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Influence of Cd^{2+} on Growth and Chlorophyll Fluorescence in a Hyperaccumulator: *Lonicera japonica* Thunb.

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Abstract Chlorophyll fluorescence is an important instrument to study the responses of plants to cadmium (Cd) stress, which in turn can provide a better understanding of Cd tolerance in plants. In the present study, the influence due to cadmium (Cd^{2+}) exposure on growth and chlorophyll fluorescence was investigated in a new hyperaccumulator—*Lonicera japonica* Thunb. Four levels of Cd^{2+} (0, 5, 25, and 125 mg kg^{-1}) were added to the soil. After 90 days of Cd^{2+} exposure, maximal photochemical efficiency of photosystem II (F_v/F_m) and effective quantum yield of photosystem II (ΦPS II) of *L. japonica* showed an increase under 5 mg kg^{-1} Cd^{2+} exposure, but decreased under higher concentrations of Cd^{2+}exposure. However, when Cd concentrations in soil were up to the highest concentrations (125 mg kg^{-1}), no significant differences of F_v/F_m, ΦPS II, photochemical quenching, chlorophyll, and carotenoid contents compared with the control were observed. These results indicate that the good tolerance of *L. japonica* to Cd might result from effective mechanisms including the capacity to maintain good growth, photosynthetic pigment composition, and chlorophyll fluorescence activity, which would be beneficial to enhance the potential for phytoremediation.

Keywords *Lonicera japonica* Thunb. · Cadmium · Hyperaccumulator · Chlorophyll fluorescence

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Introduction

Heavy metal contamination caused by human activities has become a growing problem in recent years (Chaoui and EI Ferjani 2013). Cadmium (Cd) is one of the most toxic heavy metals to all biological systems due to its high water solubility, and neurotoxic and carcinogenic effects (Tripathi and others 2013). It is accumulated in soils mainly from human activities such as sewage sludge, commercial fertilizers, and atmospheric fall-out from industrial activities. Cd is a nonessential trace element for plants and animals, and it can be easily transferred into the food chain and threatens human health (López-Millán and others 2009). It is therefore important to develop methods to remediate Cd-contaminated soils. Some traditional physical and chemical remediation methods, such as solidification/stabilization, vitrification, and electrokinetic remediation, can be expensive and ineffective for in situ Cd removal. Phytoremediation is a promising and eco-friendly technology that utilizes hyperaccumulators to extract, transform, or stabilize pollutant metals in contaminated water or soils (Regvar and others 2013). Hyperaccumulators could have the ability to accumulate extraordinarily high amounts of heavy metals in the plant organs. Presently, more than 400 species of hyperaccumulators have been reported in the world. However, within them, less than ten species have been considered as Cd-hyperaccumulators (Dahmani-Muller and others 2000). In addition, most known Cd-hyperaccumulators grow slowly with low biomass, which limits their wide use in remediation. Therefore, identifying a new Cd-hyperaccumulator that possesses rapid growth with high biomass will have phytoremediation potential for Cd-contaminated environments.

Lonicera japonica Thunb., as a popular ornamental, has become established in temperate and tropical regions

worldwide in the past 150 years. It has deep roots and its shoots may be as long as 150 cm. It possesses the characteristics of high biomass, easy cultivation, extensive competitive ability, wide geographic distribution, and strong resistance to environmental stresses (Liu and others 2009). Furthermore, *L. japonica* is also an Asian medicinal plant, and has a wide spectrum of biological and pharmacological properties. In our previous study, it was shown that *L. japonica* is a new potential Cd-hyperaccumulator (Liu and others 2011b).

Cd is known to cause chlorosis, growth inhibition, and eventually plant death which is directly or indirectly related with photosynthesis. The capacity of photosynthesis is one of the important reasons responsible for slow growth and poor survival of plants. Some studies have reported that Cd interferes with chloroplast metabolism by hampering light and dark reactions of photosynthesis (Baryla and others 2001), and other studies showed Cd-induced changes in photosynthetic apparatus, such as net photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (E) (López-Millán and others 2009). However, little information is available on the toxic Cd effects on chlorophyll fluorescence. Chlorophyll fluorescence has shown to be a useful instrument to study responses to heavy metal stress (Wodala and others 2012). Therefore, in the present study, the responses of plant growth, photosynthetic pigment composition, and chlorophyll fluorescence to different concentrations Cd^{2+} exposure were investigated, which will be helpful to have a better understanding of the Cd tolerance mechanisms in the new-found hyperaccumulator—*L. japonica*.

