Plant Soil (2019) 439:259–272
https://doi.org/10.1007/s11104-019-04057-2

REGULAR ARTICLE

# Dark septate endophytes improve the growth of host and non-host plants under drought stress through altered root development

**Xia Li · Chao He · Xueli He · Fang Su · Lifeng Hou · Ying Ren · Yiting Hou**

Received: 3 August 2018 / Accepted: 25 March 2019 / Published online: 10 April 2019


**Abstract**
*Aims* This study aimed to investigate how dark septate endophytes (DSE) from arid habitats affect host growth and their application to crops and medicinal plants in drought-prone soils.
*Methods* First, the osmotic-stress tolerance of *Paraphoma* sp., *Embellisia chlamydospora*, and *Cladosporium oxysporum*, isolated from *Hedysarum scoparium*, was tested using osmotically adjusted pure culture. Second, we examined the performance of host (*H. scoparium*) and non-host (*Glycyrrhiza uralensis* and *Zea mays*) plants inoculated with these fungi under mild (MD) and extreme drought (ED) conditions in a growth chamber.
*Results* All the DSE showed high tolerance to osmotic stress in vitro and could colonise the roots of all the plants. For *H. scoparium*, DSE improved the root biomass and length depending on DSE species, with *Paraphoma* sp. and *C. oxysporum* exhibiting positive effects under all the drought treatments. For *G. uralensis* and *Z. mays*, DSE inoculation enhanced the root development of plants under MD condition and was dependent on the plant–fungus species. However, this positive effect was weakened under extreme drought stress.
*Conclusions* DSE isolated from *H. scoparium* enhanced the root growth of the host plant under drought conditions and may also be used to promote the cultivation of agricultural and medicinal plants.

**Keywords** *Hedysarum scoparium* · Dark septate endophytes (DSE) · Root development · Drought · Symbiosis

Responsible Editor: Felipe E. Albornoz.

**Electronic supplementary material** The online version of this article (https://doi.org/10.1007/s11104-019-04057-2) contains supplementary material, which is available to authorized users.

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## Introduction

Water deficiency is becoming one of the most important factors affecting human societies and environment (Sheffield et al. 2009). Plants respond to the environmental change directly as well as indirectly; indirect response through altered interactions among species has recently received increased attention. The introduction of symbiotic fungi may influence the response of plants to drought stress and is generally considered to enhance the ability of plants to cope with environmental stresses (Azcón-Aguilar et al. 2003; Kannadan and Rudgers 2008; Kivlin et al. 2013; Shi et al. 2015). It is, therefore, of prime importance to choose fungal strains that are best for individual plant species and are well adapted to drought stressful conditions.

Dark septate endophytes (DSE) generally colonise living plant roots without causing apparent negative effects. They are characterised by dark septate hyphae and melanised microsclerotia (Jumpponen and Trappe 1998; Mandyam and Jumpponen 2005). They are found in diverse ecosystems, especially under stressful environments, such as alpine, dry, saline, and polluted habitats. DSE occur in several orders of Ascomycotina, including Helotiales, Xylariales, and Pleosporales (Ashrafi et al. 2018; Knapp et al. 2018). In some cases, several DSE were reported to display stress tolerance in vitro (Ban et al. 2012; Berthelot et al. 2016; Santos et al. 2017). Melanin, a group of complex polymeric compounds composed of indolic and phenolic monomers, has been considered to provide structural rigidity to cell walls and can increase resistance against environmental stresses, such as those caused by oxidising conditions, drought, and heavy metal (Berthelot et al. 2017a; Bloomfield and Alexander 1967; Butler and Day 1998; Zhan et al. 2011). In addition, studies on the response of DSE to heavy metal stress showed that fungal oxidation plays important roles in decreasing the oxidative damage through the biosynthesis of various antioxidant enzymes, such as superoxide dismutase and catalase activity (Ban et al. 2012; Zhang et al. 2008). The pathogenic or beneficial interactions between DSE and host plants have been discussed in several reports (Mandyam and Jumpponen 2005; Mayerhofer et al. 2013; Newsham 2011). The effects of DSE on host plants are variable and dependent on the host-symbiont combination of the plant and fungal species. Indeed, several DSE act as plant growth promoters by facilitating C, N, and P uptake (Della Monica et al. 2015; Newsham 2011; Surono 2017), and by protecting plants against biotic (pathogen) and abiotic stress (heavy metal, elevated $CO_2$, drought) (Alberton et al. 2010; Andrade-Linares et al. 2011; Likar and Regvar 2013; Santos et al. 2017; Su et al. 2013).

Previous studies on the effects of DSE inoculation on host stress resistance mostly considered heavy metal pollution (Berthelot et al. 2016, 2017b; Diene et al. 2014; Jin et al. 2018; Li et al. 2011; Likar and Regvar 2013; Wang et al. 2016) and pathogen stress (Andrade–Linares et al. 2011; Khastini et al. 2012; Su et al. 2013); the ecological roles of DSE in water deficient habitats are not well known. For example, five DSE isolates obtained from *Agropyron cristatum*, *Psathyrostachys juncea*, and *Bouteloua gracilis* were inoculated in these grasses under low water conditions resulting in positive effects on the shoot dry mass of *A. cristatum* and *P. juncea*, but had negative effect in *B. gracilis* (Perez-Naranjo 2009). In another inoculation study using rice grown in phytotron, Santos et al. (2017) evaluated the ability of DSE to reduce the effects of polyethylene glycol (PEG 6000)-induced water stress. They found that DSE isolates could promote the growth of roots and shoots of plants and the effects varied with the conditions of water deficit. In some cases, DSE can also affect the growth of non-host plants under stress treatments. For example, DSE may promote the growth of plants (Surono 2017; Vergara et al. 2017) and alleviate heavy metal and pathogen stress (Berthelot et al. 2016, 2017b; Diene et al. 2014; Khastini et al. 2012; Li et al. 2011; Wang et al. 2016). Although *Exophiala pisciphila* isolated from maize was reported to enhance the dry weight of shoots and roots in water-stressed *Sorghum bicolor* (Zhang et al. 2017), studies evaluating the efficacy of DSE on non-host plants under drought stress are rare.

