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Full Length Article

Dark Septate Endophyte Improves Drought Tolerance in Sorghum

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Abstract

Dark septate endophytes (DSE) protect host plants against a variety of environmental stresses, however our knowledge about the roles of DSE in improving drought tolerance of crops is poor. In this study, sorghum (*Sorghum bicolor* L. Moench) was inoculated with a DSE strain (*Exophiala pisciphila* GM25) under two different soil water conditions (well-watered (WW), -0.11 MPa; drought-stressed (DS), -0.69 MPa) for one month. At the end of this experiment, sorghum roots were obviously colonized by DSE with 50.5%–62.5% colonization rate. When compared with non-inoculated seedlings under both WW and DS conditions, *E. pisciphila*-inoculated sorghum had greater plant height, collar diameter, shoot dry weight, net photosynthetic rate (*Pn*), stomatal conductance (g_s), transpiration rate (*E*), maximal photochemical efficiency of PSII photochemistry (*Fv/Fm*) and actual quantum yield (*ΦPSII*), and lower intercellular CO_2 concentration (*Ci*). In addition, in comparison to non-inoculation under DS conditions, *E. pisciphila* inoculation also improved the root dry weight, non-photochemical quenching values (*NPQ*), photochemical quenching values (*qP*), increased the content of related secondary metabolites including anthocyanin, polyphenol and flavonoid and enhanced the enzymatic activities related to secondary metabolism, such as cinnamyl alcohol dehydrogenase (CAD), phenylalanine ammonia-lyase (PAL), guaiacol peroxidase (G-POD) in sorghum seedlings. Our results demonstrated that the drought resistance of sorghum seedlings were positively improved by *E. pisciphila* inoculation with better plant growth, gas exchange, photosynthesis, chlorophyll fluorescence, secondary metabolites and enzyme activities related to secondary metabolism. Inoculation with *E. pisciphila* may be an efficient strategy to survive for sorghum in drought environments. © 2016 Friends Science Publishers

Keywords: Dark septate endophyte fungi; Drought stress; Sorghum; Photosynthesis; Secondary metabolites

Introduction

Dark septate endophytes (DSE) are a diverse group of root-inhabiting endophytic fungi, and they frequently form symbiotic relationships with epidermis and cortex of plant roots (Jumpponen and Trappe, 1998; Jumpponen, 2001). These conidial or sterile ascomycetous fungi are characterized by dark septate hyphae and microsclerotium (aggregation of dark, thick-walled, closely packed inflated cells) (Peterson *et al*., 2004; Li *et al*., 2011). Ubiquitous DSE colonization in root tissues has positive effects on host plants, especially in various unfavourable or stressful environments (Rommert *et al*., 2002). More experimental evidence suggests that DSE play positive roles on producing phytohormones substances, supplying nutrients, breaking down complex carbohydrates and providing simple sugars for host plants, as a result, improving plant growth (Wu and Guo, 2008). In addition, chitin and melanin in dark hyphae may increase hardness, reduce permeability of plant cytoderm, and protect host plants against a variety of environmental stresses, including cool, heavy metal and saline (Cousin, 1996; Money *et al*., 1998; Wu *et al*., 2009). However, our knowledge about interactions between DSE and host plants against drought stress is poor.

Continuous or frequent drought is a serious environmental issue it has spread to approximately 45% of agriculture lands on earth (Singh *et al*., 2012). Drought stress often occur in parallel during rapid growth and grain filling period, which lead to about 10% loss in total grain output worldwide (Farooq *et al*., 2009; Celebi *et al*., 2010), and this situation seems to deteriorate in recent years, due to anthropogenic disturbances and climate changes (Gong *et al*., 2013). Cereal plants have specific complex adaptation strategies to cope with drought stress, these mechanisms involve changes at cellular and whole-plant levels (Boomsma and Vyn, 2008; Farooq *et al*., 2014). However, only limited studies have been done on the development of physiological approaches for improving drought tolerance of minor cereals in arid and semi-arid areas.

