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ACCUMULATION OF CADMIUM AND ITS EFFECTS ON PHYSIOLOGICAL CHARACTERISTICS IN *COIX LACRYMA-JOBI* L.

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Abstract

A wetland simulated experiment was employed to investigate the accumulation of cadmium (Cd) in *Coix lacryma-jobi* L. and the effects of Cd addition on the growth, photosynthesis characteristics and antioxidant enzymes activities of *C. lacryma-jobi* L. Results showed that lower concentrations of Cd (0.05 mg/l) increased the stem biomass but higher concentrations of Cd (0.125 and 2.5 mg/l) decreased that. The order of Cd accumulation is stems < leaves < roots. The accumulation of Cd in the above-ground parts were much higher than that of the below-ground parts under moderate concentrations of Cd (from 0.05 to 0.125 mg/l). Meanwhile, the moderate concentration of Cd not only increased the translocation factor of Cd and the effective photochemical efficiency of photosystem II in *C. lacryma-jobi* but stimulated the activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), despite a decrease was found in these characteristics at high Cd stress (2.5 mg/l). These results suggested that the oxidative stress may involve in the mechanism of Cd toxicity and that the tolerance of *C. lacryma-jobi* to Cd since the activities of SOD, POD and CAT were stimulated by moderate concentrations of Cd.

Introduction

Cadmium (Cd) is regarded as one of the most toxic heavy metals in ground water, soil and sediments due to its wide industrial application, hence posing a serious environmental concern. It can undermine the ecosystem function and human health at trace level due to its high toxicity (Zhi *et al*. 2016). Being a highly toxic metal pollutant of soil, Cd, which has detrimental effects on plant growth and nutrients uptake (Johna *et al*. 2009, Ahmad *et al*. 2015), is highly mobile between soil-plant systems and can be quickly absorbed by plants and hence transported to upper parts causing toxicity (Irfan *et al*. 2014). Hence, knowledge of Cd - plant interactions is important for the safety of the environment.

In plants, Cd is readily taken up by the cells of different plant species and induces many morphological, physiological, biochemical and structural changes (Moussa and Alla 2016, Shahid *et al*. 2017). Numerous studies indicated that excessive amount of Cd may cause nutrient deficiency, disruption of ATPase activity, decrease of photosynthesis, inhibition of various enzyme activities, induction of oxidative stress including alterations in enzymes of the antioxidant defense system, even reduce genotoxicity by reducing of nuclear DNA content and damaging DNA structure (Johna *et al*. 2009, Zhang *et al*. 2010, Douchiche *et al*. 2012, Moussa and Alla, 2016, Shahid *et al*. 2017). To avoid Cd toxicity plants adopt various defense strategies including antioxidant defense system for managing overproduction of ROS and evading oxidative stress, vacuolar compartmentalization, phytochelation and sequestration (Shahid *et al*. 2017). Previous studies also showed that subcellular accumulation of Cd in plants varied greatly not only among plant species, cultivars and genotypes but also among different environmental chemical properties

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of the same species, and the plants' Cd detoxification and tolerance mechanisms (Liu *et al* 2017, Shahid *et al*. 2017). Apparently, plant species in different environments would show a wide range of plasticity in Cd tolerance.

Coix lacryma-jobi L., belonging to Gramineae, is a very important crop. The species is widely cultivated as a food plant and as a medicinal plant in East Asia and Southeast Asia. Earlier studies on the species were mainly focused on its biological characteristics, medicinal components and their efficacy, and on its genetics and genomics (Woo *et al* 2007, Cai *et al*. 2014). Despite being widely cultivated, the effect of Cd stress on *C. lacryma-jobi* is not still clear and needs further investigations. Based on the above studies, our investigations aimed at exploring the effects of Cd stress on photosynthesis, chlorophyll fluorescence parameters and antioxidant enzymes activities of *C. lacryma-jobi* by using a simulate wetland environment since it always grows on waterlogged farmland.

Material and Methods

To simulate the wetland environment, a mixed medium of soil and water was used. The tested soil belongs to red clay. Surface soil (0 - 20 cm) sample and water was collected from the garden of the Guangxi Institute of Botany and the basic physiochemical properties were analyzed (Table 1). Tested soil (5 kg) was potted in each plastic tackle and kept 4 litres of water on soil. The treatments included control (no Cd) and five doses of Cd chloride i.e. 0, 0.01, 0.025, 0.05, 0.125 and 2.5 mg/l Cd.

Table 1. Basic physiochemical properties of tested soil and water.