*Hedysarum scoparium* is a xerophytic desert shrub found in the arid areas of northwest China, characterised by typical (semi) arid continental climate (Gong et al. 2015). It has been widely used for the recovery of vegetation in northwest China because of the vital roles it plays in the reduction of desertification (Deng et al. 2015). In a previous study, we found that in the natural habitats, the roots of *H. scoparium* were colonised by typical DSE structures and isolated several DSE strains (Xie et al. 2017). Based on the results of previous studies and considering the harsh growing environment in which *H. scoparium* occurs, the prospect that DSE could improve the growth of this plant and play

important roles in ecosystems with low water availability needs to be explored. In addition, considering the fact that drought stress is increasingly affecting the growth of food crops and medicinal plants (Chaves et al. 2003), it is intriguing to know whether DSE isolated from *H. scoparium* could colonise these plants and promote their growth in drought-prone soils.

The distribution and abundance of DSE in arid/semiarid ecosystems have been widely investigated, but knowledge about their function in relation to plants is still limited (Knapp et al. 2012, 2015, 2018; Li et al. 2015; Lugo et al. 2009, 2018; Xie et al. 2017). This study aimed to obtain insights into the ecological roles of DSE in arid environments and to extend their potential for agricultural and medicinal plants. Firstly, three DSE strains isolated from *H. scoparium* were exposed to low osmotic potentials induced by PEG 6000 in pure cultures to test their tolerance to osmotic stress. Secondly, we examined the effects of DSE inoculation on the performance of the host (*H. scoparium*) and non-host (*Glycyrrhiza uralensis*, *Zea mays*) plants in an inoculation experiment using these DSE strains under mild and extreme drought conditions. Specifically, we addressed the following questions in this study: (1) Do the DSE strains from arid habitat exhibit high tolerance to osmotic stress in vitro? (2) Does the DSE inoculation promote the growth of host and non-host plants under drought conditions? If yes, (3) does extreme drought affects the relationship between DSE and plants?

## Materials and methods

### Fungal isolates and plant materials

The three isolates (DKHB7, WHHB1, and ALSHB3) used in the experiment were obtained from *H. scoparium* and were deposited in the culture collection of the Laboratory of Plant Ecology, Hebei University, China. These fungi were identified based on phylogenetic analyses of nrDNA Internal Transcribed Spacer (ITS) sequences. Maximum parsimony analysis clustered DKHB7 (*Pa*, KU561868) with *Paraphoma* sp. (98% identity with *Paraphoma* sp. KT269033). WHHB1 (*Ec*, KU561863) was grouped in a clade with *Embellisia chlamydospora* AY956759, with a bootstrap support of 100%. ALSHB3 (*Co*, KU561865) was very closely related to *Cladosporium oxysporum* (100% identity with *Cladosporium oxysporum* HM148118) (for details, see Xie et al. 2017). Each isolate was grown on potato dextrose agar (PDA) culture medium for two weeks at 27 °C in the dark.

Mature seeds of *H. scoparium* were collected from natural populations in Inner Mongolia and stored at 4 °C. *Glycyrrhiza uralensis* is a perennial leguminous species and an important medicinal plant widely grown in northern China (Xie et al. 2018). The seeds of *G. uralensis* and *Z. mays* were provided by Hebei Agriculture University and were stored at 4 °C.

### Experiment 1

#### *Osmotic stress tolerance of DSE in vitro*

The capacity of the DSE isolates to grow under low osmotic potentials was tested in a preliminary experiment in liquid culture. The experiment was performed under sterile conditions, and the low osmotic potential was induced with PEG 6000. PEG 6000 is an inert osmoticum, widely used to simulate the effect of osmotic stress in organisms, primarily because it is chemically inert and non-toxic (Fernandez and Koide 2013; Santos et al. 2017). The basal medium was a modification of Modified Melin Norkrans (MMN) medium (pH 5.5). PEG 6000 was added to give osmotic potentials of 0, −0.45, −0.90, −1.34, −2.24, and − 3.58 MPa (Chen et al. 2003). Discs of inoculum (5 mm) were cut from the edge of actively growing 14-days-old colonies and one disc, for each isolate, was inoculated into a 250 mL Erlenmeyer flask containing 100 mL liquid medium. The cultures were incubated in the dark for 10 days with constant shaking, and each treatment was replicated four times. Upon harvest, the fungal mycelia were washed with distilled water and collected for the analyses. The fresh mycelia were randomly divided into two parts. The first part was directly used for the determination of superoxide dismutase (SOD) activity and melanin content. The remaining part was weighed before drying to a constant weight at 80 °C and then the water content was determined. The biomass production of DSE was the sum of the dry weights of these two parts.

#### *Determination of the SOD activity and melanin content*

Fresh mycelia from each isolate were homogenized and grinded in 5 mL 50 mM potassium phosphate buffer (pH 7.8), which contained 0.2 mM EDTA and 2% (*w*/*v*)

polyvinylpyrrolidone kept in ice bath. The homogenate was centrifuged at 15,000×g and 4 °C for 30 min. The supernatant liquid was decanted and used for analysis of enzyme activity. The SOD activity was determined using the photochemical method described by Elavarthi and Martin (2010), wherein the activity was determined by recording the decrease in the absorbance of nitroblue tetrazolium (NBT) complex by the enzyme. One unit of SOD was referred to as the quantity of enzyme needed to cause 50% inhibition of the reduction rate of NBT at a wavelength of 560 nm.