Sorghum (*Sorghum bicolor* L. Moench) is one of important minor cereals under crop production wordwide, it often grows well in poor soils and has a good ability of

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drought resistance compared to other cereals (Funnell-Harris *et al*., 2010). Now, it is gaining extensive attentions for their usability as food, fodder, biofuels and feedstock of alcoholic beverages. The health beneficial effects of sorghum grain for human chronic diseases such as cardiovascular diseases, cancer, diabetes and obesity, have been recognized, most likely attributed to its secondary metabolites, polyphenol, flavonoids and anthocyanin (Luthria and Liu, 2013). But this minor crop is often faced with a challenge to keep high yields under substantial drought conditions. In order to stabilize and increase global production of sorghum, enhancing the drought tolerance of sorghum should be an important objective.

In the present study, we hypothesized that DSE could improve the drought tolerance of sorghum. To test this hypothesis, *Exophiala pisciphila* GM25, one of drought resistant DSE strains, was isolated from the roots of sorghum, and used for inoculum. Greenhouse pot experiments were conducted under well-watered (WW) and drought-stressed (DS) conditions in soils. Objectives of the study were to assess whether *E. pisciphila* GM25 improved the drought tolerance of sorghum according to the growth performance, gas exchange, chlorophyll concentrations, photosynthetic capacity, maximum photochemistry efficiency and the content of secondary metabolism. The possible mechanisms of DSE enhancing the drought tolerance of sorghum were also discussed in this study. Results may enable to use future biotechnological applications of *E. pisciphila* as micro organic complex fertilizers in cultivating crops.

Materials and Methods

Experimental Design and Statistical Analysis

The experiment consisted of a randomized block design with two factors: (1) colonized treatments: *i.e*., *Exophiala pisciphila* GM25, and a non-colonized control; (2) soil water conditions *i.e*., well-watered (WW) and drought-stressed (DS) conditions. Each of the four treatments had five replicates (pots) leading to a total of twenty pots (one seedling per pot). Data were statistically analyzed using SPSS software package (version 13.0 for Windows, SPSS Inc., IL, USA) for analysis of A two-way ANOVA, and Tukey's multiple range test was used in evaluating differences among treatment means.

Preparation of DSE Colonized and Non-colonized Sorghum Seedlings

Sorghum seeds (*Sorghum bicolor* L. Moench, genotype:Yuliang 4) were kindly provided by Henan Academy of Agricultural Science (Zhengzhou, China). The seeds were surface-sterilized by using 75% alcohol for 10 min, subsequently two times with 5% NaOCl for 10 min under agitation, and were thoroughly rinsed with sterile water after each disinfection step. Sterilized seeds were placed on sterilized moist filter paper for germination in a illumination incubator at 25°C/80% relative humidity for 72 h.

River sand was sieved through a 2 mm sieve, and washed with tap water in order to remove soil silt and nutrients. Washed sand was dried and autoclaved in the 180°C oven for 5 h, germinating seeds were transplanted into sterile plastic container (15 cm in diameter and 15 cm in depth) with 1.5 kg of autoclaved sand mixed with 400 mL MS liquid medium (Li *et al*., 2011).

E. pisciphila GM25 strains were isolated from roots of maize (*Zea mays* L.) on Petri dishes containing potato dextrose agar (PDA), they were identified according to analyses of morphological characteristics and internal transcribed spacer region sequences of ribosomal DNA and were deposited in Key Laboratory of Microbial Resources Exploitation and Utilization, Henan University of Science and Tenchology, China. *E. pisciphila* GM25 strain was grown in 250 mL Erlenmeyer flasks with 120 mL liquid medium for 4 weeks at 28°C and 150 rpm agitation. Fresh mycelium was filtered and washed with sterile distilled water for inoculums. *E. pisciphila* GM25 inoculums were placed 5 cm below sorghum roots, covered with the gas-permeable plastic film at the time of transplantation, and non-fungi treatment consisted of sterilized (0.11 Mpa and 121°C for 2 h) inoculum. All cultures were carried out in a growth chamber at 25°C in the day and 18°C in the night with a photoperiod of 12 h, 80% relative humidity.

