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Full Length Article

Influences of Arbuscular Mycorrhizal Fungi and Dark Septate Endophytes on the Growth, Nutrition, Photosynthesis, and Antioxidant Physiology of Maize

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Abstract

A pot experiment was conducted to study the influences of single and dual inoculations with arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE) on maize performance. Results showed the single and dual inoculations with AMF and DSE significantly increased maize biomass and contents of nitrogen (N), phosphorus (P), potassium and glutathione; improved activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and reduced malondialdehyde (MDA) contents. Moreover, single DSE inoculation and dual inoculation with AMF and DSE improved leaf photosynthesis. Shoot biomass was significantly positively correlated with N content and SOD and CAT activity; root biomass was significantly positively correlated with P content and CAT and POD activity, while negatively correlated with MDA content. Thus the promotion of maize growth by AMF and DSE is closely related to their beneficial effects on mineral nutrition and antioxidant physiology. AMF and DSE exert a synergistic effect on the growth, P nutrition, and antioxidant physiology of maize. © 2019 Friends Science Publishers

Keywords: Fungal endophytes; Dual inoculation; Mineral nutrition; Antioxidant physiology; Synergistic effect

Introduction

Plant roots are generally colonized in different natural habitats with endophytic fungi (Rodriguez *et al*., 2009), including arbuscular mycorrhizal fungi (AMF) and dark septate endophytes (DSE). A field survey revealed that AMF and DSE share the same host and root regions (Massenssini *et al*., 2014). Seedlings of three types of pine and one hybrid spruce were found to be simultaneously colonized with AMF and DSE in an examination of their roots in a forest (Wagg *et al*., 2008). Based on an investigation on 50 weed species in different locations in Southern India, 44 weed species were colonized with AMF; of these, 25 were simultaneously colonized with AMF and DSE (Seerangan and Thangavelu, 2014).

As fungi extensively existing in soil, AMF can colonize the roots of most terrestrial plants to form a mutualistic symbiosis (Smith and Read, 2010), thereby generating positive and promoting effects on the growth, nutrient absorption, photosynthesis, and antioxidant physiology of the host plant (Smith and Smith, 2011). For example, AMF enhances the mineral nutrient absorption of host plants; increases mineral contents, including nitrogen (N), phosphorus (P) and potassium (K); improves the plant nutrient status (Hodge *et al*., 2010; Miransari, 2013; Zhao *et al*., 2015); increases the chlorophyll content and net photosynthetic rate of leaves; strengthens the photosynthesis of the host plant (Mathur *et al*., 2018; Zhang *et al*., 2018); increases the activities of antioxidant enzymes (Zhan *et al*., 2018), such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT); decreases malondialdehyde (MDA) content; relieves the harmful damage caused by reactive oxygen species (ROS) and lipid peroxidation; improves plant antioxidant capacity to promote growth; and increases plant biomass (Yang *et al*., 2015).

DSE are generally colonized in plant roots and present high colonization rates in various stress environments, such as drought and heavy metal pollution (Addy *et al*., 2005). DSE colonization in roots also promotes absorption of mineral elements by the host plant; increases the contents of N, P (Newsham, 2011; Berthelot *et al*., 2017; Vergara *et al*., 2017), antioxidant substances and photosynthetic pigments (Ban *et al*., 2017; He *et al*., 2017); improves the photosynthetic capacity and antioxidant enzyme activities of host plant leaves; strengthens the antioxidant physiology of their host (Wang *et al*., 2016; He *et al*., 2017) and increases plant growth and biomass (Li *et al*., 2011). Thus, DSE colonization usually improves plant stress resistance and promotes the growth of the host plant (Mandyam and Jumpponen, 2005; Newsham, 2011).

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Despite their many benefits, however, the above studies only consider the influence of single AMF or DSE inoculation on the growth and physiology of the host plant. Under natural conditions, AMF and DSE are simultaneously colonized in host plant roots, and their coexisting effects on physiology and ecology have attracted considerable research attention. For example, MDA concentration and chlorophyll content in leaves were significantly reduced after ryegrass was simultaneously colonized with AMF and DSE in heavy metal-polluted soil (Berthelot *et al*., 2018). Dual inoculation with AMF and DSE increased the P content and promoted the uptake and utilization of mineral nutrients in the host plant (Monica *et al*., 2015). Few studies are available on the coexistent ecological effects of AMF and DSE.

