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-glutamylcysteine-glycine (GCS), and the subsequent addition of glycine to glutathione (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.
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 EC$_{50}$ 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 HNO$_3$/HClO$_4$ (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% HNO$_3$. 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:

\[ C_{protein} = \frac{A_{test} - A_{blank}}{A_{standard} - A_{blank}} \times 0.563 \ g/L \]

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 μmol 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 H₂O₂ 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 sulphhydryl (-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 ± 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 GST-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)).
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 a significant increase in the -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.
were observed (Table 2). These observations indicate that Cd stress particularly activates GSSG 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 (Sobrino-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$_2$O$_2$ 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
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.

Acknowledgements

The authors gratefully acknowledge Professor Zhiwei Zhao (Laboratory for Conservation and Utilization of Bio-Resources and the Key Laboratory for Microbial Resources of the Ministry of Education, Yunnan University) for his help in providing the DSE strain Exophiala pisciphila ACCC32496.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by the National Natural Science Foundation of China [grant number 41461093], [grant number 41661056]; the Natural Science Foundation of Yunnan Province [grant number 2016FB032] and the Science and Technology Innovation Team of Yunnan Province [grant number 2017HC015].

ORCID

Yongmei He http://orcid.org/0000-0001-8072-7514

References

Adams PD, Gomes DS, Pinto MILC, Panek AD, Eleutherio EC. 2004. The role of glutathione transferases in cadmium stress. Toxicol Lett. 154:81–88.
Anjum NA, Ahmad I, Mohmood I, Pacheco M, Duarte AC, Pereira E, Umar S, Ahmad A, Khan NA, Iqbal M, et al. 2012. Modulation of glutathione and its related enzymes in plants’ responses to toxic metals and metalloids – a review. Environ Exp Bot. 75:307–324.
Anjum NA, Umar S, Iqbal M, Khan NA. 2011. Cadmium causes oxidative stress in mung bean by affecting the antioxidative enzyme system and ascorbate-glutathione cycle metabolism. Russ J Plant Physiol. 58:92–99.
Aravind P, Prasad MNV. 2005. Cadmium-zinc interactions in a hydroponic system using Ceratophyllum demersum L.: adaptive ecophysiology, biochemistry and molecular toxicology. Braz J Plant Physiol. 17:3–20.
Atafar Z, Mesdaghiha A, Nouri J, Homaei M, Yunesian M, Ahmadimoghaddam M, Mahvi AH. 2010. Effect of fertilizer application on soil heavy metal concentration. Environ Monit Assess. 160:83–89.
Baltruschat H, Fodor J, Harrach BD, Niemczyk E, Bana B, Gullner G, Janezko A, Kogel KH, Schäfer P, Schwarczinger I, et al. 2008. Salt tolerance of barley induced by the root endophyte Piriformospora indica is associated with a strong increase in antioxidants. New Phytol. 180:501–510.
Berch SM, Kendrick B. 1982. Vesicular-arbuscular mycorrhizae of southern Ontario ferns and fern-allies. Mycologia. 74:769–776.
Berthelot C, Blauze D, Leval C. 2017. Differential growth promotion of poplar and birch inoculated with three dark septate endophytes in two trace element-contaminated soils. Int J Phytoremediat. 1023. doi:10.1080/15226514.2017.1328392
Chen W, Lu S, Peng C, Jiao W, Wang M. 2013. Accumulation of Cd in agricultural soil under long-term reclaimed water irrigation. Environ Pollut. 178:294–299.
Chen F, Wang F, Wu FB, Mao WH, Zhang GP, Zhou MX. 2010. Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. Plant Physiol Biochem. 48:663–672.
Cuypers A, Plusquin M, Remans T, Jozefczak M, Keenen E, Gielen H, Opdenaker K, Nair AR, Munters E, Artois TJ, et al. 2010. Cadmium challenge: BioMetals. 23:927–940.
Degola F, Fattorini L, Bona E, Spremuto CT, Argese E, Berta G, di Santà TL, Santà di Toppì L. 2015. The symbiosis between Nicotiana tabacum and the endomycorrhizal fungus Funneliformis mosseae increases the plant glutathione level and decreases leaf cadmium and root arsenic contents. Plant Physiol Biochem. 92:11–18.