Growth Conditions

Thirty days after sowing, each developed seedling was transplanted into a plastic container (15 cm in diameter and 15 cm in depth) with 2 kg of autoclaved (0.11 Mpa and 121°C for 2 h) soils (pH 7.9 (1:5 soil: water ratio), available nitrogen 30.64 mg kg^{-1}, Olsen phosphorus 12.13 mg kg^{-1}, available potassium 89.31 mg kg^{-1}, organic matter17.11 g kg^{-1}). The soil was collected from experimental farm in Henan University of Science and Technology, China.

Seedlings were grown in a greenhouse under natural light between April and July 2014 at a temperature of 20-35°C, with 60-75% relative humidity. In order to make high DSE colonization rate in sorghum roots, the DS treatment began two months after transplantation. One month of drought-stressed phase was conducted in August 2014. In this phase, half of the pots were maintained in a well-watered (WW) condition at 70% field capacity (–0.11 MPa), whereas the other half were subjected to drought-stressed (DS) conditions at 30% field capacity (–0.69 MPa). Soil water content was measured gravimetrically by weighing pots, and lost water was replaced with fresh distilled water each day at 18:00 in order to keep 70 or 30% field capacity, respectively. After one month of drought-stressed phase, all physiological data were detected.

Root Colonization Analysis

DSE colonization rate of 1 cm root sections was assessed by cleared for 1 h in 10% KOH at 90°C, bleached in alkaline hydrogen peroxide for 20 min, acidified in 1% HCl, and stained with 0.05% (w/v) trypan blue in lactophenol, according to Phillips and Hayman (1970). Quantification was performed using the method of gridline intersect (Giovannetti and Mosse, 1980). Twenty five root segments were checked in the evaluation of DSE colonization in each treatments, and each treatments had four replicates. AM colonization rate was counted by the following formula:

AM colonization (%) = (infected root length/observed root length) × 100%

Plant Measurements

Physiological response parameters of sorghum seedlings were measured at the end of the drought-stressed phase. All seedlings were harvested and the growth substrate thoroughly removed from soil. At the end of the growth period, seedlings were sampled and the plant height, root length, collar diameter, plant weight were separately recorded. Shoot dry weight and root dry weight were determined by oven drying at 70°C to constant weight.

Physiological response parameters of sorghum seedlings were measured at the end of the drought stress phase, and each treatment had five plants for plant measurements. At harvest, shoots were cut 1 cm above the soil surface. Roots were gently pulled up from the pots, washed with tap water, before any dirt and soil was carefully removed using fine-tip forceps (Aggangan *et al*., 2010). Plant height and root length were measured using a steel ruler. Root and shoot biomass were determined by oven drying at 70°C for 48 h.

Gas exchange parameters, including net photosynthetic rate (*Pn*), stomatal conductance (g_s), intercellular CO_2 concentration (*Ci*) and transpiration rate (*E*), were determined by using a steady-state porometer LI-6400 (LI-COR, Inc., Lincoln, NE, USA) under irradiation 1,500 μmol m^{-2} s^{-1} (photons), air flow rate 0.5 dm^3 min^{-1}, CO_2 concentration 350 cm^3 m^{-3}, leaf temperature 25.0°C between 9:30 and 11:30 AM. Five plants with completely expanded leaves were randomly chosen for evaluation.