In the present research, maize was selected as a host plant, inoculated with single or dual strains of AMF and DSE, and then planted in a pot experiment. Non-inoculation was considered as the control, while single AMF, single DSE and dual inoculation with AMF and DSE were considered the treatments. Maize biomass, mineral nutrient contents, photosynthesis, and antioxidant physiology were determined to investigate the influences of AMF and DSE on maize performance and analyze the coexisting relationship between these fungi in plant roots. AMF and DSE are assumed to improve the mineral nutrition, photosynthesis, and antioxidant physiology of the host plant to promote plant growth.

Material and Methods

Materials

The test soil was red soil collected from a mountain (E 102° 75′ 76″, N 25° 13′ 95″) in Panlong District, Kunming City, Yunnan Province, China. This soil featured the following characteristics: pH 7.5; organic matter content of 13.87 $g \cdot kg^{-1}$; total N, P and K contents of 0.112, 0.32 and 1.98 $g \cdot kg^{-1}$, respectively; and alkaline hydrolyzable N, available P and available K contents of 26.18, 1.15 and 33.74 $mg \cdot kg^{-1}$, respectively. The soil was ground and passed through a 2 mm sieve after drying naturally in a room. River sand was also passed through a 2 mm sieve after drying naturally. The sand and soil were sufficient blended at a ratio of 3:1 (W/W), autoclaved at 121°C for 2 h and then used for the pot experiments.

The AMF inoculant used in the experiment was *Funneliformis mosseae* BGC YN05 and provided by the AMF Resources Bank of the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry. The AMF inoculant contained 60–70 spores per gram of material. The DSE strain used was *Exophiala pisciphila* and separated from *Arundinella bengalensis* roots naturally growing in Huize, a lead/zinc mining area in Yunnan Province (Zhang *et al*., 2008). The DSE strain was preserved in the laboratory, inoculated into potato sucrose agar (PDA) culture medium, and cultured under a constant temperature of 28°C for 2 weeks for activation.

The maize (*Zea mays* L.) variety used in the experiment was Huidan 4. Before seed sowing, full maize seeds with consistent sizes were selected for surface sterilization, placed under dark treatment, and then cultured for 3 d at a constant temperature of 28°C. When the seeds had germinated and a white portion of approximately 1 cm was exposed, sterile seedlings with uniform growth were selected for standby use.

Pot Experiment and Treatment

First, a maize–DSE consortium was established and placed in cylindrical glass bottles (height: 25 cm, diameter: 6.5 cm) into which 16.5 g of perlite and 20 mL of Hoagland nutrient solution were added. The bottles were sealed and autoclaved at 121°C for 30 min for standby use. A total of 15 DSE colonies with a diameter of 2 mm were placed in eight glass bottles and blended with perlite. Thereafter, two germinated maize seeds were implanted and covered with sterilized sand (thickness: 1 cm). The glass bottles were sealed using a sterilized polyvinyl alcohol membrane, and the maize seeds were cultured for 14 d under a temperature of 25°C with 1,000–8,000 lux light for 10 h per day. After 14 d, some maize seedlings were taken out and microscopic examination was performed to determine whether DSE was successfully colonized in the maize roots. Eight other glass bottles were not inoculated with DSE strains, and the growing maize seedlings were not colonized with DSE.

The pot experiment was performed using pots (height: 20 cm, diameter: 25 cm) disinfected with 75% ethyl alcohol and filled with 5.0 kg of sterilized sand–soil mixture. Four treatments were used in the experiment, namely, CK (non-inoculation), DSE (single DSE inoculation), AMF (single AMF inoculation), and AMF+DSE (dual inoculation with AMF and DSE). For the CK treatment, two maize seedlings with non-colonization and 50 g of dead AMF inoculants were planted in a pot. In the AMF treatment, two maize seedlings without DSE colonization were planted with 50 g of AMF inoculants in a pot. Two maize seedlings with DSE colonization and 50 g of dead AMF inoculants were planted for the DSE treatment. Two maize seedlings with DSE colonization and 50 g of AMF inoculants were planted for the AMF+DSE treatment. Each treatment was repeated for four times. After planting of the maize seedlings, 150 mL of 50% Hoagland nutrient solution was used for watering every 3 d, and a weighing-based moisturizing method was applied to maintain a constant water content in the sand–soil mixture.