Melanin was extracted from mycelia following the method described by Ellis and Griffiths (1974), with minor modifications. Briefly, melanin was extracted from hyphae with hot alkali solution (1 M NaOH at 100 °C) for 4 h in a water bath. The cooled cell extract was filtered through a double layer of filter paper and acidified with concentrated HCl (7 M) until precipitation at pH 2.0. The resulting dark brown precipitate was recovered by centrifugation at 10,000×*g* for 15 min and washed with distilled water. The coagulated melanin was then dissolved in 1 M NaOH and the yield of melanin was estimated. The amount of melanin was determined by a standard curve plotted from results of photometry at 459 nm.

Experiment 2

*Plant growth promotion experiment*

The experiment was performed in a growth chamber (27 °C day/ 22 °C night) using a completely randomised design in a 4 × 2 factorial arrangement with DSE inoculation treatment (non-inoculated control, *Pa*, *Ec*, *Co*) and drought treatment (mild drought, MD; extreme drought, ED) as the variables for each plant species (*H. scoparium*, *G. uralensis*, and *Z. mays*). Each treatment was replicated five times, thus, accounting for a total of 120 experimental pots.

The seeds of each plant species were surface sterilised with 70% ethanol for 3 min and 2.5% sodium hypochlorite for 10 min under agitation. The sterilised seeds were thoroughly rinsed with sterile water and then aseptically planted onto water agar medium (containing 10 g/L agar) in Petri dishes for germination at 27 °C. Following pre-germination, the seedlings were transferred to sterile pots (8 cm diameter, 24 cm height; 1 seedling for each pot) containing 500 g sand, which was collected from the natural habitats of *H. scoparium* and autoclaved for 120 min at 121 °C. The sand contained 10.89 mg/g organic matter, 46.75 mg/kg available nitrogen, and 6.32 mg/kg available phosphorus. The fungal mycelia discs (5 mm in diameter, 1 disc for each plant), cut from a 14-days-old PDA culture medium, were placed 1 cm below the roots of the plants (Ban et al. 2017). The control treatments were inoculated with plugs excised from the medium without fungus. All the inoculation processes were performed on a clean bench. All the pots were kept in a growth chamber at 27 °C during the day and 22 °C during the night under a photoperiod of 10 h, and the mean relative humidity of the air was 60%.

One week after sowing, half of the seedlings (both control and inoculation treatments) were subjected to mild drought stress (MD, 40% field water capacity), and the other half were subjected to extreme drought stress (ED, 20% field water capacity). The drought stress treatments in this study were applied according to the lower and upper limits in the natural habitat of *H. scoparium* in Northwest China (Xie 2017). The soil moisture was determined with a soil humidity recorder (L99-TWS-2, China). The dry weights of shoots and roots, as well as root colonization and morphological traits were measured at 90 days after sowing. The *Z. mays* seedlings were harvested at 40 days after sowing.

*Plant biomass and root morphology traits*

Plant shoots and roots were separately harvested and washed carefully with deionised water. Individual root sections were first floated in approximately 1 cm depth of deionised water in a plexiglass tray and scanned with a desktop scanner (EPSON Perfection V800 Photo, Japan). Several morphological traits of roots (such as, total root length and average root diameter) were determined using the WinRHIZO image analysis system (Chen et al. 2012). The roots were collected after scanning and few root samples were randomly selected to analyse the DSE colonization (see below). The remaining roots and fresh shoots were dried at 70 °C for at least 48 h prior to calculate the plant biomass.

*Microscopic observation of root colonization*

To evaluate whether the roots were colonised by DSE, the fungal structures within the roots were stained with 0.5% (*w*/*v*) acid fuchsin at 90 °C for 20 min and

observed under an optical microscope, as described previously (Biemann and Linderman 1981; Phillips and Hayman 1970). For each plant, approximately 20 randomly selected 0.5-cm segments were placed on slides and viewed under a light microscope.

*Statistical analyses*

All statistical analyses were performed with SPSS 21.0 (SPSS, Chicago). For the first experiment, two-way analysis of variance (ANOVA) was performed to analyse the effects of the DSE species and osmotic stress on the biomass, SOD activity, and melanin content of three DSE. For the second experiment, two-way ANOVA was performed to examine the effects of the DSE inoculation treatment, drought treatment, and their interactions on the dry weight and root morphology traits of each plant species. All data in each experiment were tested for normality and homogeneity of variance before statistical analyses. These statistical analyses were conducted on transformed data (standardised data) of these parameters, but untransformed values are shown. The values reported in figures are means of at least three replicates. Within significant interactions, means were compared by one-way ANOVA in each DSE fungus or plant species. The differences between the means among the different treatments were analysed by Tukey's honestly significant difference test at a probability level of 0.05.

## Results

Experiment 1

*Osmotic stress tolerance of DSE in vitro*

The antioxidant substances and biomass production of three DSE were observed after 10 days of culture. In general, all the tested DSE isolates exhibited high tolerance to osmotic stress (Table 1, Fig. 1).

In the enhanced stress treatment, all the DSE showed an increasing trend in biomass production, which declined after reaching a maximum value at an osmotic potential of −0.90 (*Pa*) or − 1.34 MPa (*Ec*, *Co*) (Fig. 1a). The highest biomass of *Pa*, *Ec*, and *Co* was 200%, 167%, and 194% of those at 0 MPa, respectively. For *Pa* and *Co*, the osmotic stress did not exhibit negative effects on the fungal biomass at all the levels of treatments, and they were able to tolerate and grow well even at −3.58 MPa (Fig. 1a). *Ec* was more sensitive to the stress treatment than *Pa* and *Co*, showing a significantly lower yield at −3.58 MPa than at 0 MPa.