Materials and Methods

Plant Culture and Cd Exposure

The soil used in the pot-culture experiment was collected from the top soil (0–20 cm) in the Shenyang Botanical Garden of Chinese Academy of Sciences (41°46′ N and 123°26′ E), which is in the temperate zone with a semi-humid monsoon climate. The soil type of the tested soil is meadow burozem and has the properties including pH of 7.01, organic matter (OM) content of 4.06 %, and cation exchange capacity (CEC) of 18.97 cmol kg^{-1}. The mean concentration of extractable Cd in the soil was 0.15 mg kg^{-1}.

The air-dried soil samples were sieved through a 3-mm mesh sieve and placed into plastic pots with a diameter of 20.0 cm and a depth of 15.0 cm, each filled with 4.0 kg soil, mixed uniformly with the specified concentration of $CdCl_2{\cdot}2.5H_2O$ solution. Three 2-month-old seedlings of similar size were transplanted into each pot. Four Cd^{2+}-level treatments were applied: 0 (CK), 5, 25, and 125 mg kg^{-1}, respectively. Each Cd concentration was repeated four times in separate pots. Time domain reflectometry (TDR200, Spectrum Technologies, USA) was used to detect the soil water content in the pots. The plants were grown under open field conditions and harvested after 90 days of exposure for analysis.

Measurements of Plant Biomass and Cd Content

The harvested plants were rinsed with distilled water, and the roots were immersed in 20 mM Na_2-EDTA for 15 min to remove Cd adhering to the root surface. Then the plants were separated into leaves, stems, and roots. These portions were then separately rinsed with distilled water and finally with deionized water, wiped with tissues, and weighed. They were then dried at 105 °C for 10 min, then at 70 °C until the weight became constant.

Dried plant materials were weighed and crushed. The powders were digested with a concentrated acid mixture of $HNO_3/HClO_4$ (3:1, v/v). The concentrations of Cd in plant tissues and soils were determined using an Optima3000 ICP-AES instrument (Perkin-Elmer, USA).

Measurements of Chlorophyll Fluorescence

Chlorophyll fluorescence parameters were ground measured with a portable FMS-2 fluorometer (*Hansatech*, UK). When using FMS-2, the measuring radiation was provided by a pulsed amber LED source, whereas the "actinic light" and saturating irradiance were both provided by operating a halogen lamp. The chlorophyll fluorescence parameters were measured under different photon flux densities (PFD). First, the leaves were dark adapted for 5 min, and the initial fluorescence yield (F_0) was measured, then the maximum fluorescence yield (F_m) emitted during a pulse of saturating irradiance (4000 μmol m^{-2} s^{-1} PFD) was measured using a weak modulated measuring beam (0.12 μmol m^{-2} s^{-1}). Variable chlorophyll (F_v) was calculated as $F_v = F_m - F_0$. Maximal photochemical efficiency of photosystem II (PS II) was expressed as F_v/F_m. After illuminating action light again, F'_m emitted after illuminating a modulated radiation and a saturating radiation pulse, were measured in turn. After action light was off and far red light (1.67 μmol m^{-2} s^{-1}) was illuminated at once, the initial fluorescence yield when action light was off (F'_0) was measured. The variable chlorophyll fluorescence under the fluorescence condition (F'_v) was calculated as $F_v = F'_m - F'_0$. F_s indicated the fluorescence under the steady state. Photochemical quenching (qP) was calculated by the formula: $qP = (F'_m - F_s)/(F'_m - F'_0)$. Nonphotochemical quenching (qN) $= (F_m - F'_m)/(F_m - F_0)$. Effective quantum yield of photosystem II (ΦPS II) $= (F'_m - F_s)/F'_m$.

Measurement of Photosynthetic Pigments

The chlorophyll (CHL) and carotenoid (Car) contents were measured in 80 % acetone extract of 0.1 g leaf tissue (Liu and others 2011a).

Statistical Analyses

Average values and standard deviations (SD) were calculated using Microsoft Office Excel 2003. One-way analysis of variance was carried out using SPSS 11.0. The significant difference was set among treatments at $p < 0.05$ or $p < 0.01$. Multiple comparisons were also made by the least significant difference (LSD) test.