Chlorophyll fluorescence were determined at the end of the experiment by using a PAM Chlorophyll Fluorometer (PAM-2000, Heinz Walz GmbH, Germany) at room temperature (25.0°C) between 9:30 and 11:30 AM. Five fully expanded leaves were random selected and clamped to a leaf section chamber, the data were recorded. Measurements for the minimum fluorescence (F_0) and maximal fluorescence (*Fm*) yields were maded for dark-adapted (20 min) leaves, while the steady-state (*Fs*) and maximal (*Fm'*) fluorescence were assessed for light-adapted leaves (Gong *et al*., 2013). The maximum fluorescence yield (*Fm*) was attained with a 2.5 s saturating pulse (1,800 μmol m^{-2} s^{-1}), following the measurement of the minimal fluorescence level in the light-adapted state (F_0') with a 2.5 s far-infrared light (5 μmol m^{-2} s^{-1}). The maximum quantum yield of PSII (*Fv/Fm*, Fv = Fm - F_0), the actual quantum yield of PSII ($\Phi PSII$ = (Fm′ - Fs)/Fm′), the non-photochemical fluorescence quenching (*NPQ* = (Fm - Fm′)/Fm′) and the photochemical fluorescence quenching (*qP* = (Fm′ - Fs)/(Fm′ - Fo′)) were calculated according to Maxwell and Johnson (2000).

Determination of Anthocyanin, Polyphenols and Flavonoid Content

Anthocyanin content was detected by using the method of (Sato *et al*., 1996). Fresh leaves and fruits (0.5 g) were pulverized and extracted with 5 mL of acidified ethanol (85:15 of 95% ethanol: 1.5 M HCl) for 1 h at room temperature. The extracting solution was centrifuged at 10,000 rpm for 10 min, and the supernatant was measured at 535 nm by using visible spectrophotometer. Anthocyanin content was calculated using the following equation:

$$\text{Amount (mg=100 g FW)} = (A_{535} \times V)/(98.2 \times W) \times 100\%$$

The results were expressed as milligrams per 100 g FW. The value 98.2 is the molar absorptivity of anthocyanin at 535 nm. Each sample had five replicates.

For the measurement of polyphenolic content, fresh leaves and fruits (1 g) were pulverized and extracted with 10 mL of 50% methanol-water (v/v) for 1 h at room temperature. The extracting solution was centrifuged at 10,000 rpm for 20 min. Total polyphenolic content were determined by using the Folin-Ciocalteu reagent method according to Ainsworth and Gillespie (2007). The absorbance of supernatant was measured at 735 nm by using UV-Vis spectrophotometer. We used gallic acid as a standard for the calibration curve, the results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g FW. Each sample had five replicates.

For determination of the flavonoid content, fresh leaves and fruits (1 g) were extracted with 20 mL of 70% methanol-water (v/v) for 30 min. After filtering, the filtrates were collected and transferred into a 50 mL volumetric flask, the volume metered to 50 mL. Rutin was used as the standard for the calibration curve. The content was detected by high performance liquid chromatography (HPLC) according to the method of Li *et al*. (2007), each sample had five replicates.

Determination of CAD, PAL, G-POD Activities

For the measurement of CAD activities, leaves and fruits (0.3 g) were homogenized in 2 mL of 100 mM K-phosphate buffer (pH 7.4), containing 0.5 mM DTT, 2 mM L-cysteine,

2 Mm EDTA, 8 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVPP). The extract was centrifuged at 14,000 rpm for 20 min. CAD activity (EC 1.1.1.195) was measured on the basis of the rate of increase in absorbance at 400 nm following the oxidation of coniferyl alcohol (Mitchell *et al*., 1994).

To determine phenylalanine ammonia-lyase (PAL, EC4.3.1.5) activity, fresh leaves and fruits (0.5 g) were extracted with 5 mL of 50 mM boracic acid buffer (pH 8.8). After centrifugation for 20 min at 14,000 rpm. PAL activity was performed as nmol cinnamic acid h^{-1} mg^{-1} protein at 290 nm according to the method of Wu and Lin (2002).