Determination of Maize Growth and Related Physiology

Height was determined 60 d after transplanting the maize plants. The maize was then harvested and divided into shoots and roots, which were cleaned using distilled water and oven-dried at 75°C for 48 h to determine dry biomass.

Before the maize harvest, an LCA-4 photosynthetic tester (Analytical Development Company Limited, ADC, Hoddesdon, England) determined the photosynthetic physiology of the maize leaves on a sunny morning. The first completely unfolded leaf was selected from the top to the bottom of the maize plant to determine its net photosynthetic rate, intercellular carbon dioxide (CO_2) concentration, transpiration rate and stomatal conductance. Fresh maize leaves (0.5 g) were sampled and ground into a homogenate in a precooled mortar. The sample homogenate was transferred to a centrifugal tube with 0.9% normal saline (10 mL) and centrifuged at a temperature of 4°C and 10,000 × *g* for 20 min. The supernatant was subjected to the corresponding assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) to determine MDA contents; activities of antioxidant enzymes, including SOD, POD and CAT and contents of reduced glutathione (GSH).

Dry maize shoots and roots were crushed. About 0.1 g of the samples was weighed and placed in a digestive tube. The samples were heated for digesting using a concentrated mixture of sulfuric acid–hydrogen peroxide. Digestion was used to determine the contents of N, P, and K by using the Kjeldahl, molybdenum antimony colorimetry and flame photometry methods, respectively (Bao, 2000).

Determination of AMF Colonization in Maize Roots

After the maize was harvested, the soil around the maize roots was collected to determine the number of AMF spores. Wet sieving, decantation and saccharose centrifugation separated the AMF spores from the soil, and the spore number was calculated under a stereomicroscope (Daniels, 1982).

Maize roots were collected randomly and dissociated using 10% (W/V) potassium hydrate (KOH) solution in thermostatic water at 90°C for 1 h. The roots were washed using distilled water with several drops of lactic acid to neutralize the residual KOH and cut into fragments at 0.5–1 cm. A total of 20 fragments were selected and placed on a glass slide for dyeing with blue ink. Then, the root samples were decolored and observed under a microscope (McGonigle *et al*., 1990). The crossing method was used to calculate the infection rates of AMF and DSE.

Statistical Analysis of Data

The test data comprise the mean of four repetitions and are expressed as mean ± standard deviation. Statistical product and service solutions (S.P.S.S.) 22.0 statistical software (S.P.S.S. Inc., Chicago, I.L., U.S.A.) was used to test significant differences at the 0.05 level by the least-significant difference method. The influences of AMF and DSE on the growth and physiology of maize, as well as their interaction, were analyzed via two-way analysis of variance (ANOVA).

Results

AMF and DSE Colonization in Maize Roots

After single inoculation with AMF or DSE and dual inoculation with AMF and DSE, AMF or DSE colonization in maize roots was observed, thus indicating that AMF and DSE successfully infected the maize roots. The number of AMF spores was 17.0 and 14.5 per gram soil and mycorrhizal infection rates were 35% and 25% in the AMF and AMF+DSE treatments, respectively. No significant difference between treatments was found. However, a significantly higher DSE infection rate (38%) was observed after single DSE inoculation than after dual inoculation with AMF and DSE (16%).

Influences of AMF and DSE on Maize Growth

After transplantation for 60 d, the two-way ANOVA demonstrated that AMF and DSE exert a very significant effect on maize height and a significant effect on shoot biomass. A significant positive interaction was determined between AMF and DSE in terms of maize height and biomass (Table 1).

Maize heights in the single AMF and single DSE inoculation treatments were significantly higher than that in the control. Single inoculation with AMF or DSE and dual inoculations with AMF and DSE significantly increased shoot biomass by 54%–59%. In addition, single AMF and single DSE inoculations significantly increased root biomasses by 54% and 45%, respectively. Thus, single AMF, single DSE, and dual AMF and DSE inoculations promoted maize growth (Fig. 1).

Influences of AMF and DSE on the Photosynthetic Physiology of Maize Leaves

The results of two-way ANOVA indicated that AMF has a significant influence on intercellular CO_2 concentration, while DSE presents significant or very significant influences on the photosynthetic and transpiration rates and intercellular CO_2 concentration of maize leaves. However, no interaction was found between DSE and AMF in terms of influencing the photosynthetic physiology of maize leaves (Table 1).

