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RESEARCH ARTICLE



Effects of a root-colonized dark septate endophyte on the glutathione metabolism in maize plants under cadmium stress

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ABSTRACT

A high Cd-tolerant dark septate endophyte (DSE), *Exophiala pisciphila*, was inoculated into maize (*Zea mays* L.) roots under Cd stress. The Cd content, enzymes activity and thiol compound content relevant to glutathione (GSH) metabolism in maize leaves were analyzed. The Cd content in maize shoots increased with increasing Cd stress, but the DSE significantly reduced the Cd content at the 40 mg/kg Cd treatment. Cd stress increased the enzyme activity of glutathione reductase (GR), glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px) as well as the thiol compound contents of sulfur, thiols (-SH) and oxidized glutathione (GSSG). The content of reduced GSH and the GSH/GSSG ratio reached a peak at the 5 mg/kg Cd treatment but then decreased with increasing Cd stress. Furthermore, the DSE significantly enhanced the GR and GSH-Px activity and increased the contents of -SH and GSH under low Cd stress (5 and 10 mg/kg), but decreased the γ -glutamylcysteine synthetase and GST activity under high Cd stress (20 and 40 mg/kg). Highly positive correlations between the Cd content with enzymes activity and enzymes activity with thiol compound content were observed. Results indicated that DSE played a role in activating GSH metabolism in maize leaves under Cd stress.

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KEYWORDS

Plant–endophyte interaction; enzyme activity; thiol compound content; abiotic stress; stress resistance physiology

Introduction

Cadmium (Cd) is one of the most toxic heavy metals in the environment. Due to human-driven production activities such as mining and smelting (Robson et al. 2014), fertilization and wastewater irrigation, increasing amounts of Cd has entered arable soils (Atafar et al. 2010; Chen et al. 2013). In particular, soil Cd pollution is always severe in farmland around mining and smelting areas (Li et al. 2014). Therefore, plants grown on polluted farmland absorb and accumulate abundant amounts of Cd in the plants (Nannoni et al. 2016). How these plants tolerate Cd toxicity has garnered much attention.

Crops have adapted molecular, physiological and biochemical mechanisms to tolerate Cd toxicity (Gallego et al. 2012). Among the Cd tolerance mechanisms, glutathione (GSH, γ -glutamate-cysteine-glycine) metabolism and its involved thiol compound have been regarded to play a key role in alleviating Cd toxicity (Sobrino-Plata et al. 2014; Hernández et al. 2015). Cd stress induces an increase in sulfate uptake depends on the enhanced expression of a high-affinity sulfate transporter (HAST) (Nocito et al. 2002). Sulfate leads to cysteine (Cys) biosynthesis by the sulfur assimilation pathway in plants. In addition, cysteine can then bond with γ -glutamate to form a peptide, catalyzed by γ -glutamylcysteine synthetase (γ -GCS), and the subsequent addition of glycine by glutathione synthetase results in the synthesis of the tripeptide GSH, which occurs by two ATP-dependent reactions (Mendoza-Cózatl et al. 2005).

GSH plays a multifunctional role in plants exposed to Cd stress. GSH not only is an important chelating compound in Cd homeostasis due to its thiol (-SH) group with a high affinity (Na and salt 2011) but also is an effective antioxidant

against Cd-induced reactive oxygen species (ROS) (Jozefczak et al. 2012; Jozefczak et al. 2014). More importantly, GSH is a major precursor of thiol compounds transformed by enzymes such as glutathione S-transferases (GST), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) (Nocito et al. 2012). For instance, GST governs the conjugation of GSH with Cd ions to yield an inactive, less toxic complex (Adamis et al. 2004). In addition, both GST and GSH-Px consume GSH directly as a reducing agent for the scavenging of H_2O_2 and organic peroxides (Anjum et al. 2012). GR converts oxidized glutathione (GSSG) to reduced GSH, thus helping to maintain a high ratio of GSH/GSSG under Cd stress (Gill et al. 2013).

In natural environments, there are many endophytes that colonize plant roots (Rodríguez et al. 2009). Root endophytes always have a beneficial relation with the host plant and increase host fitness by conferring abiotic stress tolerance to heavy metal pollution conditions (Deng and Cao 2017). Under Cd stress, endophytes can also enhance plant fitness and alleviate Cd toxicity by regulating GSH metabolism and thiol compound contents in the host plant. For instance, the endophyte *Sphingomonas* SaMR12 up-regulates the expression of GSH synthesis-relevant genes (*ATPS*, *GS* and *GSH1*), increases GSH concentrations and subsequently enhances both Cd tolerance and accumulation (Pan et al. 2016). Similarly, the AM fungus *Funneliformis mosseae* decreases leaf Cd contents with increasing GSH levels in tobacco (Degola et al. 2015).