Under low stress treatment (−0.45 MPa), a significant increase in the SOD activity was observed in *Pa* (+171%), *Ec* (+84%), and *Co* (+40%), compared to that under 0 Mpa treatment (Fig. 1b). However, the SOD activity of these fungi decreased when the osmotic potential was lower than −0.9 MPa. At −2.24 MPa, only *Ec* showed lower SOD activity than at 0 MPa (Fig. 1b). At −3.58 MPa, all the DSE displayed lower values of SOD activity than their unstressed control.

Under conditions of osmotic stress, the melanin content of all the DSE showed an increasing trend initially, after which a decline was observed with decreasing osmotic potential of the medium (Fig. 1c). At −0.45 MPa, the osmotic stress caused a significant increase in the melanin content in *Co* (+141%) compared to that at 0 MPa; however, no increase in the content was observed in the case of *Pa* and *Ec*. When the osmotic potential reached −0.90 or − 1.34 MPa, the melanin content of all the DSE was significantly higher than that at 0 MPa. The melanin content of DSE showed a decrease at −2.24 and − 3.58 MPa. At the lowest osmotic potential (−3.58 MPa), the melanin content of *Pa* and *Ec* was lower than that at 0 MPa.

**Table 1** Analysis of variance (ANOVA) for the effects of dark septate endophyte (DSE) species and osmotic stress on the biomass, superoxide dismutase (SOD) activity, and melanin content of *Paraphoma* sp., *Embellisia chlamydospora*, and *Cladosporium oxysporum*

| | Biomass (g) | | SOD (U/mg Pr) | | Melanin content (mg/g) | |
|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* |
| DSE | 2789.9 | **<0.001** | 49.3 | **<0.001** | 458.9 | **<0.001** |
| Osmotic stress | 751.6 | **<0.001** | 289.4 | **<0.001** | 520.8 | **<0.001** |
| DSE × Osmotic stress | 63.2 | **<0.001** | 18.9 | **<0.001** | 38.0 | **<0.001** |

Significant *P*-values are in bold

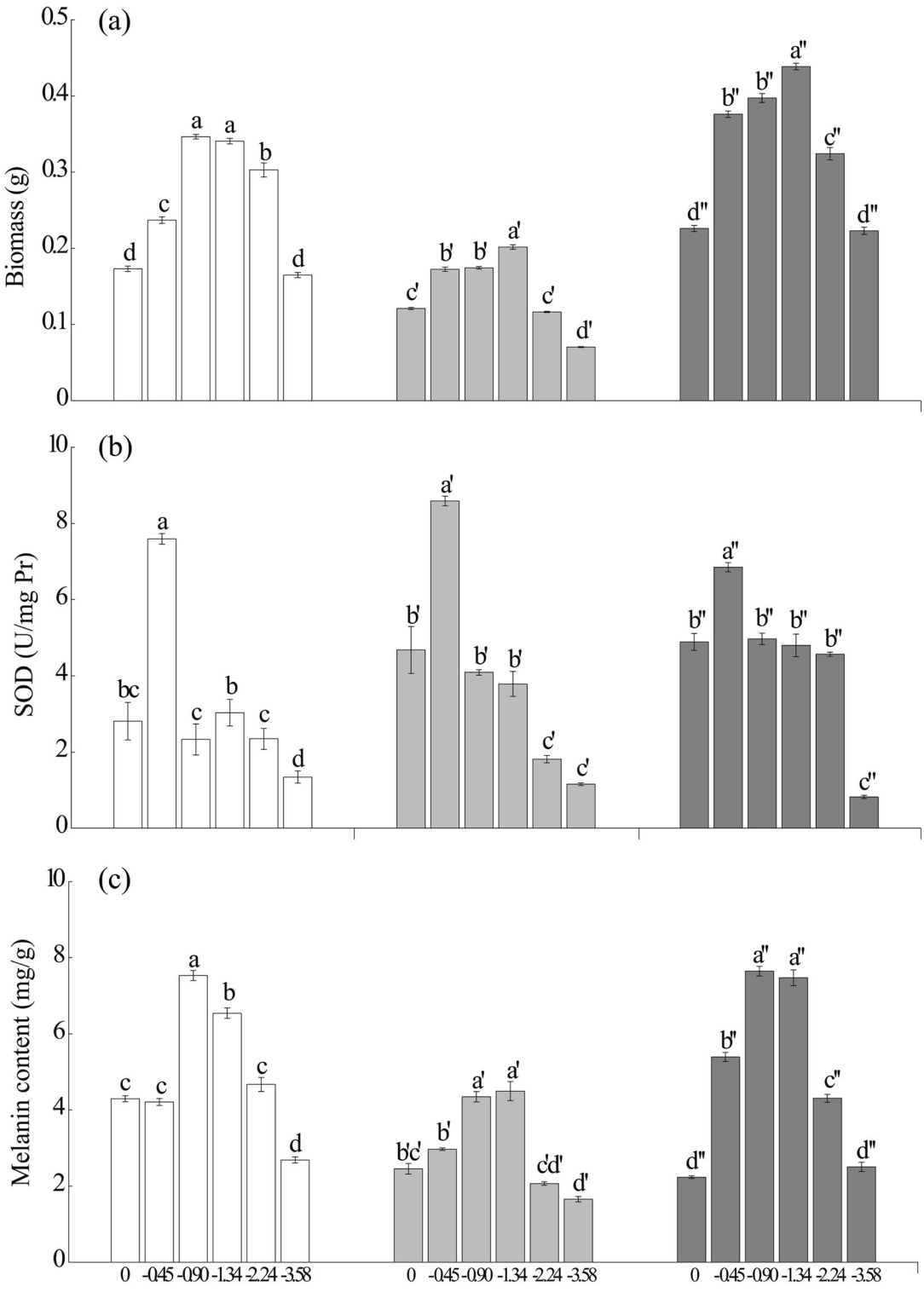
(a)
Biomass (g)
0.5
0.4
0.3
0.2
0.1
0
d c a a b d c' b' b' a' c' d' d'' b'' b'' a'' c'' d''
(b)
SOD (U/mg Pr)
10
8
6
4
2
0
bc a c b c d b' a' b' b' c' c' b'' a'' b'' b'' b'' c''
(c)
Melanin content (mg/g)
10
8
6
4
2
0
c c a b c d b'c' b' a' a' c'd' d' d'' b'' a'' a'' c'' d''
0 -0.45 -0.90 -1.34 -2.24 -3.58
0 -0.45 -0.90 -1.34 -2.24 -3.58
0 -0.45 -0.90 -1.34 -2.24 -3.58
Paraphoma sp.
Embellisia chlamydospora
Cladosporium oxysporum