However, single DSE and dual inoculations with AMF and DSE significantly elevated the photosynthetic and transpiration rates of maize leaves. Single DSE inoculation significantly increased the stomatal conductance and dual inoculation with AMF with DSE significantly reduced the intercellular CO_2 concentration of maize leaves (Fig. 2).

Influences of AMF and DSE on the Mineral Nutrition of Maize

The results of two-way ANOVA indicated that AMF exerts

Table 1: Results of two-way ANOVA between AMF and DSE inoculation on the height, biomass and photosynthesis physiology of maize

Factor	F-value and level of significance						
	Height	Shoots biomass	Roots biomass	Photosynthetic rate	Transpiration rate	Stomatal conductance	Intercellular CO_2 concentration
AMF	14.20^{**}	6.83^{*}	0.99^{NS}	2.73^{NS}	2.89^{NS}	0.07^{NS}	5.25^{*}
DSE	22.19^{**}	5.33^{*}	0.02^{NS}	24.66^{**}	10.54^{**}	4.86^{*}	5.68^{*}
AMF×DSE	59.68^{**}	4.86^{*}	16.97^{**}	0.82^{NS}	0.13^{NS}	3.87^{NS}	0.65^{NS}

**, $P < 0.01$; *, $P < 0.05$; NS: not significant

Table 2: Results of two-way ANOVA between AMF and DSE inoculation on the nutrients content of maize

Factor	F-value and level of significance					
	Shoots			Roots		
	N content	P content	K content	N content	P content	K content
AMF	9.77^{**}	111.96^{**}	0.22^{NS}	0.28^{NS}	107.46^{**}	20.86^{**}
DSE	8.74^{*}	1.06^{NS}	28.27^{**}	0.14^{NS}	55.55^{**}	13.64^{**}
AMF×DSE	4.52^{NS}	9.01^{*}	0.59^{NS}	0.42^{NS}	83.67^{**}	0.46^{NS}

**, $P < 0.01$; *, $P < 0.05$; NS: not significant

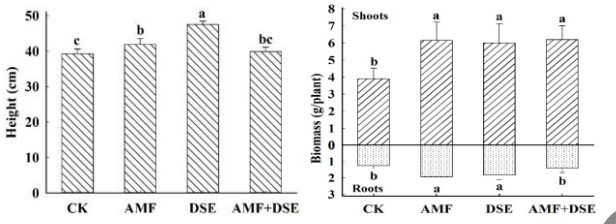

Fig. 1: Effects of AMF and DSE on height and biomass of maize. CK: the control of non-inoculation, DSE: single inoculation of *Exophiala pisciphila*, AMF: single inoculation of *Funneliformis mosseae*; AMF+DSE: co-inoculation of *Funneliformis mosseae* and *Exophiala pisciphila*

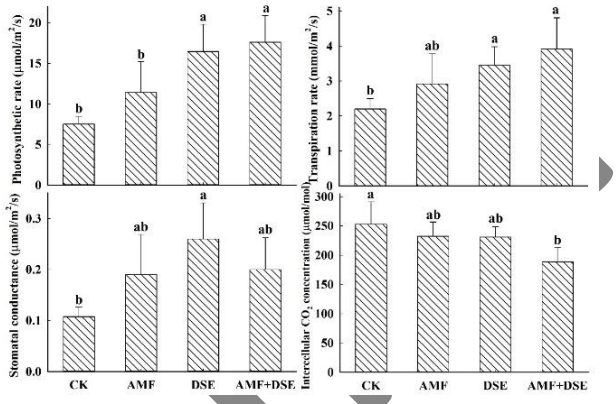

Fig. 2: Effects of AMF and DSE on photosynthesis of maize leaves. CK: the control of non-inoculation, DSE: single inoculation of *Exophiala pisciphila*, AMF: single inoculation of *Funneliformis mosseae*; AMF+DSE: co-inoculation of *Funneliformis mosseae* and *Exophiala pisciphila*

very significant influences on the contents of N and P in shoots and contents of P and K in roots. DSE, by comparison, exerted significant or very significant influences on the contents of N and K in shoots and contents of P and K in roots. Significant or very significant positive interactions existed between AMF and DSE in terms of influencing the P content in maize (Table 2).