Dark septate endophytes (DSEs) are a diverse group of ascomycetes in plant roots with dematiaceous and septate fungal hyphae (Jumpponen and Trappe 1998). In particular, DSEs are ubiquitous colonizers of plant roots in Cd-

polluted habitats (Zhang et al. 2013; Liu et al. 2017) and help to enhance Cd tolerance by improving the growth and physiology of the host plant by increasing the chlorophyll level, transpiration rate and photosynthesis (Likar and Regvar 2013; He et al. 2017); decreasing lipid peroxidation; and enhancing chlorophyll, potassium and phosphorus concentrations in birch shoots (Li et al. 2011; Likar and Regvar 2013; Berthelot et al. 2017). Also, DSE inoculation significantly enhances the activities of antioxidant enzymes and antioxidants, up-regulates the expression of Cd tolerance-relevant genes (ZIP, PCS and MTP) and promotes host plant growth (Wang et al. 2016). However, few studies have been conducted to understand the influence of DSEs on host plant physiology under Cd stress.

In the present study, the objective was to assess the effects of a DSE fungus (*Exophiala pisciphila*) on GSH metabolism in the maize leaves under Cd (0, 5, 10, 20 and 40 mg/kg) stress. We hypothesized that DSE colonization would alter the physiological changes in GSH metabolism and would benefit the enhancement of host plant tolerance to Cd stress.

Materials and methods

DSE strain and cultivation

The DSE fungus *E. pisciphila* was isolated from the roots of *Arundinella bengalensis* (Poaceae) naturally growing at a former mine smelting site in Huize County, Yunnan Province, Southwest China (103°36'E, 26°55'N) and preserved at the Agricultural Culture Collection Center of China (accession number ACCC32496). The *E. pisciphila* was highly tolerant to Cd stress, and its EC₅₀ values to Cd were 332.2 mg/L on solid medium and 111.2 mg/L in liquid medium, respectively (Zhan et al. 2015). The fungus was maintained on potato glucose agar (PDA) slants via subculture every 2 months and stored at 4°C in a refrigerator.

Preparation of DSE-inoculated and DSE-non-inoculated maize seedlings

A local maize cultivar (Huidan No. 4) in Yunnan province, China, was selected as the host plant. Maize seeds were first immersed in 75% alcohol (10 min) and then in 10% sodium hypochlorite (10 min) for surface sterilization, after which the seeds were washed in sterile water (3 times). The surface-sterilized seeds were kept in a petri dish (150 mm) at 25°C for 3 days for germination.

A glass bottle (Φ60 × 200 mm) containing 300 g of quartz sand and 25 mL of Hoagland's solution was autoclaved at 121°C for 25 min. For the DSE-inoculated treatment, 10 DSE colonies (diameter of 0.6 cm) cultured for 14 days on PDA media and 2 germinated maize seeds were simultaneously transferred to the glass bottle. During maize seedling growth, the maize roots grew and attached to the DSE colony, after which the DSE mycelia infected the maize roots. For the non-inoculated treatment, 10 autoclaved fungal disks were used instead of 10 DSE colonies. The maize seedlings grew in the glass bottles in an artificial light incubator at 25°C for 2 weeks, after which the seedlings were ready for subsequent greenhouse pot cultivation.

Greenhouse pot cultivation

Autoclaved river sand containing Cd at different concentrations (0, 5, 10, 20 and 40 mg/kg) was used as potting media. The media (5 kg) were filled into plastic pots (25 cm diameter × 20 cm height), and two maize seedlings were planted per pot. Four replicates per treatment and a total of 32 pots for the 8 treatments were prepared. All treatment pots were placed in a glasshouse at 15–28°C. Deionized water was used to irrigate the maize seedlings at a rate of 100 mL/pot every 5 days until the plants were harvested.

Measurement of fungal colonization in maize roots

The maize plants were harvested 60 days after being transplanted. Fine maize roots were randomly sampled from each pot, cleaned in water, softened in 10% (w/v) KOH in a water bath at 90°C for 2 h and then stained with 0.5% acid fuchsin (Berch and Kendrick 1982). The stained roots were cut into root fragments (0.5 cm). Ten root fragments were pressed onto slides and observed under a compound light microscope (Olympus-BX51) equipped with a 40× eyepiece. More than 300 intersections were observed to determine the fungal colonization intensity, which is the percentage of DSE mycelia at all intersections, using the magnified intersection method (McGonigle et al. 1990).