Deng Z, Cao L. 2017. Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere. 168:1100–1106.
Dixit V, Pandey V, Shyam R. 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (Pisum sativum L. cv. Azad). J Exp Bot. 52:1101–1109.
Foster JG, Hess JL. 1980. Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. Plant Physiol. 66:482–487.
Gallego SM, Pena LB, Barcia RA, Azpilcueta CE, Iannone MF, Rosales EP, Zawonzik MS, Groppa MD, Benavides MP. 2012. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environ Exp Bot. 83:33–46.
Gill SS, Anjum NA, Hasanuzzaman M, Gill R, Trivedi DK, Ahmad I, Pereira E, Tuteja N. 2013. Glutathione and glutathione reductase: a boon in disguise for plant abiotic stress defense operations. Plant Physiol Biochem. 70:204–212.
Gill SS, Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem. 48:909–930.
Gill SS, Tuteja N. 2011. Cadmium stress tolerance in crop plants: probing the role of sulfur. Plant Signal Behav. 6:215–222.
Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 249:7130–7139.
Hafeman DG, Sunde RA, Hoekstra WG. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rat. J Nutr. 104:580–587.
Hamilton CE, Gundel PE, Helander M, Saiikonen K. 2012. Endophytic mediation of reactive oxygen species and antioxidant activity in plants: a review. Fungal Divers. 54:1–10.
Hana S, Rachid R, Ibités S, Houria B, Mohammed-Réda D. 2008. Induction of anti-oxidative enzymes by cadmium stress in tomato (Lycopersicon esculentum). Afr J Plant Sci. 2:72–76.
He Y, Yang Z, Li M, Jiang M, Zhang F, Zu Y, Li T, Zhao Z. 2017. Effects of a dark septate endophyte (DSE) on growth, cadmium content and physiology in maize under cadmium stress. Environ Sci Pollut R. doi:10.1007/s11356-017-9459-6.
Hernández LE, Sobrino-Plata J, Montero-Palmero MB, Carrasco-Gil S, Flores-Cáceres ML, Ortega-Villasante C, Escobar C. 2015. Contribution of glutathione to the control of cellular redox homeostasis under toxic metal and metalloid stress. J Exp Bot. 66:2901–2911.
Hui F, Liu J, Gao Q, Lou B. 2015. Piriformospora indica confers cadmium tolerance in Nicotiana tabacum. J Environ Sci. 37:184–191.
Iqbal N, Masood A, Nazar R, Syeed S, Khan NA. 2010. Photosynthesis, growth and antioxidant metabolism in mustard (Brassica juncea L.) cultivars differing in cadmium tolerance. Agr Sci China. 9:519–527.
Jozefczak M, Keenen E, Schat H, Bilek M, Hernández L, Carleer R, Remans T, Bohler S, Vangronsveld J, Cuypers A. 2014. Differential response of Arabidopsis leaves and roots to cadmium: glutathione-related chelating capacity vs antioxidant capacity. Plant Physiol Biochem. 70:110–119.
Jumpponen A, Trappe JM. 1998. Dark septate endophytes: a review of southern Ontario ferns and fern-allies. Mycologia. 74:769–776.
Jozić M, Pau趾 G, Keenen E, Schat H, Bilek M, Hernández L, Carleer R, Remans T, Bohler S, Vangronsveld J, Cuypers A. 2014. Differential response of glutathione-related chelating capacity vs antioxidant capacity. Plant Physiol Bioch. 83:1–9.
Jozefczak M, Remans T, Vangronsveld J, Cuypers A. 2012. Glutathione is a key player in metal-induced oxidative stress defenses. Int J Mol Sci. 13:3145–3175.
Jumpponen A, Trappe JM. 1998. Dark septate endophytes: a review of facultative biotrophic root-colonizing fungi. New Phytol. 140:295–310.
Khan A, Lee JJ. 2013. Endophytic Penicillium fuscum L. HLM06 secretes gibberelins that reprograms Glycine max L. growth during copper stress. BMC Plant Biol. 13:86.
Khan NA, Singh S, Nazar R. 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (Triticum aestivum) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci. 193:435–444.

Khan AL, Waqsas M, Hussain J, Al-Harrasi A, Lee JJ. 2014. Fungal endophyte Penicillium janthinellum LK5 can reduce cadmium toxicity in Solanum lycopersicum (Sitiens and Rhe). Biol Fert Soils. 50:75–85.

Kumar M, Yadav V, Tuteja N, Johri AK. 2009. Antioxidant enzyme activities in maize plants colonized with Piriformospora indica. Microbiology. 155:780–790.

Li H, Li D, He C, Zhou Z, Mei T, Xu H. 2012. Diversity and heavy metal tolerance of endophytic fungi from six dominant plant species in a Pb-Zn mine wasteland in China. Fungal Ecol. 5:309–315.

Li T, Liu MJ, Zhang XT, Zhang HB, Sha T, Zhao ZW. 2011. Improved tolerance of maize (Zea mays L.) to heavy metals by colonization of a dark septate endophyte (DSE) Exophiala piscipila. Sci Total Environ. 409:1069–1074.