Single AMF inoculation significantly increased the

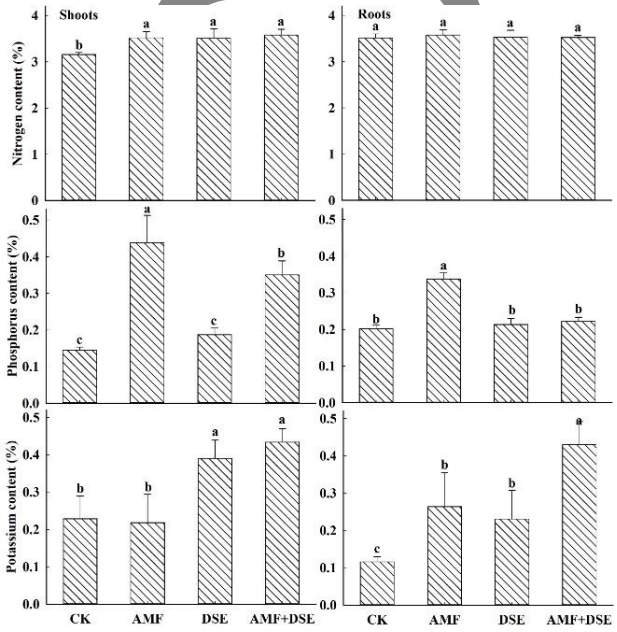

Fig. 3: Effects of AMF and DSE on nutrients contents of maize. CK: the control of non-inoculation, DSE: single inoculation of *Exophiala pisciphila*, AMF: single inoculation of *Funneliformis mosseae*; AMF+DSE: co-inoculation of *Funneliformis mosseae* and *Exophiala pisciphila*

contents of N and P in the shoots, and contents of P and K in the roots, while single DSE inoculation significantly increased contents N and K in the shoots. Dual inoculation with AMF and DSE increased contents of N, P and K in shoots and K content in roots (Fig. 3). Thus, single or dual inoculation with AMF and DSE improved the mineral nutrition of maize plants.

Influences of AMF and DSE on Antioxidant Physiology in Maize

The results of two-way ANOVA revealed that AMF exerts very significant influences on MDA content in the maize

Table 3: Effects and two-way ANOVA results of AMF and DSE on antioxidant physiology of maize

Treatment	Leaves					Roots				
	MDA content (nmol/mg protein)	SOD activity (U/mg protein)	POD activity (U/mg protein)	CAT activity (U/mg protein)	GSH content (mg/g protein)	MDA content (nmol/mg protein)	SOD activity (U/mg protein)	POD activity (U/mg protein)	CAT activity (U/mg protein)	GSH content (mg/g protein)
CK	5.78 ± 2.73 a	4.6 ± 1.3 c	318.1 ± 17.0 c	11.6 ± 3.2 c	47.7 ± 8.3 c	19.07 ± 5.21 a	18.3 ± 8.3 a	334.7 ± 19.4 c	21.8 ± 7.7 c	44.6 ± 8.1 b
AMF	2.37 ± 0.98 b	10.9±1.8 ab	693.3 ± 31.7 a	68.8 ± 5.5 b	66.8 ± 8.9 b	4.80 ± 2.46 b	18.9 ± 2.9 a	382.7 ± 20.3 b	46.8 ± 7.4 b	58.7 ± 8.6 a
DSE	5.00 ±1.83 ab	14.5 ± 4.5 a	404.1 ± 39.4 b	135.4 ± 31.5 a	107.7 ± 10.8 a	6.65 ± 1.91 b	14.6 ± 7.0 a	429.6 ± 35.3 a	70.1±1.8 a	59.5 ± 6.1 a
AMF+DSE	2.66 ± 0.99 b	9.9 ± 1.6 b	433.1 ± 51.7 b	60.7 ± 20.6 b	69.9 ± 8.8b	5.66 ± 2.46 b	21.6 ± 1.9 a	348.8±11.8 bc	39.6±4.8 b	57.3 ± 7.7 a
ANOVA (F-value and level of significance)										
AMF	10.38^{**}	0.44^{NS}	118.42^{**}	0.83^{NS}	4.09^{NS}	21.73^{**}	1.81^{NS}	1.99^{NS}	0.86^{NS}	2.41^{NS}
DSE	0.08^{NS}	11.73^{**}	22.00^{**}	36.80^{**}	46.21^{**}	12.45^{**}	0.03^{NS}	6.85^{*}	47.78^{**}	3.07^{NS}
AMF×DSE	0.35^{NS}	17.09^{**}	86.88^{**}	47.71^{**}	37.69^{**}	16.44^{**}	1.30^{NS}	30.57^{**}	87.44^{**}	4.49^{NS}