Measurement of Cd and sulfur content in maize shoots

After harvest, a dry subsample (0.50 g) of maize shoots was digested using a mixed digestive solution of HNO₃/HClO₄ (3:1) (v/v) at 200–250°C to obtain a transparent solution and then diluted into a volumetric flask (50 mL) using 0.2% HNO₃. The Cd concentration was determined using a flame atomic absorption spectrometer (TAS-990, Beijing Puxi Instrument Factory, Beijing, China). The total concentrations of S were determined using barium sulfate turbidimetry.

Measurement of enzyme activity involved in GSH metabolism

A fresh subsample (0.50 g) of maize leaves was homogenized with quartz sand in ice-cold saline water at 4°C in a prechilled mortar. The homogenate was centrifuged to obtain a supernatant at 4000 × g for 10 min at 4°C. The supernatant (50 μL) was used to determine the total soluble protein content by the absorbance of the reaction at 595 nm using a spectrophotometer (722 s model, Shanghai Precision & Scientific Instrument Co., Ltd, China) according to an assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Bovine serum albumin (BSA) was used as the standard (0.563 g/L), and double-distilled water was used as the blank. The concentration of soluble protein (g/L) was calculated using the following formula $C_{\text{protein}} = (A_{\text{test}} - A_{\text{blank}}) / (A_{\text{standard}} - A_{\text{blank}}) \times 0.563 \text{ g/L}$.

The homogenate was centrifuged at 10,000 × g at 4°C for 20 min to obtain a supernatant. The supernatant was then used to determine spectrophotometrically using assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's protocols. The γ-GCS detection kit was designed using principles described by Seelig and Meister (1985). NADH oxidation was evaluated

by following the decrease in absorbance at 340 nm at 37°C. One unit of γ -GCS activity was defined as the amount of enzyme required for the consumption of 1 μ mol of NADH per minute. The GR detection kit was designed using principles described by Foster and Hess (1980). NADPH oxidation was evaluated by measuring the decrease in absorbance at 340 nm at 37°C. One unit of GR activity equals 1 nmol of NADPH oxidized per minute.

The GST detection kits were designed using principles described by Habig et al. (1974). The conjugate formation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) was monitored by the change in absorbance at 412 nm. One unit of GST activity was defined as the amount that catalyzes the conjugation of 1 mmol/L GSH with CDNB per minute per milligram of protein. The GSH-Px detection kits were designed using principles described by Hafeman et al. (1974). The GSH-Px degraded H_2O_2 in the presence of GSH accompanying a GSH content decrease. Then the remained GSH was measured according to the specific reaction with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) by the absorbance at 412 nm. One unit of GSH-Px activity was defined as the decrease in GSH content per minute, and a correction was made for the non-enzymatic reactions. The activity of γ -GCS, GR, GST and GSH-Px was expressed as units per milligram of protein.

Measurement of thiol compound contents in maize leaves

The supernatant of a reaction mixture was used to spectrophotometrically determine the content of total sulfhydryl (-SH) contents using the assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The -SH detection kit was designed using principles described by Sedlak and Lindsay (1968). The -SH levels were spectrophotometrically measured based on the reduction of DTNB (also known as Ellman's reagent) to 2-nitro-5-thiobenzoate anion (NTP) at 412 nm.

The GSH and GSSG kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), designed using enzymatic recycling method described by Rahman et al. (2006), were used to determine the GSH and GSSG contents in maize leaves. Briefly, the leaves (0.2 g) were homogenized in 5% metaphosphoric acid. Then the homogenate was centrifuged at 3500 revolutions per minute for 10 min at 4°C to obtain a supernatant. For the total glutathione (T-GSH) assay, the enzymatic recycling method as described by Rahman et al. (2006) with DTNB, NADPH and GR was employed. The T-GSH content was quantified by spectrophotometer at 412 nm. For the GSSG measurement, the supernatant was first treated with 2-vinylpyridine to eliminate the GSH. Then the remaining GSSG was quantified in the reaction as the T-GSH assay. The GSH content was calculated by subtracting the GSSG content from the T-GSH content. The contents of -SH, GSH and GSSG were calculated according to the formula in the assay kit protocols and expressed as micromoles per gram of FW. The GSH to GSSG ratio was calculated.

