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 CO₂ 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


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-Przępióra 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-
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 Qandong 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 \( \times 4 \) Pb 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 1000 \( \mu g \ g^{-1} \)) 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\(_2\)O) 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 \( \times \) 130 mm width \( \times \) 150 mm height) was filled with 2 kg culture substrates. The Pb concentrations in culture substrates were adjusted at 50, 500, and 1000 \( \mu g \ g^{-1} \), respectively, by addition of Pb(NO\(_3\))\(_2\) solution. The treatment without Pb(NO\(_3\))\(_2\) solution 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
times with running tap water, cut into 1-cm pieces, and clarified in 10% (weight:volume) KOH at 90 °C for 20–60 min. After that the root samples were bleached with fresh alkaline H$_2$O$_2$ solution, containing 30 mL 10% (volume:volume) H$_2$O$_2$, 3 mL NH$_4$OH, and 567 mL deionized water, for 10–30 min, acidified with 2% (volume:volume) HCl for 5 min, and then soaked in 0.05% (weight:volume) trypan blue solution for 12 h at room temperature. All stained roots were transferred into acidified glycerol and incubated for 12 h at room temperature. The DSE colonization of roots was determined using the method modified by McGonigle et al. (1990) under a compound-light microscope (Olympus BX51, Japan). It was characterized by darkly pigmented or blue-stained septate hyphae and microsclerotia, and was calculated as follows:

$$\text{DSE colonization} = \left( \frac{n_1}{n_2} \right) \times 100\% \quad (1)$$

where $n_1$ is the number of intersections with fungal structure and $n_2$ is the total number of counted intersections.

**Measurements of Chl fluorescence and concentrations**

At end of the experiment, Chl fluorescence was measured on the second fully expanded leaf at room temperature using a portable chlorophyll fluorometer (model Mini-Pam, Heinz Walz, Germany). After darkening the leaf for 30 min, the minimum fluorescence ($F_0$) was recorded and the maximum fluorescence in the dark adapted state ($F_m$) was obtained by application of a saturating light pulse. The plants were illuminated with actinic light (90 μmol L$^{-1}$ s$^{-1}$) for 10 min, and then the level of modulated fluorescence during a brief interruption of actinic illumination in the presence of far-red light ($F_m'$), the maximum fluorescence yield during actinic illumination ($F_m$), and the Chl fluorescence yield during actinic illumination ($F_v$) were measured. The yield of variable fluorescence ($F_v$) was calculated as $F_m - F_0$. The maximum quantum yield of photosystem II (PS-II) photochemistry was determined as $F_v/F_m$, i.e., ($F_m - F_0$)/$F_m$. The actual quantum efficiency of PSII open centers at light adapted state (YII) was calculated as ($F_m' - F_v$)/$F_m$ (Genty et al., 1989). The coefficient of photochemical quenching (qP) is a measurement of the fraction of open centers, calculated as ($F_m' - F_v$)/($F_m' - F_0$) (Schreiber et al., 1986). The coefficient of non-photochemical quenching (qN) was calculated as $1 - (F_m' - F_v)/(F_m' - F_0)$ (Bilger and Schreiber, 1987). Calculation of quenching due to non-photochemical dissipation of absorbed light energy (NPQ) was conducted at each saturating pulse, according to the equation of $(F_m - F_m')/F_m'$ (Bilger and Björkman, 1991). The rate of linear electron transport (ETR) through PSII was calculated as ETR = $\Phi_{PSII} \times$ PPFD × 0.5 × 0.84, where $\Phi_{PSII}$ is the quantum yield of PSII photochemistry; 0.5 is used because of the supposed equal distribution of excitation between the two photosystems; PPFD is the photosynthetic photon flux density on the leaf surface; and the factor 0.84 is the fraction of the incident quanta absorbed by the leaf (Bajkán et al., 2012). Chl was extracted from the second fully expanded leaf using 1:1 (volume:volume) ethanol-acetone solution. A spectrophotometer (UVmini-1240, Shimadzu, Japan) was used to determine the absorbance of Chl a and b in the extracts at 663 and 645 nm, respectively.

