this study, a DSE fungus was isolated from the roots of the epiphytic orchid *C. viscosa* and identified as *Exophiala*. Fungi from the genus *Exophiala* have been previously reported to live in symbiosis with many plants as DSE, promoting plant growth and development and improving plant resistance against biotic and abiotic stresses (Khan et al., 2011; Zhan et al., 2015; Zhang et al., 2017). Here, we investigated the potential ecological function of HB3-1 by re-inoculating the fungus to the roots of aseptically cultured seedlings of *C. viscosa*. The results showed that fresh weight of seedlings inoculated with HB3-1 significantly increased after they had been transplanted to a medium resembling natural conditions, indicating that colonization of the roots of *C. viscosa* may promote the growth and development of seedlings in natural environments. The increase in plant weight suggests that there is more organic matter to support the plant and to survive longer periods of high temperatures and drought that characterize the environments where this epiphytic orchid naturally occurs.

Our results further showed that seedlings in the HB3-1 treatment group had higher antioxidant enzyme levels than seedlings in the control group, suggesting a greater ability to eliminate reactive oxygen species (ROS) (Amiri et al., 2015). ROS are known to cause deterioration of lipids, proteins and nucleic acids in plants when extreme environmental changes occur, such as drought, salinity, or floods, etc., and act as the critical signaling molecules throughout the cell death pathway (Kapoor et al., 2019). At the same time, the higher concentrations of soluble proteins and glucose indicate an enhanced osmotic ability of seedlings inoculated with HB3-1, and suggest that seedlings are more conducive to maintain turgor pressure under drought (Liu et al., 2021). Since PRO is involved in several
physiological processes such as regulating osmotic potential and redox reactions, affecting the transmission of energy and preventing the production of ROS (Sharma and Verslues, 2010; Azmat and Moin, 2019), accumulation of PRO is a common physiological response to various stresses. Our experiments showed that PRO levels were lower in the HB3-1-treated group than in the control group, suggesting that inoculated seedlings were less susceptible to drought stress than control seedlings.

Previous studies have already demonstrated the growth and anti-stress effect of DSE. It has been shown that DSE promote plant growth (Jumpponen et al., 1998; Jumpponen, 2001), but the mechanisms are not always clear. Narisawa et al. (2004) showed that DSE can provide nitrogen to the host plants. Similar results were obtained by Upson et al. (2009), who showed that DSE isolates increased growth of Deschampsia antarctica when the plants were supplied with an organic N source. However, no such effects were observed when plants were supplied with ammonium. Wu et al. (2021) used transcriptome sequencing of DSE-inoculated cherry and showed that DSE improved nitrate assimilation of sweet cherry through NO$_3^-$ transporters. There is also mounting evidence that plants inoculated with DSE display higher resistance to various forms of stress. Li et al. (2018), for example, reported that DSE promoted growth of Anmmopiptanthus mongolicus under drought conditions, and Exophiala pisciphila improved the Cd tolerance of maize (He et al., 2017). The mechanism by which DSE enhance drought resistance is related to the characteristics of the fungus itself. DSEs are mostly melanin-rich fungi. Melanin and chitin can protect the host in adverse environments by increasing the rigidity of cell walls and reducing permeability (Addy et al., 2005). Barrow (2003) suggested that DSE acquire and transport carbon from the host to the root surface and form polysaccharide mucus on the root surface, thus helping plants to maintain the transport of water and nutrients in arid environments.

As an epiphytic orchid, C. viscosa grows in tree canopies to obtain light (Gravendeel et al., 2004), but at the same time they have less access to water (Chase, 1987). Seasonal water shortage has led to the evolution of a pseudobulb, a storage organ that preserves water for leaves during drought. Mycorrhizal fungi have been widely reported in the past years, but it is still unclear whether these symbiotic fungi play a role in enhancing stress resistance in the host plant. However, based on the results presented in this study, it is reasonable to believe that DSE play an active role in drought resistance in the roots of C. viscosa. Additionally, the results of this research further indicate that more attention should be paid to fungal endophytes such as DSE to elucidate their role in mediating ecological adaptation of epiphytic orchids to stressful environments.

**CONCLUSION**

In this study, a DSE was isolated from the roots of C. viscosa collected in natural conditions by picking out pelotons residing in cortical cells, and reinoculation verified that this DSE could promote the growth of C. viscosa seedlings. Furthermore, our experiments showed that seedlings inoculated with DSE had higher levels of antioxidant enzyme activities, and more accumulation of osmotic regulating substances than seedlings from the control group. We therefore conclude that DSE have the potential to enhance drought resistance in epiphytic orchids and therefore play a more important role in the ecology of epiphytic orchids than previously assumed. However, the extent to which they are observed in nature and the molecular mechanisms taking place in the orchid-DSE interaction process remain to be elucidated.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

**AUTHOR CONTRIBUTIONS**

XX and HJ designed the research and revised the manuscript. NL, QL, S-CS, and GD performed the research. NL analyzed the data. XX, and HJ wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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