**Fig. 1** Biomass (**a**), superoxide dismutase (SOD) activity (**b**), and melanin content (**c**) of three dark septate endophyte (DSE) exposed to different osmotic potentials of 0, −0.45, −0.90, −1.34, −2.24, and − 3.58 MPa induced with polyethylene glycol (PEG) 6000. The effects of osmotic stress were tested by one-way analysis of variance (ANOVA) for each DSE. The error bars represent the standard error (SE). Different letters above the error bars indicate significant difference at $P < 0.05$ as assessed by Turkey test

## Experiment 2

### *Plant growth promotion experiment*

After harvesting, no DSE structures were observed in the roots of control plants. The presence of DSE hyphae and microsclerotia was observed in the stained root segments of *H. scoparium*, *G. uralensis*, and *Z. mays* (Supplementary Fig. S1). Among all treatments, the factor 'plant species (Plant)' had the highest effect on the total biomass of plants (Supplementary Fig. S2). The biomass of the respective controls of these three plants differed significantly (for example, by ~0.2–4.0 g) under the conditions used in this study. For determining the influence of DSE on the growth of host plants and for assessing their applicability for crops and medicinal plants, the data were analysed and shown separately for each plant species.

### *Plant root morphology and development*

The root morphology of *H. scoparium* was significantly affected by the DSE species as well as by the drought treatment (Table 2). Inoculation with *Pa* and *Co* increased the total root length of the host plant by 388% and 300% as compared to that of the control plants regardless of the drought treatment; however, for *Ec*, the positive effect of DSE inoculation on the root length was not observed (Fig. 2a). All the inoculated plants exhibited significantly lower values of root diameter than the control plants (Fig. 2b).

There were significant interactions between the DSE species and drought treatment for the total root length of *G. uralensis* (Table 2). Under MD conditions, inoculation with *Pa* and *Co* increased the total root length of *G. uralensis* by 153% and 101% as compared to that of the control plants (Fig. 2c). Under ED conditions, extreme drought stress decreased the positive effects of *Pa* inoculation on the root length, whereas the values were increased by 180% when inoculated with *Co* as compared to those of the control plants. For plants inoculated with *Ec*, the total root length showed no difference with respect to the control plants under both the MD and ED treatments. For all the three DSE, inoculation also resulted in significantly lower root diameter than that observed for the control plants (Fig. 2d).

The root morphology of *Z. mays* was significantly affected by the DSE species, drought treatment, and their interactions (Table 2). Under MD conditions, plants inoculation with *Co* had a positive effect on the total root length of *Z. mays* (+22%) relative to that of the control plants (Fig. 2e). Under ED conditions, extreme drought stress decreased the positive effect of DSE inoculation. For the inoculated and non-inoculated plants, the total root length of plants did not show any difference. Unlike *H. scoparium* and *G. uralensis* plants, DSE inoculation did not affect the root diameter of *Z. mays* seedlings (Fig. 2f).

### *Plant biomass production*

The biomass production of *H. scoparium* was significantly affected by the DSE species regardless of the drought treatment (Table 2). The inoculation of *Pa* and *Co* resulted in significant increases in root (+200% and + 112%) and root/shoot ratio (+147% and + 91%) of hosts compared to the corresponding values for the control plants (Fig. 3a,b). There were no significant differences in the biomass production between the *Ec* and control plants.

The DSE species significantly influenced the shoot biomass of *G. uralensis* regardless of drought treatment (Table 2). Compared to the control plants, inoculation with *Pa* improved the shoot biomass of the host plants by 15%, whereas *Ec* and *Co* had no effect (Fig. 3c). The root biomass and root/shoot ratio were significantly affected by the DSE species, drought treatment, and their interactions (Table 2). Under MD conditions, inoculation with *Pa* and *Co* increased the root biomass of hosts by 42% and 29% as compared to those of the control plants, whereas inoculation with *Ec* had a neutral effect (Fig. 3c). Under ED conditions, only the plants inoculated with *Co* displayed higher root biomass than that of control plants. The root/shoot ratio of *G. uralensis* plants was increased by *Pa* under MD conditions, as well as by *Co* under ED conditions compared to that in the control treatment (Fig. 3d).