**Measurements of photosynthetic parameters**

At end of the culture period, photosynthetic parameters (including net photosynthetic rate, stomatal conductance, intercellular CO$_2$ concentration, and transpiration rate) were determined on fully developed leaves between 8:30–11:30 a.m. using a portable open-flow gas exchange system (LI-6400, LI-COR, Inc., USA) according to the manufacturer’s instructions. The system maintained a constant photosynthetically active radiation (1000 μmol m$^{-2}$ s$^{-1}$), 25 °C leaf temperature, 50% relative humidity, and 0.5 dm$^3$ min$^{-1}$ flow rate of atmosphere.

**Measurements of plant biomass and Pb concentration in plants**

Plant height and root length (the length of the longest root) were measured by precision straight edge, and basal diameter was measured with a vernier caliper (ECV150C, Insize Co., China). Shoots (leaves and stems) and roots were collected, respectively, and the roots were thoroughly washed by tap water to remove the silt. The shoots and roots were then placed in an oven at 80 °C, dried to constant weight, and weighed after cooling in vacuum desiccators. After that, the shoots and roots were ground separately by a minivestegation disintegrator (FZ102, Tianjing Test Instrument Co., China) and then digested in a mixture of concentrated HNO$_3$ and HClO$_4$. The Pb content was determined with an atomic absorption spectrophotometer (Solaar Mk2-M6, Thermo, USA). The translocation factor (TF) of Pb was defined as the ratio of Pb content in shoot to that in root.

**Statistical analyses**

The determinations of physiological and biochemi-
cal parameters were performed with 4 replicates (randomly selected from 10 plants per treatment), and data are presented as means ± standard errors. All data were tested for normality and analyzed by one- and two-way analyses of variance using SPSS 16.0 software (SPSS Inc., USA). Comparisons between means were carried out using Duncan’s multiple range tests at $P < 0.05$. Graphical work was carried out using SigmaPlot for Windows version 10.0 software packages.

RESULTS

DSE colonization and plant biomass

No DSE structures were observed in the roots of non-inoculated maize after harvesting. In the inoculated treatments, typical structures of \textit{G. cylindrosporus} were observed under four concentrations of Pb. The colonization intensity of \textit{G. cylindrosporus} increased nonlinearly with the increasing Pb concentrations and peaked at 63.6\% in the roots of maize grown in the substrates applied with 1 000 μg g$^{-1}$ Pb (Fig. 1). Inoculation with \textit{G. cylindrosporus} increased the height, basal diameter, root length, and total biomass of maize seedlings under different Pb concentrations stress (Table I). Under the Pb concentration of 1 000 μg g$^{-1}$, the shoot and root biomass of inoculated maize were 1.35 and 2.81 times, respectively, higher than those of the non-inoculated maize. Inoculation with \textit{G. cylindrosporus} significantly alleviated the negative effects of increasing toxicity of heavy metals, and the differences of growth indicators between the inoculated and non-inoculated plants were more significant under high Pb concentration stress. Based on the results of interaction between the factors (Table II), both of Pb concentration and inoculation treatment had significant ($P < 0.05$) effects on the growth of maize.

<table>
<thead>
<tr>
<th>Pb concentration (μg g$^{-1}$)</th>
<th>Inoculation</th>
<th>Height (cm)</th>
<th>Basal diameter (cm)</th>
<th>Root length (cm)</th>
<th>Shoot biomass (g)</th>
<th>Root biomass (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No GC</td>
<td>41.22 ± 1.17$^{a}$$^{b}$</td>
<td>0.35 ± 0.01ab</td>
<td>34.53 ± 1.08c</td>
<td>2.17 ± 0.04bc</td>
<td>2.11 ± 0.10b</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>43.60 ± 0.21b</td>
<td>0.36 ± 0.01ab</td>
<td>48.24 ± 1.07a</td>
<td>3.81 ± 0.16b</td>
<td>4.05 ± 0.18a</td>
</tr>
<tr>
<td>50</td>
<td>No GC</td>
<td>42.87 ± 0.85b</td>
<td>0.36 ± 0.01ab</td>
<td>39.21 ± 2.02b</td>
<td>2.24 ± 0.11b</td>
<td>2.30 ± 0.13b</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>52.75 ± 1.19a</td>
<td>0.37 ± 0.00a</td>
<td>50.21 ± 0.89a</td>
<td>3.84 ± 0.15a</td>
<td>3.86 ± 0.10a</td>
</tr>
<tr>
<td>500</td>
<td>No GC</td>
<td>39.19 ± 1.69bc</td>
<td>0.34 ± 0.02b</td>
<td>30.04 ± 1.67cd</td>
<td>1.50 ± 0.06d</td>
<td>1.23 ± 0.07c</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>43.90 ± 2.16b</td>
<td>0.35 ± 0.00ab</td>
<td>42.50 ± 1.49b</td>
<td>2.17 ± 0.09bc</td>
<td>2.31 ± 0.04b</td>
</tr>
<tr>
<td>1 000</td>
<td>No GC</td>
<td>37.79 ± 1.78c</td>
<td>0.29 ± 0.01c</td>
<td>27.21 ± 1.51d</td>
<td>1.39 ± 0.02d</td>
<td>0.78 ± 0.034</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>42.98 ± 2.46b</td>
<td>0.35 ± 0.02ab</td>
<td>30.21 ± 1.64cd</td>
<td>1.88 ± 0.03c</td>
<td>2.19 ± 0.13b</td>
</tr>
</tbody>
</table>