The enzyme extracts of guaiacol peroxidase (G-POD, EC1.11.1.7) was carried out as described by Lamikanra and Watson (2001). Fresh leaves and fruits (1 g) was ground with 2 mL potassium-phosphate buffer (0.1 mol L^{-1}, pH 7.3), the supernatant centrifugated at 14,000 rpm for 10 min at 4℃, enzyme activity was measured by the increase of absorbance caused by guaiacol oxidatio at 420 nm, and quantified spectrophotometrically as the amount of formed tetraguaiacol per minute per gram of fresh plant tissue. All protein content was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Results

AM Colonization

Abundant dark separate mycelia and microsclerotia were detected in roots of *E. pisciphila*-inoculated seedlings, and the typical DSE structures were not observed in stained roots of non-inoculated sorghum after harvesting. Drought stress significantly restrained DSE colonization in sorghum roots. The colonization rate in inoculated sorghum roots was 62.5% under WW conditions, and 50.5% under DS conditions, respectively (Table 1).

Growth Parameters

Drought stress restrained significantly the plant height, root length, collar diameter, shoot dry weight and root dry weight in sorghum both in *E. pisciphila*-inoculated and non-inoculated treatments (Table 1). *E. pisciphila* inoculation alleviated the negative effects of drought stress, it was shown by the significantly higher sorghum biomass in *E. pisciphila*-inoculated sorghum than those of non-inoculated sorghum. *E. pisciphila*-inoculated sorghum showed higher plant height, collar diameter and shoot dry weight, compared with non-inoculated seedlings under both WW and DS conditions (Table 1). Root dry weight and root length were significantly increased by *E. pisciphila* inoculation under DS conditions, but not under WW conditions.

Gas Exchange and Chlorophyll Fluorescence

Drought stress decreased *Pn*, *gs* and *E*, and increased *Ci* in sorghum seedlings (Fig. 1). *E. pisciphila* inoculation enhanced *Pn*, *gs* and *E* and reduced *Ci* in sorghum leaves, relative to non-inoculation treatments under both WW and DS conditions.

Drought stress inhibited *Fv/Fm*, $\Phi PS\,II$ and *qP*, and increased *NPQ* in sorghum seedlings (Fig. 2). The *Fv/Fm* and $\Phi PS\,II$ in mycorrhizal seedlings was significantly higher than that in non-inoculated seedlings under both WW and DS conditions. *NPQ* was obviously lower, and *qP* was significantly higher in inoculated seedlings than that in non-inoculated seedlings under DS conditions, however, *qP* and *NPQ* were not affected by *E. pisciphila* inoculation under WW conditions.

Secondary Metabolite Content

Drought stress increased the contents of anthocyanin, polyphenol and flavonoids in the leaves and grains of sorghum (Table 2). In sorghum leaves, only the phenols content in *E. pisciphila*-inoculated seedlings was higher than that in the non-inoculated control under both WW and DS conditions. The contents of anthocyanin and flavonoids in sorghum leaves were increased by *E. pisciphila* inoculation under DS conditions, but not under WW conditions. In sorghum grains, the contents of anthocyanin, phenols and flavonoids were obviously improved by *E. pisciphila* inoculation under WW and DS conditions, except for anthocyanin under WW conditions.

Secondary Metabolism-related Enzyme Activities

The activities of secondary metabolism-related enzymes in the leaves and grains of sorghum were significantly improved by drought stress (Table 3). Under both WW and DS conditions, *E. pisciphila* inoculation enhanced the enzyme activities of PAL and G-POD in sorghum leaves, compared to non-inoculation treatments, but had no obvious effect on the enzyme activity of CAD. In sorghum grains under both WW and DS conditions, the enzyme activities of CAD, PAL and G-POD in *E. pisciphila* inoculated seedlings were obvously higher than that in the non-inoculated control, but not the enzyme activity of PAL under both WS conditions.

Discussion

Dark septate endophytes (DSE) are common fungi over broad hosts and geographical ranges (Jumpponen and Trappe, 1998), but the number of studies investigating DSE associations in plant roots under experimental drought conditions is considerably low. In the present study, dark separate hyphae and microsclerotia were observed in sorghum roots after a month of *E. pisciphila* inoculation, and they were morphologically similar to typical DSE described by other reporters (Jumpponen, 2001). DSE colonization rate in sorghum roots was from 50.5% to 62.5% in this study, it was showed that *E. pisciphila*