**Table 2** Analysis of variance (ANOVA) for the effects of dark septate endophyte (DSE) inoculation and drought treatment (Drought) on the root morphology and biomass production of *Hedysarum scoparium*, *Glycyrrhiza uralensis*, and *Zea mays*

| | Root length | | Root diameter | | Shoot biomass | | Root biomass | | Root: shoot ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* | *F* | *P* |
| *Hedysarum scoparium* | | | | | | | | | | |
| DSE | 106.0 | **<0.001** | 26.6 | **<0.001** | 1.5 | 0.233 | 25.4 | **<0.001** | 45.1 | **<0.001** |
| Drought | 47.2 | **<0.001** | 1.6 | 0.208 | 28.3 | **<0.001** | 48.0 | **<0.001** | 9.8 | **0.004** |
| DSE × Drought | 1.6 | 0.212 | 1.7 | 0.181 | 2.2 | 0.105 | 2.8 | 0.057 | 0.9 | 0.439 |
| *Glycyrrhiza uralensis* | | | | | | | | | | |
| DSE | 47.9 | **<0.001** | 30.6 | **<0.001** | 4.9 | **0.007** | 15.0 | **<0.001** | 8.1 | **<0.001** |
| Drought | 34.8 | **<0.001** | 0.0 | 0.899 | 61.3 | **<0.001** | 111.0 | **<0.001** | 18.2 | **<0.001** |
| DSE × Drought | 22.6 | **<0.001** | 0.4 | 0.727 | 0.7 | 0.562 | 6.1 | **0.002** | 8.8 | **<0.001** |
| *Zea mays* | | | | | | | | | | |
| DSE | 4.4 | **0.011** | 1.5 | 0.223 | 10.1 | **<0.001** | 16.1 | **<0.001** | 5.7 | **0.003** |
| Drought | 320.7 | **<0.001** | 50.1 | **<0.001** | 664.8 | **<0.001** | 341.6 | **<0.001** | 338.9 | **<0.001** |
| DSE × Drought | 3.7 | **0.022** | 1.2 | 0.311 | 10.7 | **<0.001** | 4.8 | **0.007** | 4.2 | **0.013** |

Significant *P*-values are in bold

The effects of DSE species, drought treatment, and their interactions on the biomass of *Z. mays* plants were significant (Table 2). Under MD conditions, the shoot biomass of *Z. mays* was decreased by the *Pa* (−23%) and *Ec* (−29%) inoculation compared to that of the control plants (Fig. 3e). The plants inoculated with *Ec* and *Co* showed an increase in the root biomass (+39% and + 42%), and inoculation with *Ec* also increased the root/shoot ratio by 101% as compared to that in the control treatment (Fig. 3e,f). No significant difference on the biomass production was observed between the control and inoculated plants under the ED conditions.

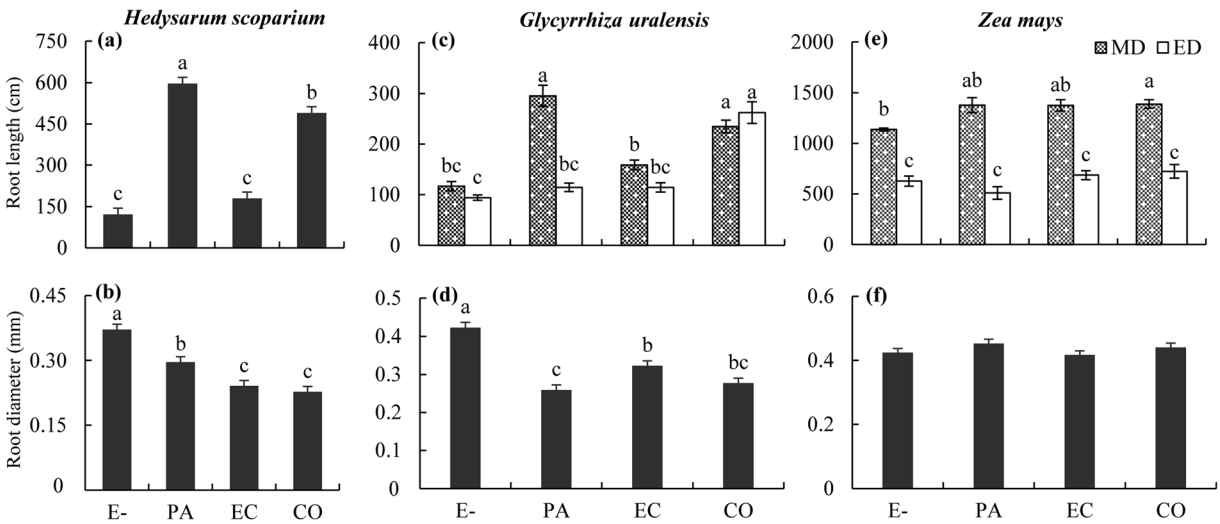


**Fig. 2** Effects of dark septate endophyte (DSE) inoculation and drought treatment on the root morphology of *Hedysarum scoparium*, *Glycyrrhiza uralensis*, and *Zea mays*. Data of these parameters were analysed separately for each plant species. The error bars represent the standard error (SE). Different letters above the error bars indicate significant difference at $P < 0.05$ as assessed by Turkey test. The estimated means were presented when interactions were not significant. E– indicates non-inoculated control. PA, EC, and CO indicate plants inoculated with *Paraphoma* sp., *Embellisia chlamydospora*, *Cladosporium oxysporum*, respectively. MD, ED, indicate mild drought stress and extreme drought stress