$^{a}$Means ± standard errors ($n = 4$).

$^{b}$Means followed by the same letter(s) within each column are not significantly different at $P < 0.05$ according to Duncan’s multiple range test.

Fig. 1 Dark septate endophytic (DSE) colonization (by \textit{Gaeumannomyces cylindrosporus}) in roots of maize under stress of different Pb concentrations. Vertical bars indicate standard errors of the means ($n = 4$). Bars with the same letter(s) are not significantly different at $P < 0.05$ according to Duncan’s multiple range test.

Effect of inoculation with \textit{G. cylindrosporus} on photosynthesis

Under different Pb concentrations, Chl \textit{a} content of maize seedlings inoculated with \textit{G. cylindrosporus} was higher than that of the non-inoculated seedlings (Table III). However, the difference was not significant except under stress of high Pb concentration (1 000 μg g$^{-1}$), which was consistent with the results of total Chl content. Under Pb concentration of 1 000 μg g$^{-1}$, the Chl \textit{a} and Chl (\textit{a} + \textit{b}) contents were recorded 66.9\% and 59.5\% higher in the inoculated plants than in the non-inoculated plants. In addition, Pb concentrations in substrates and inoculation treatments showed no significant ($P > 0.05$) effects on Chl \textit{a}/\textit{b} value of maize seedlings (Table II).

Effect of inoculation with \textit{G. cylindrosporus} on Chl

$$
\begin{align*}
\text{Pb concentration (μg g}^{-1}) & \quad \text{Inoculation} & \quad \text{Height (cm)} & \quad \text{Basal diameter (cm)} & \quad \text{Root length (cm)} & \quad \text{Shoot biomass (g)} & \quad \text{Root biomass (g)} \\
0 & \quad \text{No GC} & \quad 41.22 ± 1.17^{a}^{b} & \quad 0.35 ± 0.01 & \quad 34.53 ± 1.08 & \quad 2.17 ± 0.04 & \quad 2.11 ± 0.10 \\
 & \quad + GC & \quad 43.60 ± 0.21 & \quad 0.36 ± 0.01 & \quad 48.24 ± 1.07 & \quad 3.81 ± 0.16 & \quad 4.05 ± 0.18 \\
50 & \quad \text{No GC} & \quad 42.87 ± 0.85 & \quad 0.36 ± 0.01 & \quad 39.21 ± 2.02 & \quad 2.24 ± 0.11 & \quad 2.30 ± 0.13 \\
 & \quad + GC & \quad 52.75 ± 1.19 & \quad 0.37 ± 0.00 & \quad 50.21 ± 0.89 & \quad 3.84 ± 0.15 & \quad 3.86 ± 0.10 \\
500 & \quad \text{No GC} & \quad 39.19 ± 1.69 & \quad 0.34 ± 0.02 & \quad 30.04 ± 1.67 & \quad 1.50 ± 0.06 & \quad 1.23 ± 0.07 \\
 & \quad + GC & \quad 43.90 ± 2.16 & \quad 0.35 ± 0.00 & \quad 42.50 ± 1.49 & \quad 2.17 ± 0.09 & \quad 2.31 ± 0.04 \\
1 000 & \quad \text{No GC} & \quad 37.79 ± 1.78 & \quad 0.29 ± 0.01 & \quad 27.21 ± 1.51 & \quad 1.39 ± 0.02 & \quad 0.78 ± 0.034 \\
 & \quad + GC & \quad 42.98 ± 2.46 & \quad 0.35 ± 0.02 & \quad 30.21 ± 1.64 & \quad 1.88 ± 0.03 & \quad 2.19 ± 0.13 \\
\end{align*}
$$

$^{a}$Means ± standard errors ($n = 4$).