Table 1: Effects of *Exophiala pisciphila* on growth parameters of sorghum under well-watered (WW) and drought-stressed (DS) conditions

Water conditions	Inoculation	DSE colonization (%)	Plant height (cm)	Root length (cm)	Collar diameter (mm)	Shoot dry weight (g)	Root dry weight (g)
WW	Non−DSE	0 c	73.1 b	40.3 a	11.9 ab	41.4 b	31.2 a
	E. pisciphila	62.5 a	82.8 a	43.6 a	12.3 a	52.4 a	34.7 a
DS	Non−DSE	0 c	54.5 d	30.2 c	8.7 c	24.4 d	20.9 d
	E. pisciphila	52.5 b	63.3 c	37.3 b	9.8 b	32.3 c	24.6 c
Significance							
DS		*	**	*	*	*	*
DSE		*	**	NS	*	*	NS
DS × DSE		**	NS	*	NS	NS	*

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test

Table 2: Effects of *Exophiala pisciphila* on anthocyanin, phenols and flavonoid content in sorghum under well-watered (WW) and drought-stressed (DS) conditions

Water conditions	Inoculation	Leaves			Grains		
		Anthocyanin (mgg^{-1} FW)	Polyphenol (mgg^{-1} FW)	Flavonoid (mgg^{-1} FW)	Anthocyanin (mgg^{-1} FW)	Polyphenol (mgg^{-1} FW)	Flavonoid (mgg^{-1} FW)
WW	Non−DSE	35.1 c	7.14 c	3.72 c	21.4 c	10.7 d	14.7 d
	E. pisciphila	36.4 c	10.2 b	4.24 c	22.3 c	14.8 c	20.5 c
DS	Non−DSE	42.5 b	11.2 b	8.23 b	29.7 b	24.5 b	29.1 b
	E. pisciphila	54.7 a	15.3 a	11.1 a	34.4 a	31.3 a	34.7 a
Significance							
DS		*	*	*	*	**	**
DSE		NS	*	*	NS	**	**
DS × DSE		*	NS	NS	*	NS	NS

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test

Table 3: Effects of *Exophiala pisciphila* on secondary metabolism-related enzyme activities in sorghum under well-watered (WW) and drought-stressed (DS) conditions

Water conditions	Inoculation	Leaves			Grains		
		CAD; (nmol min^{-1} mg^{-1} protein)	PAL; (Unit mg^{-1} protein)	G-POD; (μmol min^{-1} mg^{-1} protein)	CAD; (nmol min^{-1} mg^{-1} protein)	PAL; (Unit mg^{-1} protein)	G-POD; (μmol min^{-1} mg^{-1} protein)
WW	Non−DSE	10.2 b	2.45 d	6.24 c	18.2 c	2.42 c	3.21 d
	E. pisciphila	11.3 b	3.78 c	9.78 b	24.9 b	2.77 c	6.25 c
DS	Non−DSE	16.7 a	5.24 b	10.6 b	25.4 b	3.61 b	8.24 b
	E. pisciphila	17.1 a	7.85 a	15.4 a	32.1 a	4.72 a	10.2 a
Significance							
DS		*	**	*	*	*	*
DSE		NS	**	*	*	*	NS
DS × DSE		*	NS	*	*	NS	*

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test

could well form mutualistic association with sorghum. The high root colonization rate of DSE was also found in some plants from drought desert ecosystems in Argentina (Fracchia *et al*., 2011). Most of DSE are lack of conidia or other reproductive structures for dispersal and survival, intracellular-melanized hyphal aggregation acts as dispersal propagules for DSE colonization within epidermis and cortex of plant roots, which reflects an adaptation to abiotic factors in different stress environment (Hambleton and Currah, 1997). In the present study, drought had negative effects on DSE colonization in sorghum roots, drought stress depressed the hyphal spread, microsclerotia development and dispersal propagules for DSE, which lead to the decrease of DSE colonization rate (Wu and Zou, 2009).