CK: the control of non-inoculation, DSE: single inoculation of *E. pisciphila*, AMF: single inoculation of *F. mosseae*; AMF+DSE: co-inoculation of *F. mosseae* and *E. pisciphila*. Means followed by the same letter do not differ significantly at $P < 0.05$ by LSD test. **, $P < 0.01$; *, $P < 0.05$; NS: not significant

Table 4: Correlation coefficients between biomass with photosynthesis, mineral nutrition and antioxidant physiology of maize

	Photosynthetic rate	Transpiration rate	Stomatal conductance	Intercellular CO_2 concentration	N content	P content	K content	MDA content	SOD content	POD content	CAT content	GSH content
Shoots biomass	0.438	0.410	0.382	-0.326	0.946^{**}	0.474	0.445	-0.288	0.521^{*}	0.440	0.599^{*}	0.410
Roots biomass	0.308	0.370	0.563^{*}	0.119	0.017	0.530^{*}	0.246	-0.519^{*}	-0.055	0.522^{*}	0.633^{**}	0.288

"*" and "**" means $P < 0.05$ and $P < 0.01$, respectively

plant and POD activity in leaves. DSE showed significant or very significant influences on the activities of the three antioxidant enzymes and GSH content in leaves, as well as MDA content and activities of POD and CAT in roots. Very significant positive interactions were observed between AMF and DSE in terms of influencing the activities of POD and CAT in leaves and roots and SOD activity and GSH and MDA contents in leaves (Table 3).

Single or dual inoculation with AMF and DSE significantly improved the activities of SOD and POD in maize leaves and CAT activity in roots and leaves, increased GSH content in leaves and roots and reduced MDA content in roots. In addition, single inoculation with AMF or DSE significantly improved POD activity in roots. These results indicate that single or dual inoculation with AMF and DSE improves the antioxidant physiology of maize (Table 3).

Correlation Analysis

Shoot biomass had a very significant positive correlation with N content and significant positive correlations with the activities of SOD and CAT in shoots. Root biomass presented a very significant positive correlation with CAT activity, significant positive correlations with P content and POD activity, and a significant negative correlation with MDA content in roots (Table 4). Thus, the ability of AMF and DSE to promote maize growth is related to their effects on improving plant mineral nutrition and antioxidant physiology.

Discussion

AMF can promote plant growth by improving various aspects of plant physiology, such as mineral nutrition and photosynthesis. In the present study, AMF inoculation significantly increased maize biomass and contents of N, P and K. The AMF mycelia played an important role in nutrients acquisition for the host plant. AMF grown in the soil and extended considerable amounts of extraradical mycelia into it (Krishnakumar *et al.*, 2013). Extraradical mycelia improved the physiological properties of the soil in the rhizosphere of the host plant and absorbed and transported mineral nutrients from the soil to the host plant, thereby enhancing the absorption of mineral nutrients (Miransari, 2013). Many studies confirmed that AMF acquired nutrients, including N and P, in different forms from the soil through extraradical mycelia, transported the nutrients to the host plant, and increased its contents of N and P (Leigh *et al*., 2009; Behie and Bidochka, 2014; Bagyaraj *et al*., 2015). In addition, AMF mycelia increased the magnesium uptake of maize roots, increased the chlorophyll content in leaves and significantly improved the photosynthetic capacity of maize leaves (Mathur *et al*., 2018).

DSE had an ecological function similar to that of AMF. It can generally improve the mineral nutrition, photosynthetic physiology, and growth of the host plant (Newsham, 2011). In the present study, DSE inoculation led to a significant increase on the maize biomass and contents of N, P and K. DSE stretched viscid mycelia into the soil from the plant roots and helped the plant absorb nutrients from the soil (Addy *et al.*, 2005). For instance, inoculation with two DSE strains hydrolyzed protein and ribonucleic acid as N and P sources, thereby contributing to the absorption and uptake of nutrients by the host plant (Caldwell *et al*., 2000). DSE inoculation of *Phialocephala fortinii* significantly increased the contents of N and P and promoted the growth and biomass of the host plant (Newsham, 2011). DSE inoculation and colonization in maize roots improved photosynthesis in leaves and promoted growth and plant biomass of maize

(He *et al*., 2017). In brief, the present experiment also confirmed that AMF and DSE improved the mineral nutrition and growth of maize. While DSE inoculation improved the photosynthetic physiology of maize leaves, AMF inoculation exerted an insignificant influence on the photosynthetic physiology of the plant.