$^{b}$Means followed by the same letter(s) within each column are not significantly different at $P < 0.05$ according to Duncan’s multiple range test.
Gaeumannomyces cylindrosporus

Effect of inoculation with 

TABLE III

<table>
<thead>
<tr>
<th>Pb concentration</th>
<th>Inoculation</th>
<th>Chl a content</th>
<th>Chl b content</th>
<th>Chl a/b value</th>
<th>Total Chl content</th>
</tr>
</thead>
<tbody>
<tr>
<td>μg g⁻¹</td>
<td></td>
<td>mg g⁻¹ FW</td>
<td>mg g⁻¹ FW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>No GC</td>
<td>2.048 ± 0.14(ab)</td>
<td>0.559 ± 0.02b</td>
<td>3.684 ± 0.38a</td>
<td>2.607 ± 0.13a</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>2.188 ± 0.11a</td>
<td>0.603 ± 0.02ab</td>
<td>3.638 ± 0.26a</td>
<td>2.791 ± 0.10a</td>
</tr>
<tr>
<td>50</td>
<td>No GC</td>
<td>2.102 ± 0.08a</td>
<td>0.505 ± 0.02c</td>
<td>4.186 ± 0.27a</td>
<td>2.607 ± 0.07a</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>2.318 ± 0.14a</td>
<td>0.618 ± 0.01a</td>
<td>3.750 ± 0.19a</td>
<td>2.936 ± 0.15a</td>
</tr>
<tr>
<td>500</td>
<td>No GC</td>
<td>1.244 ± 0.14b</td>
<td>0.334 ± 0.02e</td>
<td>3.741 ± 0.44a</td>
<td>1.579 ± 0.14b</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>1.533 ± 0.20b</td>
<td>0.429 ± 0.01d</td>
<td>3.597 ± 0.55a</td>
<td>1.963 ± 0.19b</td>
</tr>
<tr>
<td>1000</td>
<td>No GC</td>
<td>0.732 ± 0.11c</td>
<td>0.242 ± 0.00f</td>
<td>3.039 ± 0.51a</td>
<td>0.974 ± 0.11c</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>1.222 ± 0.27b</td>
<td>0.331 ± 0.02e</td>
<td>3.744 ± 0.92a</td>
<td>1.554 ± 0.26b</td>
</tr>
</tbody>
</table>

a) Fresh weight.

b) Means ± standard errors (n = 4).

c) Means followed by the same letter(s) within each column are not significantly different at P < 0.05 according to Duncan’s multiple range test.

TABLE II

<table>
<thead>
<tr>
<th>Growth indicator</th>
<th>Pb</th>
<th>GC</th>
<th>Pb × GC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height</td>
<td>8.54***</td>
<td>24.25***</td>
<td>1.95NS(b)</td>
</tr>
<tr>
<td>Basal diameter</td>
<td>8.04**</td>
<td>6.08*</td>
<td>3.69*</td>
</tr>
<tr>
<td>Root length</td>
<td>45.20***</td>
<td>93.95***</td>
<td>5.42**</td>
</tr>
<tr>
<td>Shoot biomass</td>
<td>115.54***</td>
<td>253.93***</td>
<td>19.32***</td>
</tr>
<tr>
<td>Root biomass</td>
<td>119.07***</td>
<td>371.99***</td>
<td>5.35**</td>
</tr>
<tr>
<td>Chl a content</td>
<td>28.28***</td>
<td>6.50*</td>
<td>0.46NS</td>
</tr>
<tr>
<td>Chl b content</td>
<td>173.05***</td>
<td>61.73***</td>
<td>1.84NS</td>
</tr>
<tr>
<td>Chl a/b value</td>
<td>0.46NS</td>
<td>0.00NS</td>
<td>0.49NS</td>
</tr>
<tr>
<td>Total Chl content</td>
<td>46.60***</td>
<td>11.79**</td>
<td>0.58NS</td>
</tr>
<tr>
<td>Pn</td>
<td>73.55***</td>
<td>101.30***</td>
<td>2.92NS</td>
</tr>
<tr>
<td>TR</td>
<td>4.90*</td>
<td>37.48***</td>
<td>0.29NS</td>
</tr>
<tr>
<td>Gs</td>
<td>20.18***</td>
<td>3.30NS</td>
<td>0.23NS</td>
</tr>
<tr>
<td>Ci</td>
<td>68.32***</td>
<td>378.50***</td>
<td>34.46***</td>
</tr>
<tr>
<td>Pb content in root</td>
<td>248.69***</td>
<td>18.17***</td>
<td>9.45**</td>
</tr>
<tr>
<td>Pb content in shoot</td>
<td>173.76***</td>
<td>36.26***</td>
<td>7.13**</td>
</tr>
<tr>
<td>Pb content in plant</td>
<td>283.49***</td>
<td>12.31**</td>
<td>7.64**</td>
</tr>
<tr>
<td>TF of Pb</td>
<td>14.29***</td>
<td>19.80***</td>
<td>0.27NS</td>
</tr>
</tbody>
</table>