Statistical analysis

All the data are presented as the means of four replicated measurements. The results are expressed as the means \pm

standard deviations (SDs). Duncan's new multiple range test at the .05 probability level was used to detect differences between treatments using the SPSS 22.0 statistical software package (SPSS Inc, Chicago, IL, USA). Two-way ANOVA was used to evaluate the effects of the DSE inoculation, Cd stress and their interactions. Pearson's correlation coefficient analysis of the mean data was applied to determine the relationships between GSH metabolic enzyme activity and the contents of Cd, sulfur and thiol compounds.

Results

DSE colonization

After checking the morphology of the maize roots, we did not observe DSE colonization in non-inoculated treatments. In DSE-inoculated treatments, some typical structures (hyphae and melanized microsclerotia) of DSE colonization were observed (Figure 1). These observations indicated a successful DSE colonization in the maize roots of inoculated treatments.

Cd content in maize shoots

The increased Cd stress in the media resulted in an increase in Cd content in the maize shoots. However, the DSE inoculation induced a significant decrease in Cd content in the maize shoots by 25.1% in the 40 mg/kg Cd treatment compared with the non-inoculated treatment (Figure 2).

Enzyme activity involved in GSH metabolism

There were increases in the activity of GR, GST and GSH-Px induced by Cd stress; an increase in γ -GCS activity in the 20 mg/kg Cd treatment; and a decrease in the γ -GCS activity in the 40 mg/kg Cd treatment. Furthermore, DSE colonization induced significant increases both in GR activity in the 5, 10 and 20 mg/kg Cd treatments and in GSH-Px activity in the 10 mg/kg Cd treatment compared with the non-inoculated treatment, but DSE colonization induced significant decreases in the γ -GCS and GST activity in the 20 and 40 mg/kg Cd treatments (Figure 3).

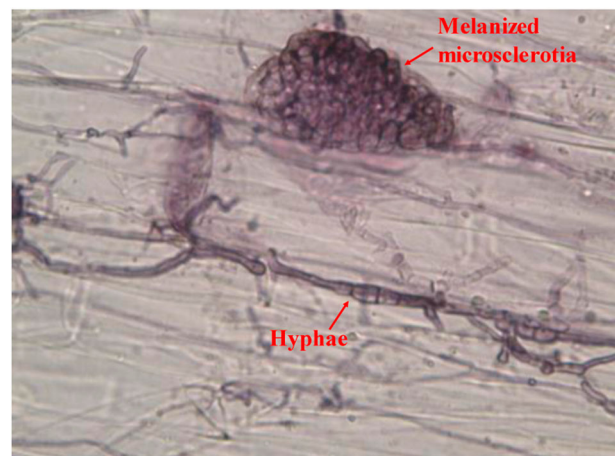


Figure 1. The morphology of DSE colonization in maize roots [hyphae and melanized microsclerotia (40x)].

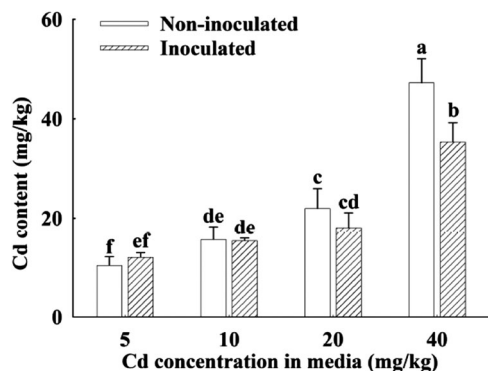


Figure 2. The Cd content in maize shoots. All values represent the means \pm SDs, $n = 4$. Different letters indicate significant differences ($P < .05$) according to Duncan's new multiple range test.

Contents of sulfur and thiol compounds

Under Cd stress, the contents of sulfur, -SH and GSSG in the maize leaves significantly increased. However, there were no clear effects of DSE colonization on sulfur and GSSG content in the maize leaves. Cd stress promoted the GSH content in the maize leaves. The GSH content reached its peak in the 5 mg/kg Cd treatment but then decreased with increasing Cd stress. These results indicate that the GSH contents in the maize leaves reached their maxima under low Cd stress before decreasing with increasing Cd stress. Furthermore, DSE colonization caused significant increases both in -SH content in the 10 mg/kg Cd treatment and in GSH contents in the 5 and 10 mg/kg Cd treatments compared with the non-inoculated treatment (Figure 4).