	Physiochemical properties				
Soil	PH	TN(mg/l)	TP(mg/l)	TK(g/kg)	T-Cd (mg/kg)
	6.70	1.167	0.802	0.782	0.011
Water	PH	DO(mg/l)	SpC (μs/cm)	$[Ca^{2+}]$(mg/l)	T-Cd (mg/l)
	7.38	1.56	235.00	53.00	0.001

TN =Total nitrogen. TP = Total phosphorus. TK = Total Kalium. T-Cd = Total cadmium. DO = Dissolved oxygen. SpC = Specific conductance.

After being cultivated for 120 days, chlorophyll fluorescence parameters, photosynthetic gas exchange parameters, and the activity of antioxidant enzymes were determined. After that, the plants were harvested; carefully washed with tap water and deionized water; separated into leaves, stems, and roots; the parts cut into bits with a pair of stainless steel scissors, and dried at 40°C for 48 hrs for elemental analysis. Total Cd in the plant material was estimated, following the protocol of Liu *et al.* (Liu *et al*. 2017), after digesting the oven-dried samples (100 mg each).

Chlorophyll fluorescence parameters were determined by the method described by Lichtenthaler *et al*. (2005) using a portable fluorometer (Monitoring-PAM, Walz, Germany) separately. The topmost fully expanded leaves of treated and control plants were first light- and dark-adapted for 20 min to obtain F and Fo. The Fm'and Fm values (maximum fluorescence yield of light- and dark-adapted leaves, respectively) were calculated with a saturation pulse, and then the maximum photosystem II quantum yield was calculated by the formula [(Fm - Fo)/ Fm=Fv/Fm]. The quantum yield of PSII electron transport (yield), yield = (Fm - F)/Fm, was

determined according to Genty *et al*. (1989). All measurements were taken from five plants of each replication during 8:00 to 11:00 a.m.

The activity of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) was assayed by following the protocols of Asthir *et al*. (2010) with a slight modification. Leaves (samples of 0.3 g each) were homogenized in 5 cm^3 of ice-cold 50 mM phosphate buffer (pH 6.5 for POD and SOD and 7.5 for CAT). The extracts were centrifuged at 10 000 g for 20 min at 0 - 4 °C in a Beckmann refrigerated centrifuge, and the supernatants were used for the enzyme activity assays.

Data analyses were performed using SPSS (18.0). Variance analysis (ANOVA) was performed on experimental data. For mean separations, Duncan's multiple range test (DMRT) was used at $p < 0.05$ (Patterson 1981).

Results and discussion

In general, Cd application decreased the biomass of roots (except at 0.01 mg/l), while no effects on the leaves biomass (except at 0.025 mg/l) (Table 2). Although lower level Cd treatments (from 0.01 to 0.025 mg/l) did not show statistically significant differences compared with control, moderate concentrations of Cd (0.05 mg/l) increased the stems biomass but higher concentrations of Cd (0.125 and 2.5 mg/l) decreased that (Table 2). Especially, the low level (0.025 mg/l) and moderate (0.05 mg/l) level Cd increased the leaves and stems biomass, respectively (Table 2). These results suggest that appropriate application of Cd can stimulated the growth of *C. lacryma-jobi* by increasing its leaf biomass or stem biomass disputing its inhibited to root of this species in a simulate the wetland environment. However, higher level Cd impacted the growth of *C. lacryma-jobi* by preventing its root and stems growth. That is in agreement with the results reported in other plant species, by reducing soil microbes, damage root tips, reduce nutrient and water uptake by plants and impair photosynthesis, leading to growth inhibition of plants (Ahmad *et al*. 2015, Jali *et al*. 2016).

Table 2. The biomass of *C. lacryma-jobi* after four months of growth in the Cd-contaminated soil-water medium.

Concentration (mg/l)		0.00	0.001	0.025	0.05	0.125	2.50
Biomass (g)	Leaves	7.60^{b}	10.75^{ab}	13.82^{a}	9.35^{bc}	7.94^{b}	8.14^{b}
	Stems	16.88^{b}	19.74^{ab}	13.93^{bc}	21.47^{a}	10.76^{cd}	6.71^{d}
	Roots	12.28^{a}	11.00^{a}	5.30^{b}	6.31^{b}	5.59^{b}	5.01^{b}

Data with different superscript letters indicate a significant difference at $p < 0.05$.