*: Significant at P < 0.05, P < 0.01, and P < 0.001, respectively.

**: Chl is the chlorophyll; Pn is the net photosynthetic rate; TR is the transpiration rate; Gs is the stomatal conductance; Ci is the intercellular CO₂ concentration; TF is the translocation factor.

***: Not significant.

fluorescence of maize seedlings under four different Pb concentrations is shown in Fig. 2. The value of Fv/Fm decreased with the increasing Pb concentrations both in the inoculated and non-inoculated treatments. However, the Fv/Fm values of inoculated seedlings under Pb concentrations of 500 and 1000 μg g⁻¹ were both significantly higher (P < 0.05), being 1.12 and 1.51 times, respectively, that of the non-inoculated seedlings. The Y(II) value increased firstly and then decreased significantly with the increase of Pb concentration. The results showed that the Y(II) value was improved by colonization of G. cylindrosporus; however, the differences between the inoculated and non-inoculated treatments under 50, 500, and 1 000 μg g⁻¹ Pb stress were not significant (P > 0.05). Under four concentrations of Pb, there were no significant differences for the qP values between the inoculated and non-inoculated treatments. The qN value decreased when Pb concentration of substrates was up to 50 μg g⁻¹, and then increased with the increasing Pb concentration. There were no significant differences for the NPQ values between the non-inoculated and the inoculated seedlings under Pb stress of 50, 500, and 1 000 μg g⁻¹. The changing trend of ETR with the increasing Pb concentration was consistent with that of Y(II); however, the differences between the inoculated and non-inoculated treatments were significant only under no Pb stress.

Effect of inoculation with G. cylindrosporus on photosynthetic characteristics of maize seedlings under Pb stress is shown in Table IV. The net photosynthetic rate (Pn) was improved under light Pb stress (50 μg g⁻¹), and reduced obviously under the concentrations of 500 and 1 000 μg g⁻¹. However, Pn of inoculated seedlings was 2.14 and 4.11 times higher than that of the non-inoculated seedlings under the two Pb concentrations. Transpiration rate (TR) was improved by the colonization of G. cylindrosporus under Pb stress. Under Pb stress levels of 50, 500, and 1 000 μg g⁻¹, TR of inoculated seedlings increased by 88.0%, 76.2%, and 114.3%, respectively, compared with the un inoculated seedlings. Based on the results of interaction between the factors (Table II), inoculation treatment had no significant effects on stomatal conductance (Gs) of maize (P > 0.05), but the effect of Pb stress was obvious. The differences of intercellular CO₂ concentration (Ci) between the inoculated and non-inoculated
seedlings under four Pb concentrations were significant \((P < 0.05)\), and \(C_i\) of inoculated maize were only 45.3\% and 39.0\% of that of non-inoculated seedlings, respectively, when the Pb concentration was up to 500 and 1000 \(\mu g\) g\(^{-1}\).