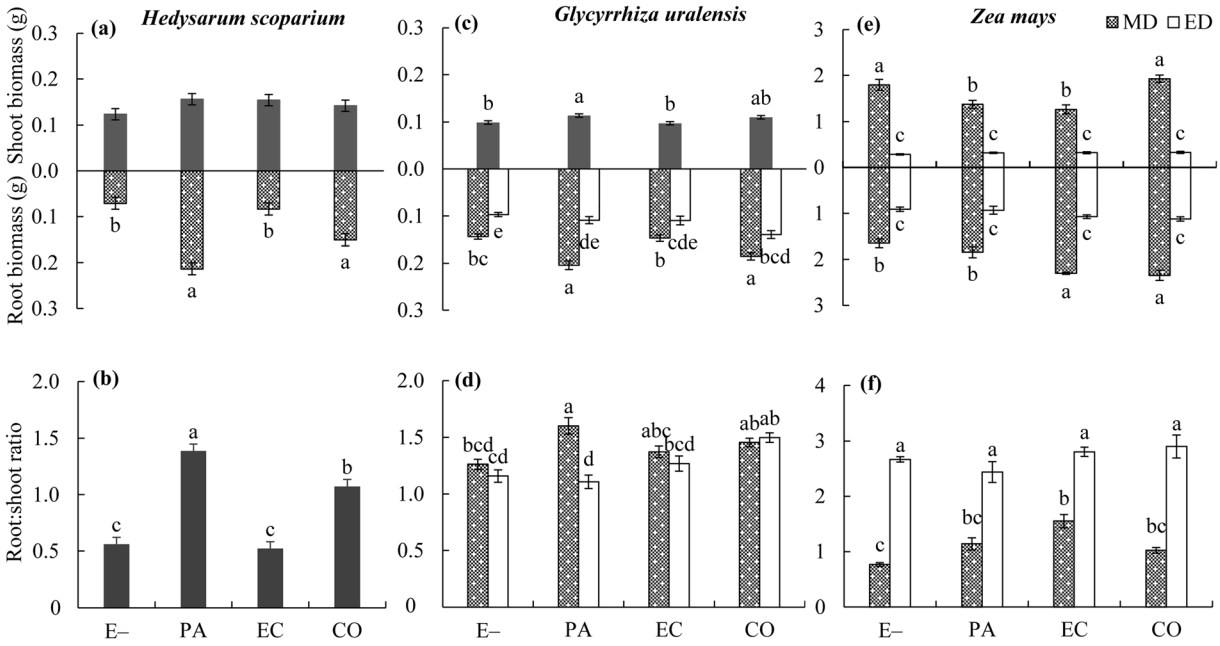


**Fig. 3** Effects of dark septate endophyte (DSE) inoculation and drought treatment on the biomass production of *Hedysarum scoparium*, *Glycyrrhiza uralensis*, and *Zea mays*. The error bars represent the standard error (SE). Different letters above the error bars indicate significant difference at $P < 0.05$ as assessed by Turkey test. The estimated means were presented when interactions were not significant. E– indicates non-inoculated control. PA, EC, and CO indicate plants inoculated with *Paraphoma* sp., *Embellisia chlamydospora*, *Cladosporium oxysporum*, respectively. MD, ED, indicate mild drought stress and extreme drought stress

## Discussion

### Osmotic stress tolerance of the DSE isolates

The results obtained using the pure cultures revealed that no significant decline in the biomass production of *Pa* and *Co* occurred when exposed to any of the stress treatments, whereas *Ec* was negatively affected at −3.58 MPa. In addition, our observations showed that the intermediate stress treatment (−1.34 or − 2.24 MPa) was more suitable for the DSE growth. A similar result was obtained by Santos et al. (2017), who found that the growth of DSE isolated from wild rice was increased by 50% at −0.8 MPa than that of at 0 MPa. We speculate that the preference of DSE for low osmotic potentials in this study might be related to their arid habitats and the low water potential of *H. scoparium* in deserts (Bai et al. 2008; Gong et al. 2015; Xie et al. 2017). Under this condition, these DSE might have adapted well to the arid environments.

Osmotic stress usually exerts negative effects on organisms and causes oxidative damage in cells (Li et al. 2008; Liu et al. 2010). In this study, we determined the SOD activity and melanin content in these three fungi to detect the response of antioxidant substances to osmotic stress. SOD has been reported as one of the most important enzymes for the removal of reactive oxygen species (ROS) (Collin-Hansen et al. 2005). The increased SOD activity in DSE at high osmotic potential (−0.45 MPa) indicated that SOD was synthesised to remove ROS under intensified stress treatment. Similar phenomenon was also reported in the response of DSE exposed to heavy metals, with enhancement in the SOD activity observed under Pb stress (Ban et al. 2012). The SOD activity declined at osmotic potentials below −0.90 MPa, indicating that other components might contribute to the fungal response to enhanced stress treatment. For all the DSE used in the present study, the melanin content was significantly increased with the decrease in osmotic potentials from −0.90 to −2.24 MPa. Melanin is considered to be an important trait for the survival of DSE under stressful environments because it can act as an antioxidant agent to relieve oxidative damage (Ban et al. 2012; Zhan et al. 2011). Besides, melanin production has also been reported to contribute to osmotic stress tolerance of an ectomycorrhizal fungus induced by PEG (Fernandez and Koide 2013). Thus, increased melanin content in

DSE under −0.90 to 2.24 MPa treatments, as observed in this study, might contribute to their high tolerance to osmotic stress.

## Effects of DSE inoculation on host plants

Although there is accumulating evidence regarding the diversity and distribution of DSE (Barrow 2003; Knapp et al. 2012; Lugo et al. 2015; Porras-Alfaro et al. 2008; Xie et al. 2017; Zhang et al. 2010), their effects on plants are not well understood, especially under drought conditions (Santos et al. 2017). The results of existing studies on the effects of DSE inoculation on plant growth under drought conditions are variable. The inoculation of rice or *B. gracilis* plants with DSE isolates in soils exposed to drought resulted in neutral to negative effects on plant growth (Perez-Naranjo 2009; Santos et al. 2017). On the other hand, sorghum plant grew better in soils exposed to drought upon inoculation with *E. pisciphila* (Zhang et al. 2017). In the present study, typical DSE hyphae and microsclerotia were observed in roots of *H. scoparium* after harvest in all the treatments, which indicated that all these DSE are effective root colonizers even under extreme conditions of drought. Moreover, the host response of *H. scoparium* to DSE colonization was strain-dependent but was independent of drought treatment. Specifically, *H. scoparium* plants inoculated with *Pa* and *Co* showed significantly higher root biomass production than the control plants, regardless of drought stress conditions, whereas *Ec* did not have any influence on the growth of the host. Our observations were consistent with those of previous studies showing that the species of DSE may influence the DSE-plant interaction (Mandyam and Jumpponen 2005; Mayerhofer et al. 2013; Newsham 2011).