The growth parameter of host plants is one of important indicators for the efficiency of DSE mutualistic association. Root colonization by DSE fungi was reported to cause a variety of host growth responses (Jumpponen and Trappe, 1998). By using meta-analyses of plant responses to DSE from 18 research articles, Newsham (2011) concluded that DSE inoculation had positive effects on total, shoot and root biomass with increases of 79%, 45% and 71% in these parameters for host plants, compared to non-inoculated controls. In this study, *E. pisciphila* inoculation improved plant height, collar diameter and shoot dry weight in sorghum under DS conditions, the possible reason was that DSE mutualistic association effected on the regulation of plant hormone, and this phenomenon was more obvious under drought stress conditions. A kind of phytohormones substances (IAA) was identified in culture medium from two DSE strains *Helgardia ericae* (Gay and

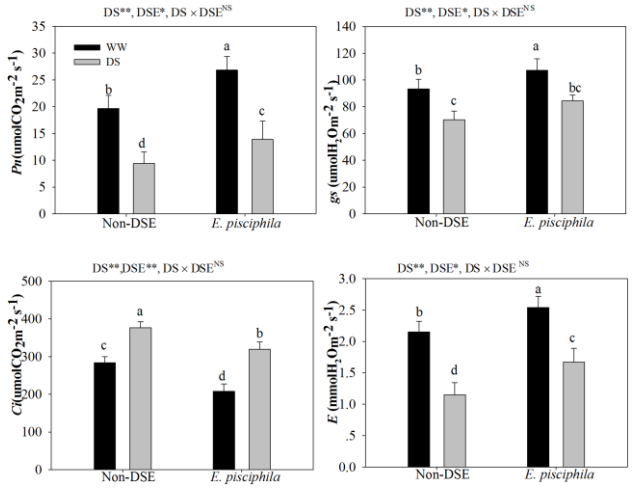

Fig. 1: Effects of *Exophiala pisciphila* on net photosynthetic rate (*Pn*), stomatal conductance (g_s), intercellular CO_2 concentration (*Ci*), and transpiration rate (*E*) in sorghum leaves under well-watered (WW) and drought-stressed (DS) conditions

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; Values are means ± SD, n = 5; *$P < 0.05$, **$P < 0.01$, NS not significant

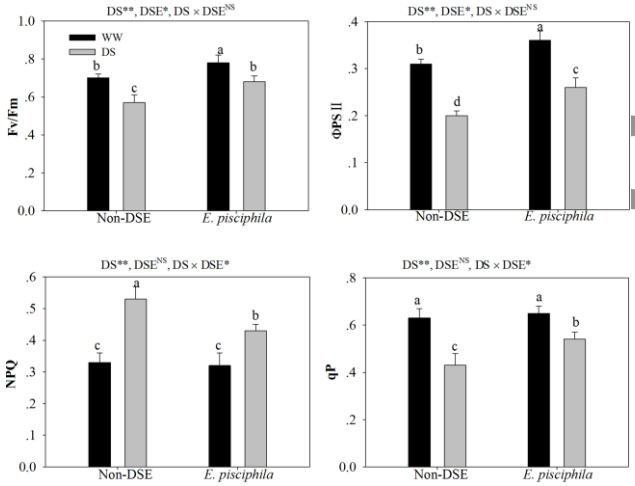

Fig. 2: Effects of *Exophiala pisciphila* on maximum quantum yield in the dark-adapted state (*Fv/Fm*), actual quantum yield in the light-adapted steady state (*ΦPSII*), nonphotochemical quenching values (*NPQ*), and photochemical quenching values (*qP*) in sorghum leaves under well-watered (WW) and drought-stressed (DS) conditions

The same letter in each column indicates no significant difference among treatments at $P < 0.05$ using Tukey's test; Values are means ± SD, n = 5; *$P < 0.05$, **$P < 0.01$, NS not significant

Debaud, 1987) and *Phialophora* sp. (Rommert *et al*., 2002), Andrade-Linares *et al*. (2011) also found that DSE indirectly improved hormone signal transduction and hormone-regulated gene expression in host plants. Other possible reason was that DSE fungi produced a wide range of enzymatic capabilities, which increased the efficient utilization of organic matter and other available nutrients for host plants (Caldwell *et al*., 2000).