Results and Discussion

When the concentration of Cd^{2+} exposure in soil was 5 mg kg^{-1}, total biomass dry weight, height, and root length increased, indicating that low concentrations of Cd^{2+} exposure might have a stimulating effect on plant growth (Table 1). With the concentrations of Cd^{2+} exposure in soil increasing, Cd concentrations in roots and shoots of the plant all increased significantly (Fig. 1, $p < 0.01$). It seemed that the highest concentrations (125 mg kg^{-1}) did not affect the normal growth of *L. japonica*, and the Cd concentrations accumulated in shoots under 125 mg kg^{-1} Cd^{2+} exposure were 221.70 μg g^{-1} DW, above 0.01 % dry tissue (100 μg g^{-1}), which are considered as Cd hyperaccumulators (Baker and Brooks 1989). Furthermore, a positive correlation between Cd accumulated in roots and shoots of the plant and Cd^{2+} concentrations in soil indicated that *L. japonica* had a good potential in accumulating Cd. It is represented by the following Eqs. (1) and (2):

$$Y = 7.70X + 90.80\,(R^2 = 0.96,\ p\ <0.01) \quad (1)$$

$$Y = 1.67X + 19.26\,(R^2 = 0.94,\ p\ <0.01) \quad (2)$$

where Y is Cd accumulation in roots and shoots of the plant respectively, and X is Cd concentration in soil.

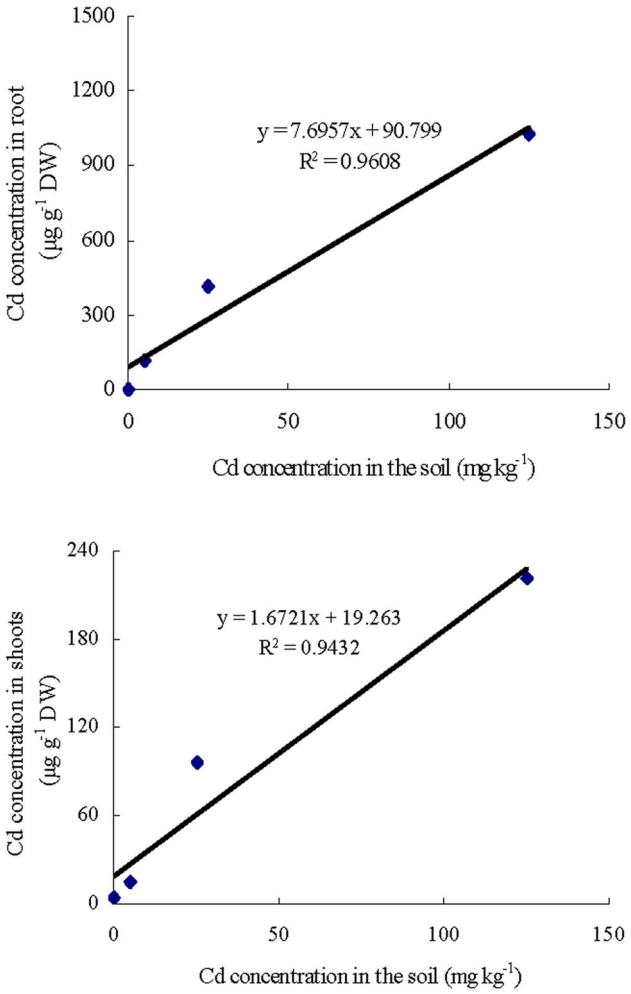

Fig. 1 Influence of Cd^{2+} in soil on root and shoot Cd concentrations in *L. japonica* after 90 days of exposure

Chlorophyll fluorescence was shown to be very sensitive to Cd^{2+} stress. According to Wodala and others (2012), PSII is one of the primary targets of Cd^{2+} stress, and chlorophyll fluorescence is widely used to monitor PSII photochemistry. After 90 days of Cd^{2+} exposure, maximal photochemical efficiency of photosystem II (F_v/F_m) and effective quantum yield of photosystem II (ΦPS II) of *L. japonica* under low concentrations Cd^{2+} exposure all

Table 1 Influence of Cd^{2+} in soil on growth in *L. japonica*

Cd concentration in the medium (mg kg^{-1})	Total biomass dry weight (g)	Height (cm)	Root length (cm)
0	16.22 ± 2.51^{a,b}	22.13 ± 3.08^{a}	53.69 ± 7.12^{a}
5	18.81 ± 4.27^{a}	23.95 ± 2.89^{ab}	55.02 ± 7.04^{a}
25	16.77 ± 6.29^{a,b}	22.36 ± 4.11^{a}	54.83 ± 5.97^{a}
125	15.69 ± 3.42^{a,b}	21.52 ± 3.25^{ab}	52.35 ± 8.93^{a,b}