The GSH/GSSG ratio significantly increased in the 5 mg/kg Cd treatment but then decreased with increasing Cd stress. Moreover, DSE colonization caused a significant increase in

the GSH/GSSG ratio in the 10 mg/kg Cd treatment compared with the non-inoculated treatment (Figure 5).

Interaction and correlation analyses

Two-way ANOVA indicated that the Cd stress had very significant effects on the enzymes activity and thiol compounds' content involved in GSH metabolism in maize leaves ($P < .01$). The DSE inoculation had very significant effects on the GR activity and -SH content ($P < .01$), and significant effects on the GSH content and the GSH/GSSG ratio in maize leaves ($P < .05$). Moreover, the Cd stress and DSE inoculation exhibited strong interactions on the γ -GCS, GST and GSH-Px activity ($P < .01$), the GSH content and the GSH/GSSG ratio ($P < .05$) in maize leaves (Table 1).

Correlation analyses were conducted between the Cd content and sulfur metabolism in the maize leaves. There were significant negative correlations between Cd content and the γ -GCS activity and very significant positive correlations between the Cd content and GR, GST and GSH-Px activity. In addition, there were significant positive correlations between GR activity and the contents of sulfur, -SH and GSSG; GST activity and the -SH content; and GSH-Px activity and the sulfur content, and there were very significant positive correlations between GSH-Px activity and the contents of -SH and GSSG (Table 2).

There were especially significant positive correlations between the Cd content and the sulfur and GSSG contents and a significant negative correlation between the Cd content and the GSH/GSSG ratio. Furthermore, a very significant positive correlation between the GSSG content and the sulfur content, a significant positive correlation between the GSSG content and -SH content, and a very significant negative correlation between the sulfur content and the GSH/GSSG ratio

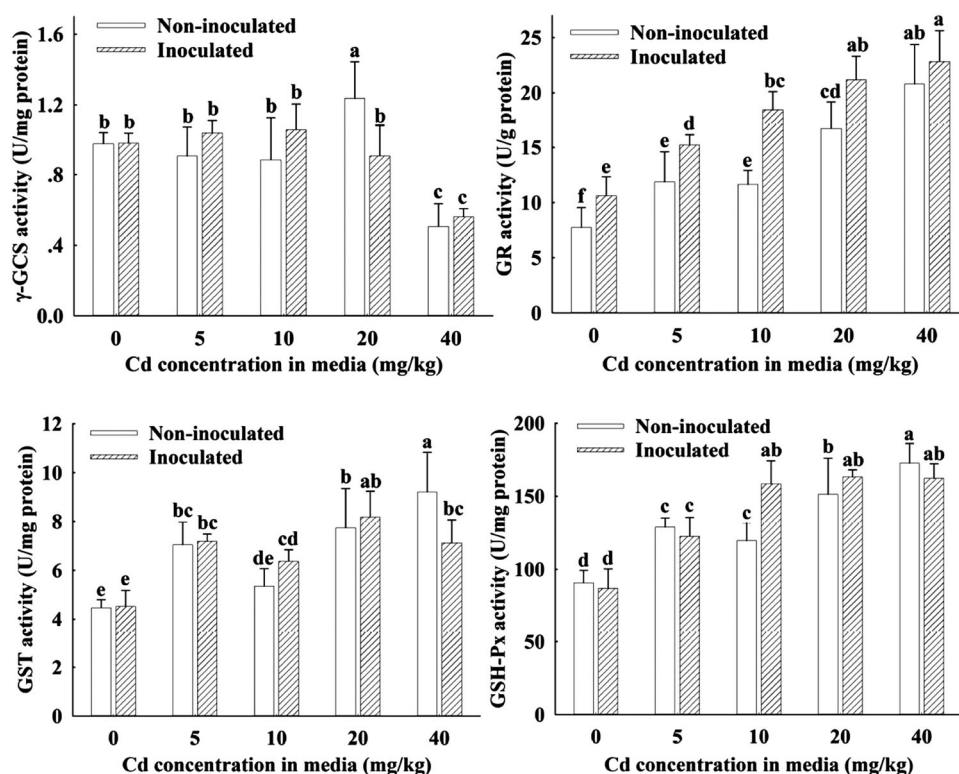


Figure 3. The enzyme activity involved in sulfur metabolism in maize leaves. All values represent the means \pm SDs, $n = 4$. Different letters indicate significant differences ($P < .05$) according to Duncan's new multiple range test.