The order of Cd accumulation is stem < leaves < roots. The accumulation of Cd in the roots (at all Cd level), leaves and stems (both only from 0 to 0.125 mg/l) showed a positive correlation with Cd concentration. The maximum accumulation of Cd in parts above the ground (including leaves and stems) occurred at 0.125 mg/l of Cd and its translocation factor can up to 1.57, the actual amounts being 20.03 mg/kg in stems, 32.26 mg/kg in leaves, and 35.74 mg/kg in roots (Fig. 1). In addition, the accumulation of Cd in soil also showed a positive correlation with Cd concentration (Fig. 1). Obviously, the accumulation of Cd in the parts below the ground was much higher than those of the parts above the ground under lower levels Cd (from 0.01 to 0.025 mg/l) and the highest level (2.5 mg/l). On the contrary, the accumulation of Cd in the parts above the ground was much higher than that in the below-ground parts under moderate levels of Cd (from

0.05 to 0.125 mg/l). Although many workers have reported that Cd is accumulated more in the roots than that of shoots of plants such as soybean (Shamsi *et al*. 2010), some Asteraceae plants (De Maria *et al*. 2013) and Solanaceae plants (Çikili *et al*. 2016), Song *et al*. (2014) found that, at solutions containing 200 mM Cd, the concentration of Cd in shoots was 3 times more than that of the roots. This suggests that *C. lacryma-jobi* could have a higher capacity to uptake Cd and have different mechanisms of tolerance, physiology of transport, and accumulation to Cd.

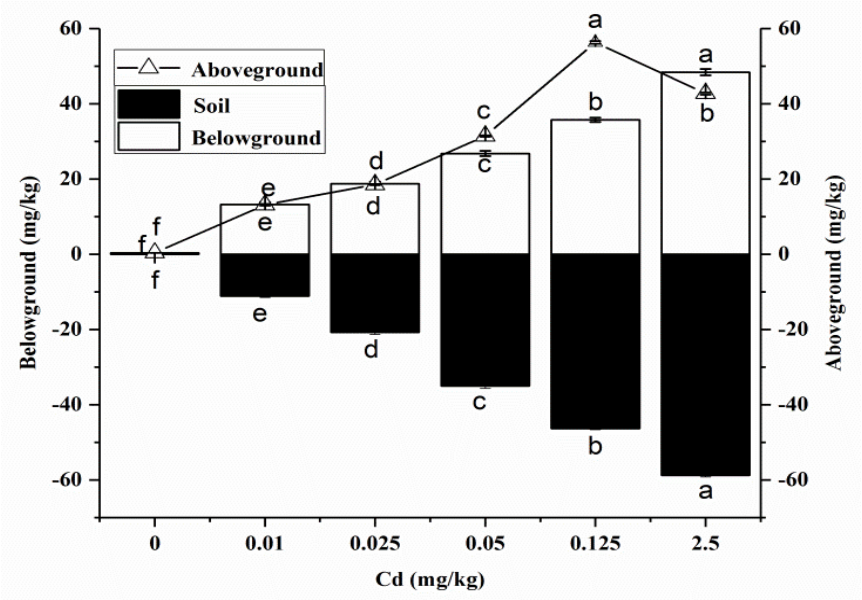

Fig. 1. Accumulation of Cd in soil, the and parts above and below the ground of *C. lacryma-jobi* after 4-months of cultivation. Different lowercase letters on the top of the bars denote significant differences ($p < 0.05$, $n = 4$) among different Cd treatments in the same part of *C. lacryma-jobi*.

There were no differences on relative chlorophyll content compared to control except that at 2.5 mg/l (Table 3). Moderate concentrations of Cd (0.05 and 0.125 mg/l) increased the yield while high concentrations of Cd (2.5 mg/l) decreased it. Cd application signifficantly decreased Fv/Fm while only higher concentrations of Cd (0.125 and 2.5 mg/l) decreased Fv/Fo (Table 3). Apparently, high level Cd (2.5 mg/l) treatments not only decreased the biomass of *C. lacryma-jobi* L. and its translocation factor to Cd, but affected photosynthesis systems of this plant. The possible reasons may be that, in Cd treated plants, reduction occurred in chlorophyll of plants which is mainly connected to its biosynthesis (Stobart *et al*. 1985). Since F_0 fluorescence originates from chlorophyll a antennae associated with the PS II light harvesting complex, yield provides a more realistic impression of the overall leaf photosynthetic condition when the plant is under heavy metals stress (Wang *et al*. 2016), and plants are exposed to metal/metalloid stresses, decline in Fv/Fm and Fv/Fo indicates a disturbance in or damage to the photosynthetic apparatus (Li *et al*. 2015). These results indicated PSII reaction centers were seriously damaged when Cd concentrations were 2.5 mg/l in soil-water medium. However, high photosynthetic activity, measured as yield and Fv/Fm, was observed, indicating that the photoactivation of PSII was stimulated by moderate concentrations Cd toxicity.