Different letters within the columns indicate significant differences at the 5 % level according to the LSD test

increased, which may show an improved growth and was proposed as hormesis by de la Rosa and others (2004). With the concentrations of Cd^{2+} exposure in soil increasing, F_v/F_m, ΦPS II, and qP showed a slight decrease, and qN showed an increasing trend compared with the control (Fig. 2). When Cd^{2+} concentrations in soil were up to the highest concentrations (125 mg kg^{-1}), F_v/F_m had no significant differences compared with the control, which indicated that *L. japonica* had a good tolerance to maintain the normal functional state of PSII. Effective quantum yield of photosystem II (ΦPS II) was slightly improved under low concentrations Cd^{2+} exposure and decreased under higher concentrations Cd^{2+} exposure, which might be attributed to a reduction of PSII electron output by Cd^{2+} exposure. This is in good agreement with other studies (Burzyński and Kłobus 2004; Hattab and others 2009). In addition, qP was decreased, and qN was increased with Cd^{2+} concentrations in soil increasing, which suggested that Cd^{2+} exposure may limit ATP and NADPH consumptions, cause a high pH-gradient to inhibit electron transport capacity of PSII, and increase the proportion for nonphotochemical reactions such as heat dissipation (Krupa and others 1993).

As already known, Cd^{2+} exposure often induces visual phytotoxic symptoms and affects photosynthetic pigment composition (Valentovičová and others 2010). In the present study, after 90 days of Cd^{2+} exposure, *L. japonica* did not show any visual leaf symptoms under low concentrations (5 and 25 mg kg^{-1}) of Cd^{2+} exposure, which was in accordance with the changes of Chl and Car contents (Fig. 3). The Chl and Car contents were increased under 5 mg kg^{-1} Cd^{2+} exposure, which was related to the changes of chlorophyll fluorescence parameters (Fig. 2). According to Nyitrai and others (2003), the Chl and Car contents, as light-harvesting pigments, may have some effects on the efficiency of light capture in the antennae. With the concentrations of Cd^{2+} exposure in soil increasing, Chl and Car contents showed a slight decrease in plants, which could result from the inhibition of protochlorophyllide reduction (Stobart and others 1985) or