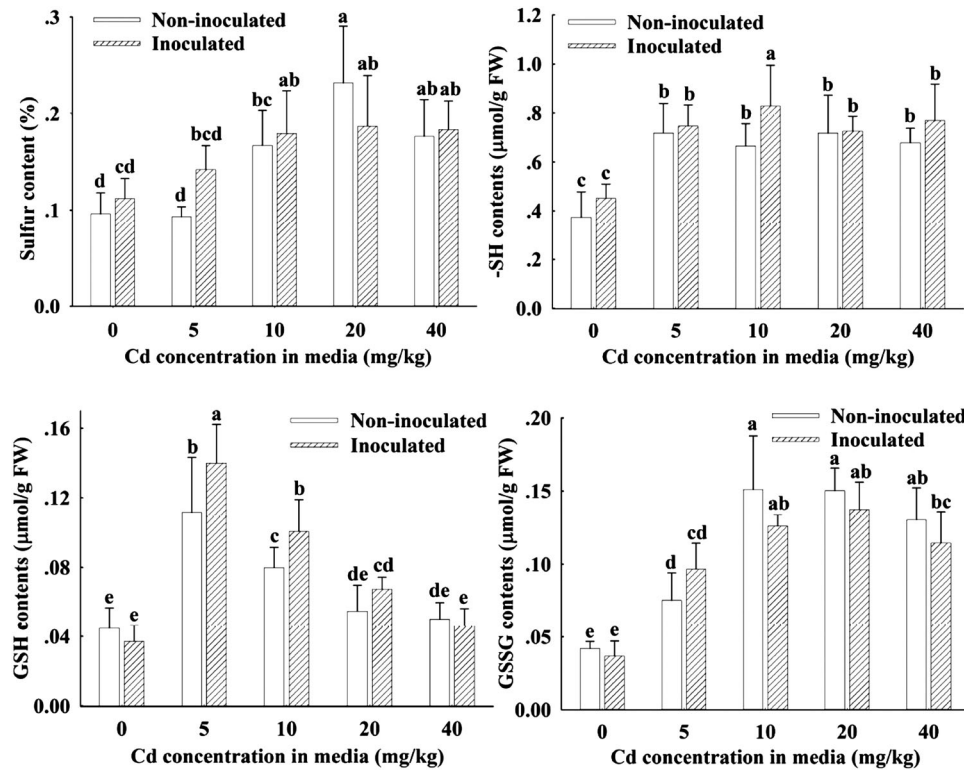


Figure 4. The contents of sulfur and thiol compounds in maize leaves. All values represent the means \pm SDs, $n = 4$. Different letters indicate significant differences ($P < .05$) according to Duncan's new multiple range test.

were observed (Table 2). These observations indicate that Cd stress particularly activates GSH generation in the maize leaves.

Discussion

Cd stress is an abiotic stress that increases with increasing pollution in soils. Cd has a strong biological toxicity to plants and causes severe harmful effects to plant growth (Gallego et al. 2012). In response to Cd stress, sulfur uptake and assimilation play a major role in determining plant resistance (Gill and Tuteja 2011). Generally, Cd stress activates sulfate uptake and the enzymes involved in thiol compound biosynthesis (Nocito et al. 2002; Liang et al. 2016); therefore, Cd stress promotes thiol compound contents in plants (Mishra et al. 2009).

Among thiol compounds, GSH is a tripeptide with a -SH group and acts as a key compound in mitigating Cd-induced damage by playing a fundamental dual role as an antioxidant

and a ligand peptide (Sobrinho-Plata et al. 2014; Hernández et al. 2015). There are several important enzymes involved in GSH metabolism, such as GSH-Px, GST and GR. GSH-Px directly employs GSH as a reducing agent to catalyze the reduction of H_2O_2 and other hydroperoxides (Anjum et al. 2012). GST catalyzes the conjugation of GSH with the Cd ion to form inactive and less toxic GSH-Cd conjugates (Adamis et al. 2004). In addition, GR maintains GSH regeneration and the GSH/GSSG ratio in plants (Gill et al. 2013).

In the present study, we found that the Cd stress activates the GR, GST and GSH-Px activity involved in GSH metabolism and enhances sulfur and thiol compound contents in the maize leaves. Similarly, significant or very significant increases have been observed regarding GSH-Px activity in wheat and barley leaves (Khan et al. 2007; Chen et al. 2010), GST activity in pea and rice leaves (Dixit et al. 2001; Zhang and Ying 2008) and GR activity in mustard (Iqbal et al. 2010) when the plants were exposed to Cd stress in the soil or in hydroponic culture experiments. However, one study reported nearly unaltered GSH-Px activity in pea leaves exposed to Cd (Dixit et al. 2001). GST activity increased under low concentrations of Cd (20–80 μM) but decreased under high Cd levels (100 and 200 μM) in tomato seedlings (Hana et al. 2008). Even the GR activity dramatically decreased in *Ceratophyllum demersum* treated with 10 μM Cd (Aravind and Prasad 2005). All these studies suggest that the GR, GST and GSH-Px enzymes involved in GSH metabolism in plant leaves actively respond to Cd stress and contribute to Cd tolerance.