**Pb uptake and translocation in maize seedlings**

The Pb contents in root and shoot and the translocation factor of Pb in the inoculated and non-inoculated maize seedlings growing in all test substrates are summarized in Table V. Pb was absorbed and accumulated increasingly by roots and shoots of both inoculated and non-inoculated seedlings with the increasing Pb concentrations. However, inoculation with *G. cylindrosporus* increased total accumulation of Pb in maize under Pb stress. Under Pb concentration of 1000 \(\mu g\) g\(^{-1}\), the sum of Pb content in root and shoot of the inoculated seedlings was 1.36 times \((P < 0.05)\) that in the non-inoculated seedlings. More importantly, root colonization by *G. cylindrosporus* changed the translocation of Pb in maize. Under the inoculation treatment, Pb was accumulated mainly in the roots, and Pb content in shoot was decreased compared with that in the non-inoculated treatments, thus the ratio of Pb content in shoot to that in root \((i.e.,\ TF)\) of inoculated seedlings being lower than that of the non-

![Fig. 2](image-url)

*Fig. 2* Effect of inoculation with *Gaeumannomyces cylindrosporus* (+ GC) on the chlorophyll fluorescence kinetic parameters of maize seedlings under stress of different Pb concentrations. Vertical bars indicate standard errors of the means \((n = 4)\). Bars with the same letter(s) are not significantly different at \(P < 0.05\) according to Duncan’s multiple range test. \(F_v/F_m\) represents the maximum quantum yield of photosystem II photochemistry, where \(F_v\) is the yield of variable fluorescence and \(F_m\) is the maximum fluorescence in the dark adapted state; \(Y(II)\) is the actual quantum efficiency of photosystem II open centers at light adapted state; \(q_P\) is the coefficient of photochemical quenching; \(q_N\) is the coefficient of non-photochemical quenching; NPQ is the quenching due to non-photochemical dissipation of absorbed light energy; ETR is the rate of linear electron transport.
TABLE IV
Effect of inoculation with *Gaeumannomyces cylindrosporus* (+ GC) on photosynthetic characteristics\(^a\) of maize seedlings under stress of different Pb concentrations

<table>
<thead>
<tr>
<th>Pb concentration (\mu g , g^{-1})</th>
<th>Inoculation</th>
<th>(P_{n}) (\mu mol , CO_2 , m^{-2} , s^{-1})</th>
<th>TR (\mu mol , H_2O , m^{-2} , s^{-1})</th>
<th>(G_s) (mol , H_2O , m^{-2} , s^{-1})</th>
<th>(C_i) (\mu mol , CO_2 , mol^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No GC</td>
<td>2.34 ± 0.20(^b)(^c)</td>
<td>0.26 ± 0.08</td>
<td>0.02 ± 0.01</td>
<td>163.3 ± 6.77</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>4.87 ± 0.29a</td>
<td>0.42 ± 0.04ab</td>
<td>0.02 ± 0.00bc</td>
<td>115.5 ± 13.12def</td>
</tr>
<tr>
<td>50</td>
<td>No GC</td>
<td>3.33 ± 0.26b</td>
<td>0.25 ± 0.05cde</td>
<td>0.03 ± 0.00ab</td>
<td>190.3 ± 7.97c</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>5.08 ± 0.15a</td>
<td>0.47 ± 0.01a</td>
<td>0.04 ± 0.00a</td>
<td>106.7 ± 3.28f</td>
</tr>
<tr>
<td>500</td>
<td>No GC</td>
<td>1.36 ± 0.23d</td>
<td>0.21 ± 0.04de</td>
<td>0.02 ± 0.00dec</td>
<td>311.3 ± 2.33b</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>2.91 ± 0.41bc</td>
<td>0.37 ± 0.02abc</td>
<td>0.02 ± 0.01cde</td>
<td>141.0 ± 10.26de</td>
</tr>
<tr>
<td>1000</td>
<td>No GC</td>
<td>0.36 ± 0.02e</td>
<td>0.14 ± 0.01e</td>
<td>0.01 ± 0.00e</td>
<td>360.0 ± 8.08a</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>1.48 ± 0.21d</td>
<td>0.30 ± 0.04bcd</td>
<td>0.01 ± 0.00e</td>
<td>140.3 ± 15.82de</td>
</tr>
</tbody>
</table>

\(^a\)\(P_{n}\) is the net photosynthetic rate; TR is the transpiration rate; \(G_s\) is the stomatal conductance; \(C_i\) is the intercellular \(CO_2\) concentration.

\(^b\)Means ± standard errors \((n = 4)\).

\(^c\)Means followed by the same letter(s) within each column are not significantly different at \(P < 0.05\) according to Duncan’s multiple range test.