In order to keep a water balance between availability and demand during soil drought, plants normally need to close stoma to lower leaf transpiration rate (*E*) and intercellular CO_2 concentration (*Ci*), and consequently decrease photosynthetic rate (*Pn*) in plant leaves (Gong *et al*., 2013). In this study, *E. pisciphila*-inoculated sorghum had higher *Pn*, *gs* and *E*, and lower *Ci*, compared with non-inoculated seedlings under both WW and DS conditions; the effects of *E. pisciphila* inoculation were more pronounced under DS conditions. It indicated that *E. pisciphila*-inoculation improved gas exchange and photosynthesis in sorghum leaves through keeping stomatal open and increasing transpiration fluxes. It also meaned that host plants may alleviate more environmental stress than non-inoculated plants, by maintaining higher water status and hence more opening stomata. Wu *et al*. (2009) showed that DSE could decompose photosynthate and prevente the feedback inhibition of photosynthate to photosynthesis in host plants. Likar and Regvar (2013) also revealed that DSE improved the general physiology of *Salix caprea* via increasing chlorophyll concentrations and transpiration rates under heavy metals stress.

The measurement of chlorophyll fluorescence is considered to be a fast, nondestructive and reliable technique for monitor the responses of of the photosynthetic apparatus (Bolhar-Nordenkampf *et al*., 1989; Souza *et al*., 2010). Light energy absorbed by chlorophyll molecules can be partly used to drive photosynthesis (photochemistry), but it can also be dissipated through chlorophyll fluorescence (nonphotochemistry) (Maxwell and Johnson, 2000; Gong *et al*., 2013). *E. pisciphila*-inoculation obviously increased *qP*, *Fv/Fm* and *ΦPS Ⅱ*, and decrease *NPQ* in sorghum leaves, compared with non-inoculation under DS conditions. *Fv/Fm*, a parameter of the potential quantum efficiency of PSII, indicates plant photosynthetic performance, when photosynthetic apparatus of plants under greenhouse conditions was subjected to photodamage or environmental stresses , this value was often below 0.80 (Bagheri *et al*., 2011). In this study, values of *Fv/Fm* below 0.800 were found in non-inoculation, but not in *E. pisciphila*-inocultaion under DS conditions. Our results implied that *E. pisciphila*-inocultaion could improve the efficiency of excitated energy capture by chloroplasts and the energy cycling among photosynthetic apparatus in sorghum leaves under drought stresses.

The colonization process by symbiotic fungi can cause a series of resistance reactions in host plants, for example stimulating secondary metabolite, such as phenols, alkaloid, terpenoid, and so on (Brundrett, 2002). Promoted synthesis of secondary metabolism in plants is one of important defense mechanisms some evidences also showed that secondary metabolisms in plants were associated with

DSE mutualistic association under drought stress (Nascimento and Fett-Neto, 2000). Wu *et al*. (2009) found that a DSE strain EF-37 enhanced the content of flavonoids in *Saussurea involucrata* seedlings. Consistently with previous report, our results showed that the contents of anthocyanin, phenols and flavonoids in sorghum grains and leaves were obviously inproved by *E. pisciphila* inoculation under drought stress.

Mycorrhizal symbioses improving drought tolerance of host plants are well known, although, DSE are common as mycorrhizas in terrestrial ecosystems, the DSE association under drought stress remains relatively understudied (Newsham, 2011). Our results suggested that *E. pisciphila* inoculation could improve the growth performance, photosynthetic capacity, secondary metabolism and enzymatic activities related to secondary metabolism in sorghum seedlings. As a result, DSE inoculation should be served as an effective measure in improving crop production under drought stress. To our knowledge, this study is the first report hitherto involving crop responses to DSE inoculation under drought conditions, and a more comprehensive study to research the mechanism of mutualistic association between DSE and crops under drought conditions is necessary.

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