In plants exposed to Cd, the activity of SOD, POD and CAT was all stimulated by moderate concentrations of Cd (from 0.025 to 0.125 mg/l) but inhibited by high concentrations of Cd (2.5

mg/l) (Fig. 2). Obviously, the activity of the three antioxidant enzymes has same responses to the toxicities of Cd within the tested concentration of Cd. In general, the activity of SOD coordinated with the activity of CAT and POD play a central protective role in the $O_2^{\cdot-}$ and H_2O_2 scavenging process since SOD catalyzes the disproportionation of $O_2^{\cdot-}$ to H_2O_2 and O_2, and both of the POD and CAT are two of the major antioxidant enzymes that eliminate hydrogen peroxide by converting it into oxygen and water. In present study, moderate concentrations of Cd (from 0.025 to 0.125 mg/l) stimulated all the activities of SOD, POD and CAT indicating that *C. lacryma-jobi* has a strong tolerance to the Cd stress in the soil-water medium since plant tolerance to heavy metal stress having been correlated with efficient antioxidative defense system (Asthir *et al*. 2010, Irfan *et al*. 2014). These results indicate that oxidative stress is involved in the mechanism of Cd toxicity and that the tolerance of *C. lacryma-jobi* to Cd is achieved, at least in part, through the increased activity of antioxidant enzymes.

Table 3. Chlorophyll fluorescence parameters and gas exchange parameters of *C. lacryma-jobi* in different level Cd treatments.

Concentration (mg/l)	Chlorophyll fluorescence parameters			
	SPAD	Y	Fv/F_0	Fv/Fm
0.00	26.59^{a}	0.77^{b}	4.15^{a}	0.92^{a}
0.01	22.18^{a}	0.79^{b}	4.13^{a}	0.87^{ab}
0.025	27.78^{a}	0.75^{b}	4.29^{a}	0.74^{b}
0.05	28.87^{a}	0.81^{ab}	4.06^{ab}	0.77^{b}
0.125	23.77^{a}	0.85^{a}	3.30^{bc}	0.71^{bc}
2.50	20.81^{b}	0.57^{c}	3.10^{c}	0.68^{c}

Data with different superscript letters indicate a significant difference at $p < 0.05$. Abbreviations: SPAD, the relative chlorophyll content; Y, the effective photochemical efficiency of photosystem II; Fv/F_0, the effective photochemical efficiency of photosystem II; Fv/Fm, the potential photochemical efficiency of photosystem II.

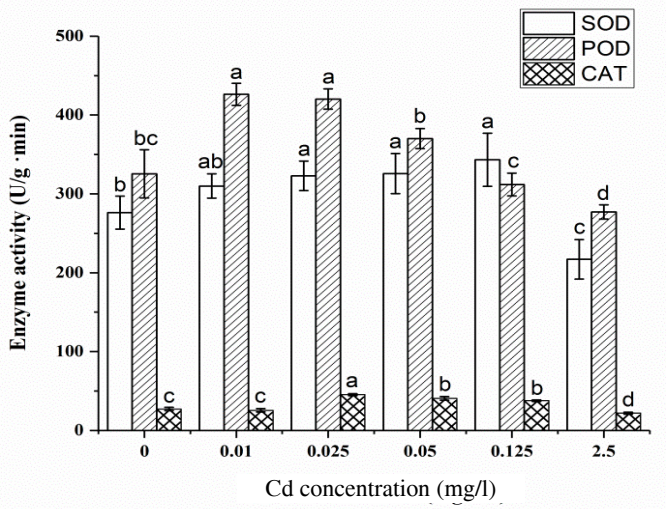

Fig. 2. Effect of Cd stress on superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity in leaf of *C. lacryma-jobi* different lower case letters on the top of the bars denote significant differences ($p < 0.05$) among different Cd treatments.

Based on the analysis of the above, appropriate application of Cd can stimulated the growth of *C. lacryma-jobi* by increasing its leaf biomass or stem biomass disputing its inhibited to root of this species in the simulate wetland environment. Increased yield value may involve the increasing of leaf biomass or stem biomass since photoactivation of PSII was stimulated by moderate concentrations Cd toxicity supported by increased Yield and Fv/Fm. Furthermore, the oxidative stress may involve in the mechanism of Cd toxicity and that the tolerance of *C. lacryma-jobi* to Cd since the activities of SOD, POD and CAT were stimulated by moderate concentrations of Cd (from 0.025 to 0.125 mg/l).

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