TABLE V
Effect of inoculation with *Gaeumannomyces cylindrosporus* (+ GC) on Pb uptake and translocation in maize seedlings under stress of different Pb concentrations

<table>
<thead>
<tr>
<th>Pb concentration (\mu g , g^{-1})</th>
<th>Inoculation</th>
<th>Pb content (mg , kg^{-1} , DW)^(a)</th>
<th>Translocation factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>Shoot</td>
</tr>
<tr>
<td>0</td>
<td>No GC</td>
<td>1.80 ± 0.08(^b)(^c)</td>
<td>0.62 ± 0.12e</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>1.70 ± 0.01d</td>
<td>0.41 ± 0.03e</td>
</tr>
<tr>
<td>50</td>
<td>No GC</td>
<td>24.30 ± 1.01d</td>
<td>7.20 ± 0.55cd</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>26.25 ± 1.07d</td>
<td>5.50 ± 0.87cd</td>
</tr>
<tr>
<td>500</td>
<td>No GC</td>
<td>65.64 ± 6.62c</td>
<td>14.35 ± 1.09b</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>85.48 ± 10.44c</td>
<td>8.34 ± 0.41c</td>
</tr>
<tr>
<td>1000</td>
<td>No GC</td>
<td>162.36 ± 12.38b</td>
<td>23.60 ± 1.36a</td>
</tr>
<tr>
<td></td>
<td>+ GC</td>
<td>235.82 ± 13.79a</td>
<td>16.43 ± 1.41b</td>
</tr>
</tbody>
</table>

\(^a\)Dry weight.

\(^b\)Means ± standard errors \((n = 4)\).

\(^c\)Means followed by the same letter(s) within each column are not significantly different at \(P < 0.05\) according to Duncan’s multiple range test.

Inoculated seedlings under four different concentrations of Pb (Table V). This indicated that inoculation with *G. cylindrosporus* decreased Pb translocation from roots to shoots and that the Pb toxicity to maize was also alleviated with the decrease of Pb content in shoots.

**DISCUSSION**

Recently, numerous surveys have proved that many dominant plant species in heavy metal-contaminated land are widely associated with DSE fungi (Deram *et al.*, 2008; Likar and Regvar, 2009; Li *et al.*, 2012; Ban *et al.*, 2015). High tolerance of DSE fungi to heavy metal pollution and their great abundance in stress habitats suggest that DSE fungi might have important ecological functions. The DSE fungi can be readily isolated from heavy metal-contaminated sites. Zhang *et al.* (2008) isolated 3 DSE strains from a waste smelter site in Southwest China and found that *E. pisciphila* H93 had high tolerance to Cd. In the present study, *G. cylindrosporus* used in the inoculation experiment was isolated from the roots of *A. adsurgens* growing at a metal-enriched site. After determination of the sensitivity of the isolate to heavy metal ions, the EC50 (the effective concentration of heavy metal that inhibits 50% of mycelial growth) and MICs (the minimum inhibitory concentrations) of Pb ions were up to 1.83 and 4.5 mg mL\(^{-1}\), respectively (Ban *et al.*, 2012).

In the present study, maize seedlings were successfully inoculated with *G. cylindrosporus*. Colonization intensity of maize roots by *G. cylindrosporus* increased with the increase of Pb concentrations, indicating that *G. cylindrosporus* had a high inherent tolerance or a low sensitivity to Pb. Arbuscular mycorrhizal fungi are...
known to have beneficial effects on the growth and photosynthesis of host plants under heavy metals stress (Shahabivand et al., 2012; Rozpydek et al., 2014), whereas the knowledge of how these plants respond to inoculation with DSE fungi is presently limited. In the present study, we found that the biomass and other growth indicators of the inoculated maize increased compared with those of the non-inoculated one under stress of different Pb concentrations. Host plant growth caused by inoculation with DSE fungi may be a result of improved nutrient absorption (Jumpponen and Trappe, 1998; Narisawa et al., 2007). In this study, inoculation with *G. cylindrosporus* increased the ratio of root to shoot biomass compared with the non-inoculated plants. It indicated that the inoculated plants invested more biomass on root production. The infection of DSE fungi could change root morphology and structures and promote the development of lateral and adventitious roots (Wu et al., 2010), followed by the improvement of root activity and the enhancement of its ability to absorb nutrients and water. Secretion and secondary metabolites of DSE fungi may also alter rhizosphere microenvironment and directly influence the growth of roots. Bartholdy et al. (2001) found that *Phialocephala fortinii* could synthesize hydroxamate siderophore, which increased the uptake of Fe(III) by roots. Other researchers suggested that the increased synthesis of phytohormones (Oelmüller et al., 2009; Andrade-Linares et al., 2011) and enzymatic degradation of main organic nutrients (Mandyam and Jumpponen, 2005; Newsham, 2011) may contribute to the increase of root biomass.