Li Z, Ma Z, van der Kuijp TJ, Yuan Z, Huang L. 2014. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. Sci Total Environ. 468–469:843–853.

Liang T, Ding H, Wang G, Kang J, Pang H, Lv J. 2016. Sulfur decreases cadmium translocation and enhances cadmium tolerance by promoting sulfur assimilation and glutathione metabolism in Brassica chinensis L. Ecotox Environ Safe. 124:129–137.

Likar M, Regvar M. 2013. Isolates of dark septate endophytes reduce metal uptake and improve physiology of Solis caprea L. Plant Soil. 370:593–604.

Liu H, Li T, Ding Y, Yang Y, Zhao Z. 2017. Dark septate endophytes colonizing the roots of non-mycorrhizal plants in a mine tailing pond and in a relatively undisturbed environment, Southwest China. J Plant Interact. 12:264–271.

McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115:495–501.

Mendoza-Cázatl D, Loza-Taveria H, Hernández-Navarro A, Moreno-Sánchez R. 2005. Sulfur assimilation and glutathione metabolism under cadmium stress in yeast, protists and plants. FEMS Microbiol Rev. 29:653–671.

Mishra S, Tripathi RD, Srivastava S, Dwivedi S, Trivedi PK, Dhankher OP, Khare A. 2009. Thiol metabolism play significant role during cadmium detoxification by Ceratophyllum demersum L. Bioresource Technol. 100:2155–2161.

Na G, Salt DE. 2011. The role of sulfur assimilation and sulfur-containing compounds in trace element homeostasis in plants. Environ Exp Bot. 72:18–25.

Nannoni F, Rossi S, Protano G. 2016. Potentially toxic element contamination in soil and accumulation in maize plants in a smelter area in Kosovo. Environ Sci Pollut Res. 23:11937–11946.

Nocto FF, Pirovano L, Cocucci M, Sacchi GA. 2002. Cadmium-induced sulfate uptake in maize roots. Plant Physiol. 129:1872–1879.

Noctor G, Mhamdi A, Chaouch S, Han YI, Neukermans J, Marquez-Garcia B, Queval G, Foyer CH. 2012. Glutathione in plants: an integrated overview. Plant Cell Environ. 35:454–484.

Pan F, Meng Q, Wang Q, Luo S, Chen B, Khan KY, Yang X, Feng Y. 2016. Endophytic bacterium Sphingomonas SaMR12 promotes cadmium accumulation by increasing glutathione biosynthesis in Sedum alfredii Hance. Chemosphere. 154:338–366.

Rahman I, Kode A, Biswas SK. 2006. Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. Nat Protoc. 1:3139–3165.

Robson TC, Braungardt CB, Rieuwerts J, Worsfold P. 2014. Cadmium contamination of agricultural soils and crops resulting from sphalerite weathering. Environ Pollut. 184:283–289.

Rodriguez RJ, White JJr, Arnold AE, Redman RS. 2009. Fungal endophytes: diversity and functional roles. New Phytol. 182:314–330.

Sedlak J, Lindsay RH. 1968. Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman’s reagent. Anal Biochem. 25:192–205.

Seelig GF, Meister A. 1985. Glutathione biosynthesis; γ-glutamylcysteine synthetase from rat kidney. Method Enzymol. 113:379–390.

Sobrino-Plata J, Meyssen D, Cuypers A, Esocbar C, Hernández LE. 2014. Glutathione is a key antioxidant metabolite to cope with mercury and cadmium stress. Plant Soil. 377:369–381.

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, et al. 2005. The endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease resistance, and higher yield. Proc Natl Acad Sci USA. 102:13386–13391.

Wang J, Li T, Liu G, Smith JM, Zhao ZW. 2016. Unraveling the role of dark septate endophyte (DSE) colonizing maize (Zea mays) under cadmium stress: physiological, cytological and genic aspects. Sci Rep. 6:3.

Zhan FD, He YM, Li T, Yang Y, Toor GS, Zhao ZW. 2015. Tolerance and antioxidiant response of a dark septate endophyte (DSE), Exophiala piscipila, to cadmium stress. B Environ Contam Tox. 94:96–102.

Zhang Y, Li T, Zhao Z. 2013. Colonization characteristics and composition of dark septate endophytes (DSE) in a lead and zinc slag heap in southwest China. Soil Sediment Contam Int J. 22:532–545.

Zhang CH, Ying GE. 2008. Response of glutathione and glutathione S-transferase in rice seedlings exposed to cadmium stress. Rice Sci. 15:73–76.