The positive effects of DSE inoculation on *H. scoparium* appeared to occur below the ground as indicated by the fact that the most important and consistent plant response to DSE inoculation was the increase in root growth. Even though DSE displayed no influence on the shoot biomass of the host, the inoculation of *Pa* and *Co* in *H. scoparium* improved the root biomass under drought conditions. It has been well documented that larger biomass allocation to roots is a key mechanism for enhancing plant survival in arid environments (Alvarez-Flores et al. 2014; González-Teuber et al. 2018). Thus, our results suggest that colonization by *Pa* and *Co* was able to promote the growth of *H. scoparium* under drought stress, probably through biomass adjustments. This might be an important survival strategy for *H. scoparium* in natural habitats, where water deficiency is always a common phenomenon (Deng et al. 2015; Gong et al. 2015).

The DSE inoculation also regulated the root architecture of *H. scoparium* to improve the performance of plants under drought conditions. In this study, plants inoculated with *Pa* and *Co* exhibited higher length of roots than the control plants, indicating positive effects on the root growth. The development of a deep and extensive root system can regulate the absorption of water and nutrients in soil, which ultimately influences the biomass production (Hund et al. 2009). Several plant growth-promoting microbes, including DSE, have also been shown to influence the root architecture of plants (Junges et al. 2016; López-Coria et al. 2016; González-Teuber et al. 2018; Villarreal-Ruiz et al. 2004; Wu et al. 2010). For example, the DSE could promote the root development of an endangered Chinese medicinal plant under unstressed conditions (Wu et al. 2010). Moreover, the average root diameter of DSE-inoculated *H. scoparium* decreased compared to the root diameter of the control plants. The roots with small diameters have been reported to exhibit faster growth and allocation of more nutrients for increasing their length, which is beneficial for plants under drought conditions (Comas et al. 2013; Palta et al. 2011). Therefore, the longer root length and finer root diameter of *H. scoparium* in the present study may be advantageous to the plants for drought adaptation (Awad et al. 2018).

## Effects of DSE inoculation on non-host plants

To gain further insights into the application of DSE on agriculture and medicinal plants, we analysed the growth promotion ability of DSE in *G. uralensis* and *Z. mays*. As a plant well-adapted to low-fertility soil and arid environments, *G. uralensis* is expected to be used for ecological restoration of degraded ecosystems in (semi) arid regions (Xie et al. 2018). Moreover, *Z. mays* is usually used as a compatible host plant for DSE inoculation experiments (Li et al. 2011; Wang et al. 2016). A few studies have reviewed the use of non-host DSE as potential agents capable of enhancing the growth of crops, such as cabbage, maize, *Asparagus officinalis*, tomato, and rice (Andrade-Linares et al. 2011; Ban et al. 2017; Diene et al. 2014; Surono 2017;

Vergara et al. 2017; Wang et al. 2016). For medicinal plants, the existing reports have generally focused on the growth promoting ability of DSE on host plants (Wu et al. 2010; Zhang et al. 2012; Zhu et al. 2015). In this study, all the DSE used could colonise the roots of *G. uralensis* and *Z. mays* and showed a positive effect on the root development of plants. This observation, therefore, agrees with the initial prediction that DSE could act as non-host colonizers and enhance the growth of the non-host plants. Our results further suggested that DSE respond differently depending on stress conditions and the plant-fungus species. Under MD conditions, *Pa* and *Co* improved the root growth of *G. uralensis*; however, for *Z. mays* plants, *Ec* and *Co* were more prominent. Thus, *Co* exerted the best effects on non-host plants under MD conditions. This promotion effect was similar to that reported by Zhang et al. (2017), showing that DSE inoculation improved the dry weight of roots in sorghum under drought condition. Under ED conditions, only *Co* showed an obvious enhancement of the root biomass in *G. uralensis*, indicating that the positive effects of DSE inoculation were decreased by extreme drought stress. As *Co* exhibited positive effects on root growth in *G. uralensis* plants under both MD and ED conditions, it was considered to be the best fungus for this plant species.

## Conclusion

In this study, three DSE isolated from *H. scoparium* showed high tolerance to osmotic stress in vitro and were able to colonise the host and non-host plants. Despite originating from the same habitats, the three DSE showed a strong interspecific variation in osmotic-stress tolerance and displayed considerable functional differences on plant growth. The response of plants to DSE varied from neutral to beneficial depending on the plant and fungus species as well as on the drought treatment. For the host plant, two of the three DSE improved the root growth in *H. scoparium* under both the MD and ED treatments. For the non-host plants, the positive effects of DSE inoculation on *G. uralensis* and *Z. mays* were mainly significant under the MD treatment. Overall, DSE from arid habitat enhanced the root growth of host plants under drought conditions and may also be used to promote the cultivation of agricultural and medicinal plants.

**Acknowledgements** This study was financially supported by the National Natural Science Foundation of China (project no. 31470533, 31770561, 31800345). We greatly appreciate the support of Experimental Center of Desert Forestry, CAF for their invaluable assistance on this experiment.

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