In addition, the single and dual inoculations with AMF and DSE reduced the MDA contents with an increase on the activities of antioxidant enzymes (SOD, POD and CAT). MDA is an important marker for judging whether a plant was subjected to oxidative stress and damage during the growth process, and its content reflect the extent of oxidative stress caused to the plant (Demidchik, 2015). Antioxidant enzymes constitute an important component of the antioxidant system to scavenge ROS and reduce MDA content in the plant (Sharma *et al*., 2012). SOD, POD, and CAT are the main enzymes for scavenging ROS, and their activities reflect the antioxidant capacity of the plant (Das and Roychoudhury, 2014). Non-enzymatic antioxidant substances include GSH, ascorbic acid, and carotenoids (Gill and Tuteja, 2010).

Similar to the results of this experiment, AMF and DSE colonized in the plant roots usually strengthened the antioxidant physiology of their host plant. AMF inoculation improved the activities of SOD, POD and CAT in leaves, alleviated membrane lipid peroxidation and reduced MDA contents in leaves (Zhu *et al*., 2010; Latef and He, 2011; Zhan *et al*., 2018). DSE mycelia contain a large quantity of melanin, which had a strong antioxidant activity (Zhan *et al*., 2011). Thus, DSE colonization in the plant roots improved antioxidant capacity, reduces ROS generation and alleviated injuries of epidermic cells in the roots of the host plant (Santos *et al*., 2017). DSE inoculation of *Phialophora mustea* increased the activities of SOD and POD and reduced the MDA content in host plant leaves (Zhang *et al*., 2017). Therefore, AMF and DSE improved the antioxidant physiology of their host plants and relieved the damage caused by oxidative stress (Nath *et al*., 2016). Inoculation with AMF and DSE strengthened the antioxidant physiology to varying degrees and reduced MDA content in maize roots.

Furthermore, there were significant positive correlation between shoot biomass with N content and SOD and CAT activity, root biomass with P content and CAT and POD activity in the present experiment. AMF alleviated *Tagetes erecta* L. from Cd stress by improving the activities of SOD, POD and CAT which had a great influence on the host plant biomass (Liu *et al*., 2011). AMF alleviated Cd toxicity of *Lonicera japonica* through the improvement of P nutrition, activities of antioxidant enzymes (glutathione reductase (GR), ascorbate peroxidase (APX) and CAT) and then improved plant biomass (Jiang *et al*., 2016). Similarly, DSE increased the plant growth and Cd tolerance by triggering antioxidant systems with increases on the activities of SOD, CAT and POD and glutathione (GSH) contents (Wang *et al*., 2016; Zhu *et al*., 2018). These results supported that AMF and DSE alleviated the host plant from Cd stress by improving the antioxidant enzymes activity and nutrients content.

Nevertheless, the relationship was complicated between the AMF and DSE in the plant roots. Field surveys have indicated that AMF and DSE simultaneously colonized in the roots of wild plants, presenting collaborative, competitive, or neural relationships. For instance, AMF promoted transportation of phosphoric substances from the rhizosphere to the host plant, while DSE promoted mineralization of organic P in plant roots (Monica *et al*., 2015). As a result of their collaborative relationships, dual inoculation with AMF and DSE significantly increased the P content and relieved oxidative stress in the host plant and significantly reduced MDA content in the leaves (Berthelot *et al*., 2018). However, some studies found that coexistence of AMF and DSE in the roots of sorghum did not present a collaborative effect (Priyadharsini *et al*., 2012). In fact, DSE mycelial secreta had been observed to generate a certain negative influence on the early symbiosis of AMF mycelia (Scervino *et al*., 2009). In the present experiment, AMF and DSE presented a positive interaction and collaborative effect on the growth, P nutrition, and antioxidant physiology of maize according to the results of two-way ANOVA. But the interaction between AMF and DSE can be complicated by various strains, host plant species and environmental conditions. Thus, more studies on this topic need to be performed.

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