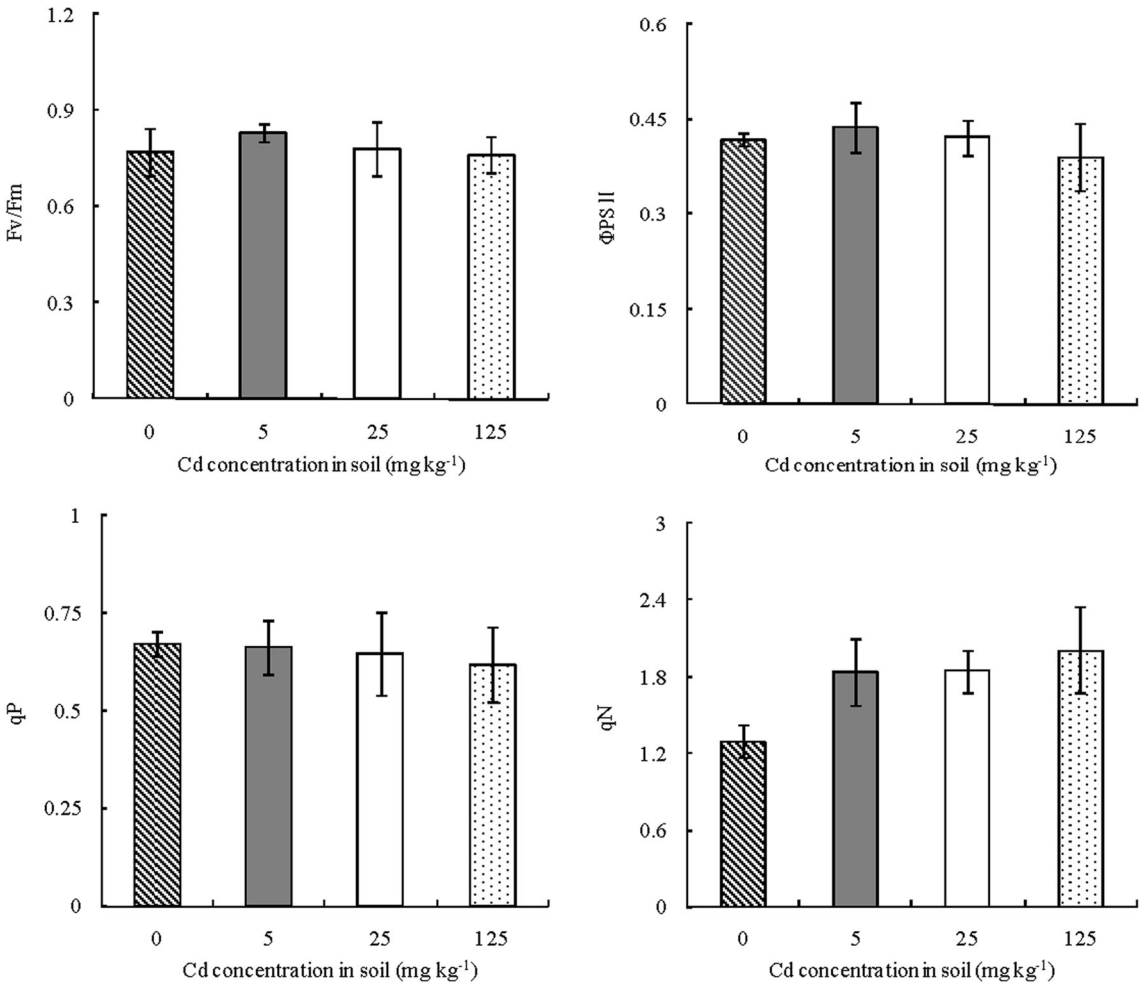

Fig. 2 Influence of Cd^{2+} in soil on maximal photochemical efficiency of photosystem II (F_v/F_m), effective quantum yield of photosystem II (ΦPS II), photochemical quenching (qP), and nonphotochemical quenching (qN) in *L. japonica*

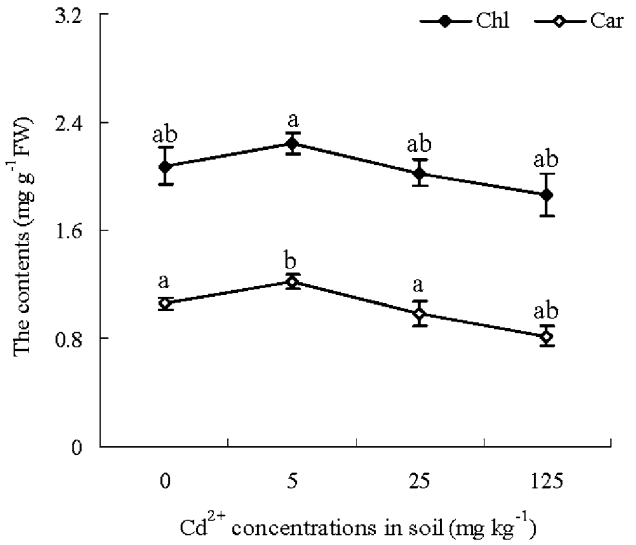

Fig. 3 Influence of Cd^{2+} in soil on chlorophyll (Chl) and carotenoid (Car) contents (mg g^{-1}FW) in leaves of *L. japonica*

Mg^{2+} substituted by Cd in the chlorophyll molecule (Küpper and others 1998).

In conclusion, in the present study, a positive correlation between Cd accumulated in roots and shoots of the plant and Cd^{2+} concentrations in soil indicated that *L. japonica* had a good capacity in hyperaccumulating Cd. With the concentrations of Cd^{2+} exposure in soil increasing, no significant differences of F_v/F_m and ΦPS II, Chl, and Car contents compared with the control were observed, indicating that *L. japonica* could develop effective tolerance mechanisms to avoid the damage caused by Cd^{2+} exposure to chlorophyll fluorescence and photosynthetic pigment composition. A stimulating effect of 5 mg kg^{-1} Cd^{2+} on total biomass dry weight, height, root length, and F_v/F_m showed an improved growth and was proposed as hormesis, which may be beneficial to enhance the potential for phytoremediation, because plants more often face low concentrations Cd^{2+} exposure in actual contaminated soils.

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Conflict of interest The authors declare that they have no conflict of interest.

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