Heavy metals have negative impacts on the physiology of plants, which can result in a decrease in the concentrations of photosynthetic pigments, and damage the process of photosynthesis (Pajević et al., 2009; Borisev et al., 2012). In this study, inoculation of *G. cylindrosporus* increased the contents of Chl a and total Chl in the leaves of maize under Pb stress. Similar results were observed by Likar and Regvar (2013). However, the combination of Pb ions in roots by DSE was the key mechanism for improving Pb tolerance of maize, in comparison with the protection of Chl, according to the results of Table V.

As so far, no research focused on the effect of inoculation with DSE fungi on Chl fluorescence parameters of host plants under heavy metal stress. Thus, it is a first report that DSE fungi could mitigate the toxic influence of heavy metal on PSII reaction center. $F_{v}/F_{m}$ is a measure of the capacity of PSII primary photochemistry, which itself is particularly sensitive to a variety of environmental stress-inducing factors; thus, it was considered to be a reliable indicator of stress (Figueroa et al., 1997). In this study, $F_{v}/F_{m}$ in the leaves of inoculated plants was found to be significantly higher than that in the leaves of non-inoculated plants. It might indicate that inoculation of *G. cylindrosporus* could improve the resistance of maize to Pb stress and that the toxicity of heavy metal to plant body was decreased obviously. qP and Y(II) have been used as photochemical quenching parameters for monitoring the general trends in the energy dissipation adjustments in PSII (Maxwell and Johnson, 2000). The increases of qP and Y(II) in the inoculated maize suggested that inoculation with *G. cylindrosporus* improved the efficiency of PSII photochemistry. The parameter qN reflects activation of the non-photochemical processes during the light period, mostly leading to the non-radiative energy dissipation to heat (Björkman and Demmig-Adams, 1995; Roháček, 2002). qN will increase when the environmental conditions become worse. For this study, qN of non-inoculated seedlings was higher than that of inoculated seedlings under different Pb concentrations, indicating that inoculation by *G. cylindrosporus* could protect plant body from heavy metal toxicity, and the effects were significant. NPQ is an important parameter that measures a change in the efficiency of heat dissipation relative to the dark-adapted state (Maxwell and Johnson, 2000; Sheng et al., 2008). In the present study, NPQ of inoculated maize was lower than that of non-inoculated maize, which indicated that the inoculated plants have higher ability to protect leaves from light-induced damage.

Arbuscular mycorrhizal fungi are known to have significant effects on the uptake and accumulation of heavy metals of host plants. However, the knowledge of the effects of DSE inoculation on this field is presently limited. In this study, there was a decline in TF with increasing concentrations of Pb in the inoculated and non-inoculated plants. It was believed that most plants had the ability to transfer heavy metal ions from root to shoot; however, the translocation efficiency of heavy metal was decreased with the increasing concentrations of heavy metals. Heavy metals have negative impacts on the physiology of plants, which can result in decreased transpiration and photosynthesis (Pajević et al., 2009; Borisev et al., 2012). Some researchers suggested that heavy metal translocation was driven by transpiration (Kuzkovina et al., 2004; Likar and Regvar, 2013), so the decreased transpiration that caused by the toxicity of heavy metals may lead to the decline of TF. In this study, the TF of Pb in the inoculated plants was significantly decreased.
FUNGUS INOCULATION EFFECT ON MAIZE UNDER PB STRESS

CONCLUSIONS

Maize seedlings inoculated with G. cylindrosporus had higher growth performance, compared with the non-inoculated seedlings, under stress of different Pb concentrations. According to the measured results of Chl content, photosynthetic characteristics, and Chl fluorescence parameters in leaves of maize under different Pb concentrations, colonization of G. cylindrosporus could effectively decrease the damage of photosynthetic system caused by Pb stress. Colonization of G. cylindrosporus could also decrease Pb accumulation in the leaves of host plants under Pb stress, which mainly caused by the Pb biosorption of hyphae and the physiological change of host plants, resulting in the improvement of Pb tolerance of host plants.

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