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Effect of Dark Septate Endophytic Fungus *Gaeumannomyces cylindrosporus* on Plant Growth, Photosynthesis and Pb Tolerance of Maize (*Zea mays* L.)

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ABSTRACT

Dark septate endophytic (DSE) fungi are ubiquitous and cosmopolitan, and occur widely in association with plants in heavy metal stress environment. However, little is known about the effect of inoculation with DSE fungi on the host plant under heavy metal stress. In this study, *Gaeumannomyces cylindrosporus*, which was isolated from Pb-Zn mine tailings in China and had been proven to have high Pb tolerance, was inoculated onto the roots of maize (*Zea mays* L.) seedlings to study the effect of DSE on plant growth, photosynthesis, and the translocation and accumulation of Pb in plant under stress of different Pb concentrations. The growth indicators (height, basal diameter, root length, and biomass) of maize were detected. Chlorophyll content, photosynthetic characteristics (net photosynthetic rate, transpiration rate, stomatal conductance, and intercellular CO_2 concentration), and chlorophyll fluorescence parameters in leaves of the inoculated and non-inoculated maize were also determined. Inoculation with *G. cylindrosporus* significantly increased height, basal diameter, root length, and biomass of maize seedlings under Pb stress. Colonization of *G. cylindrosporus* improved the efficiency of photosynthesis and altered the translocation and accumulation of Pb in the plants. Although inoculation with *G. cylindrosporus* increased Pb accumulation in host plants in comparison to non-inoculated plants, the translocation factor of Pb in plant body was significantly decreased. The results indicated that Pb was accumulated mainly in the root system of maize and the phytotoxicity of Pb to the aerial part of the plant was alleviated. The improvement of efficiency of photosynthesis and the decrease of translocation factor of Pb, caused by DSE fungal colonization, were efficient strategies to improve Pb tolerance of host plants.

Key Words: chlorophyll fluorescence, fungal colonization, growth indicator, heavy metal stress, Pb accumulation, Pb translocation, photosynthetic characteristics

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Soil pollution with heavy metals is a pressing issue worldwide. The continued increase of metal levels in soil poses a health risk to humans and animals through food chain. Heavy metals in the soil can lead to toxicity symptoms and inhibit the growth of most plants, especially the nonessential heavy metals, such as Pb, Cd, Cr, *etc.* (Nagajyoti *et al.*, 2010). Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus tolerance to metal stress (Hall, 2002). Endophytic fungi not only have the ability to protect against heavy metal toxicity but also increase nutrient acquisition of host plants and enhance their metabolic activity to combat stress (Selosse *et al.*, 2004; Gadd, 2007). Thus, the alleviation of heavy metal toxicity to host plants by endophytic fungi could be an efficient strategy to improve heavy metal tolerance in plants.

Dark septate endophytic (DSE) fungi, which are one of the groups of endophytic fungi, can colonize nearly 600 plant species representing about 320 genera and 114 families (Jumpponen and Trappe, 1998; Mandyam and Jumpponen, 2005). According to the results of recent surveys of DSE colonization, the range of host plant species was obviously enlarged (Zhang *et al.*, 2013; Massenssini *et al.*, 2014; Gucwa-Przepióra *et al.*, 2016). DSE fungi are ubiquitous in various stressful environments, especially common in heavy metal-contaminated soils (Likar and Regvar, 2009; Regvar *et al.*, 2010; Li *et al.*, 2012). Some typical DSE fungi isolated from heavy metal-contaminated soils, such as *Exophiala pisciphila* McGinnis & Ajello (Li *et al.*, 2011), *Gaeumannomyces cylindrosporus* Hornby, Slo-