In the present study, both the GSH content and GSH/GSSG ratio in the maize leaves increased under low Cd (5 mg/kg) stress, and the GSH content and GSH/GSSG ratio decreased with increasing GSSG content under high Cd (10–40 mg/kg) stress. The increasing Cd stress always promoted the generation of ROS due to the biological toxicity

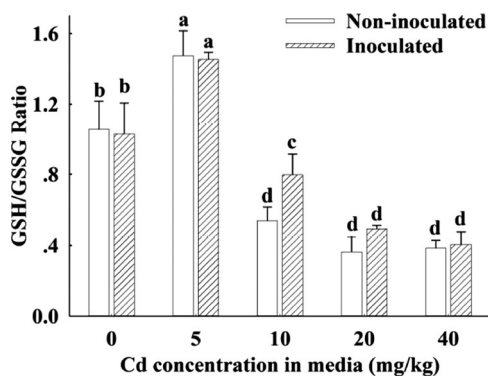


Figure 5. The GSH/GSSG ratio in maize leaves. All values represent the means \pm SDs, $n = 4$. Different letters indicate significant differences ($P < .05$) according to Duncan's new multiple range test.

Table 1. Results of two-way ANOVA between Cd stress and DSE inoculation on the enzymes' activity, and thiol compounds' content involved in GSH metabolism in maize leaves.

Factors	F (p)								
	γ -GCS activity	GR activity	GST activity	GSH-Px activity	Sulfur content	-SH content	GSH content	GSSG content	GSH/GSSG ratio
Cd	25.43 (<.01)	58.17 (<.01)	30.55 (<.01)	64.48 (<.01)	11.77 (<.01)	12.48 (<.01)	55.37 (<.01)	60.01 (<.01)	177.90 (<.01)
DSE	0.02 (.895)	46.90 (<.01)	0.10 (.751)	3.12 (.087)	0.49 (.491)	13.87 (<.01)	5.75 (.023)	2.14 (.154)	6.00 (.020)
Cd \times DSE	5.56 (<.01)	2.11 (.104)	4.48 (<.01)	7.02 (<.01)	1.70 (.176)	2.27 (.085)	2.80 (.043)	2.46 (.067)	3.24 (.025)

Table 2. Correlation coefficients between the Cd content, enzyme activity and thiol compound contents involved in GSH metabolism in maize leaves.

	Cd content	γ -GCS activity	GR activity	GST activity	GSH-Px activity	Sulfur content	-SH content	GSH content	GSSG content
γ -GCS activity	-0.691*								
GR activity	0.810**	-0.458							
GST activity	0.796**	-0.348	0.794**						
GSH-Px activity	0.842**	-0.385	0.926**	0.870**					
Sulfur content	0.647*	0.014	0.658*	0.516	0.694*				
-SH content	0.542	-0.089	0.730*	0.673*	0.800**	0.589			
GSH content	-0.188	0.305	0.052	0.154	0.044	-0.145	0.535		
GSSG content	0.644*	-0.081	0.649*	0.624	0.773**	0.838**	0.736*	0.124	
GSH/GSSG ratio	-0.667*	0.330	-0.607	-0.378	-0.609	-0.771**	-0.221	0.619	-0.692*

* Means significant difference ($P < .05$), $n = 10$.

** Means very significant difference ($P < .01$).

(Cuyppers et al. 2010). In order to scavenge the ROS induced by Cd stress, the GST and GSH-Px catalyze the conversion of reduced GSH to GSSG, and GR in turn regenerates GSH from GSSG using NADPH as an electron donor in the GSH metabolism (Gill and Tuteja 2010). Therefore, the Cd stress induced a decrease in the GSH content in *C. demersum*, while the GSSG content increased; hence, the GSH/GSSG ratio decreased (Aravind and Prasad 2005). In a soil/pot experiment, high Cd (100 mg/kg) treatment resulted in significant decreases in the GSH content and GSH/GSSG ratio in two mung beans (Anjum et al. 2011). Although there were other results that indicated different responses of GSH, GSSG content and the GSH/GSSG ratio, including increases, and there were factors that were unaltered in response to the Cd stress (Anjum et al. 2012). The homeostasis between GSH and GSSG, i.e. the GSH/GSSG ratio, regulates plant tolerance to the Cd stress (Anjum et al. 2012).

In Cd-polluted habitats, the vast majority of plant roots are colonized by endophytic fungi (Li et al. 2012). Endophytic fungi have a significant influence on plant growth and tolerance to Cd stress by different mechanisms (Wang et al. 2016; He et al. 2017). Therefore, an improvement in GSH metabolism in plants induced by endophytic fungi is considered to play an important role. For instance, the inoculation of endophytic *Sphingomonas* SaMR12 increased the GSH concentration and subsequently improved Cd tolerance and accumulation in the roots of *Sedum alfredii* (Pan et al. 2016). The endophytic *F. mosseae* reduced the Cd content in the leaves and roots by increasing the GSH content in tobacco plants (Degola et al. 2015). In addition, the endophytic fungi colonization altered the heavy metals' distribution in the host plant. The DSE always restricted heavy metals' transfer from roots to shoots in maize (Li et al. 2011), sequestered Cd in roots and decreased the Cd content in shoots (Likar and Regvar 2013; Hui et al. 2015; Wang et al. 2016). In the present experiment, the DSE colonization also decreased Cd content in maize leaves at 40 mg/kg Cd treatment. At high (50 and 100 mg/kg) Cd stresses, the DSE colonization decreased the water-soluble Cd content and promoted the Cd adsorption by pectate and protein in roots, which contributed to restrict the Cd migration into the shoots (Wang et al. 2016). But the

DSE colonization had no significant effects on the Cd content in maize leaves at low Cd treatments; the reason was not clear.

In the present study, DSE colonization increased the -SH content in the 10 mg/kg Cd treatment and the GSH contents in the 5 and 10 mg/kg Cd treatments. At the same time, DSE colonization enhanced the GR activity in the 5, 10 and 20 mg/kg Cd treatments and increased the GSH-Px activity in the 10 mg/kg Cd treatment. Furthermore, there were significant positive correlations between GR activity and -SH content and very significant positive correlation between GSH-Px activity and the -SH content. These results indicate that the thiol compound content increase is related to the activation of the GR and GSH-Px activity in the maize leaves, induced by DSE colonization. Similarly, the GSH content increase was considered relevant to the up-regulated expression of relevant genes such as *ATPS*, *GS* and *GSH1*, which were induced by the endophytic *Sphingomonas* SaMR12 (Pan et al. 2016).

One of the most important ecological functions of endophytic fungi is to enhance plant tolerance to stress by regulating GSH metabolism in the host plant (Hamilton et al. 2012). The endophyte *Piriformospora indica* causes resistance to salt stress by enhancing both the GR activity and GSH content in barley leaves (Waller et al. 2005; Baltruschat et al. 2008) and improves disease resistance by enhancing the activities of GR and GST in maize roots (Kumar et al. 2009). Similarly, *P. indica* significantly improves the expression of genes relevant to GSH metabolism (*GSH2* and *GPX*) in tobacco roots and promotes Cd retention in the roots (Hui et al. 2015). The endophyte *Penicillium funiculosum* LHL06 promotes GSH content in soybean plants under copper stress (Khan and Lee 2013), and *Penicillium janthinellum* LK5 enhances the GSH content in the tomato plants under Cd stress (Khan et al. 2014).

Therefore, both the present and previous studies indicate that endophytic fungi can strengthen GSH metabolism under stress, which is considered an important mechanism for improving plant tolerance to environmental stress by endophytic fungi in roots (Waller et al. 2005; Baltruschat et al. 2008; Pan et al. 2016). However, the physiological mechanisms by which DSEs enhance the Cd tolerance of their host plant still are poorly known and require additional study.

Conclusions

The Cd content in the maize shoots increased with increasing Cd stress, but DSE inoculation significantly decreased the Cd content under high Cd stress (40 mg/kg). The Cd stress activated enzyme activity and induced increases in the thiol compound contents relevant to GSH metabolism in the maize leaves. The DSE inoculation significantly enhanced the GR and GSH-Px activity and increased the -SH and GSH contents under low Cd stress (5 and 10 mg/kg), indicating that DSE inoculation plays a role in activating the GSH metabolism in maize leaves, therefore enhancing the plant tolerance to the Cd stress.

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Disclosure statement

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