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pe, Gutteridge & Sivanesan (Ban *et al.*, 2012), and *Phialophora/Cadophora* complex (Likar and Regvar, 2013), have been proven to have significant tolerance to heavy metal ions. The high tolerance of DSE fungi to heavy metals and their great abundance in heavy metal-polluted habitats suggested that DSE fungi may have an important function for host survival in extreme environments (Likar, 2011). However, little research has been focused on the effect of inoculation with DSE fungi on host plants under heavy metal stress in controlled pot culture conditions. Zhang *et al.* (2012) demonstrated that inoculation with a DSE isolate LBF-2 increased the total biomass of *Lycium barbarum* L. seedlings and the concentration of chlorophyll (Chl), and also enhanced Chl fluorescence. However, the study was conducted without heavy metal stress, and it was not clear that the positive effects of inoculation with DSE fungi would be enhanced or completely reversed with the addition of heavy metals. The effect of DSE fungi on the photosynthetic system of host plants may be one important way to alter the sensibility of plant to heavy metals, but so far it has not been confirmed. There still exist different results about the influence of inoculation with DSE on the uptake and accumulation of heavy metals in host plants. Li *et al.* (2011) indicated that Pb accumulation of maize (*Zea mays* L.) seedlings colonized by a DSE fungus was higher than the non-inoculated controls under four concentrations of Pb. However, the results of Likar and Regvar (2013) showed that DSE fungi reduced heavy metal uptake by *Salix caprea* L. This difference may result from various factors, such as fungus and host plant specificity, cultural conditions, *etc.* Thus, the relationship between DSE fungi and the absorption and translocation of metals in plants deserves further research.

The aim of this work was to study the effect of inoculation with *G. cylindrosporus* on the growth of maize, the Chl concentration, photosynthetic characteristics, and Chl fluorescence parameters of plant leaves, and the uptake, translocation, and accumulation of Pb in plant under different Pb concentrations.

MATERIALS AND METHODS

Experimental design

G. cylindrosporus isolated from the roots of *Astragalus adsurgens* Pall., which grew naturally on the Pb-Zn mine tailings in Qiandong Mountain of Shaanxi Province, China, was used as inoculum (Ban *et al.*, 2012). The experiment was installed under greenhouse conditions and consisted of a completely randomized factorial design (2 inoculation treatments × 4 Pd concentrations) with 10 replicates. Four plants per treatment were randomly selected for measurements of plant biomass and Pb content in plant body, and the leaves and roots of residual plants were used for the determinations of Chl concentration, photosynthetic characteristics, Chl fluorescence parameters, and DSE colonization. The treatments were either inoculation or non-inoculation of *G. cylindrosporus* along with the addition of four Pb concentrations (0, 50, 500, and 1 000 μg g^{-1}) into the substrates. River sand was used as pot culture substrates. After being washed with tap water and air-dried, river sand was passed through a 2-mm sieve and then autoclaved for 2 h at 121 °C. The basic physico-chemical characteristics of the substrates were as follows: pH (H_2O) 7.4, organic matter 0.3 g kg^{-1}, available P 0.31 mg kg^{-1}, alkali-hydro N 0.79 mg kg^{-1}, and available K 1.32 mg kg^{-1}. Each plastic pot (150 mm length × 130 mm width × 150 mm height) was filled with 2 kg culture substrates. The Pb concentrations in culture substrates were adjusted at 50, 500, and 1 000 μg g^{-1}, respectively, by addition of $Pb(NO_3)_2$ solution. The treatment without $Pb(NO_3)_2$ (*i.e.*, 0 μg g^{-1}) was set as the control. Planting was carried out after the plastic pots were placed without moving for one week to reach Pb equilibrium.

Maize seeds (cv. Zhengdan 958) were purchased from Northwest A&F University Seed Co., Shaanxi Province, China. The seeds were surface-sterilized by dipping in 75% (volume:volume) ethanol for 5 min and then in 10% (volume:volume) sodium hypochlorite for 10 min under agitation. Sterilized seeds were gently washed by deionized water for several times at room temperature, and then placed on the sterile moist filter papers (Xinhua No. 101, China) in Petri dishes for germination at 25 °C. The germinated seeds were transplanted into the plastic pots (2 seeds for each pot) at a depth of 2 cm for fungal inoculation. Inoculum of *G. cylindrosporus*, as 5-mm plugs excised from an edge of an actively growing colony on potato dextrose agar (PDA), was inoculated close to the roots of maize seedlings. Fungus-free treatments were mock-inoculated with sterile PDA plugs. The experiment was carried out at 25 °C in a greenhouse with a photoperiod of 12 h per day for a culture period of 6 weeks. Each pot was irrigated with 100 mL Hoagland's nutrient solution (Hoagland and Arnon, 1950) every week.

DSE colonization

Both non-inoculated and inoculated plant roots were prepared according to the method of Phillips and Hayman (1970). Root samples were washed several