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Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard

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ABSTRACT. In this study, we determined the effects of different concentrations of soil cadmium (0-24 $mg \cdot kg^{-1}$) on growth and photosynthetic activities in leaves of pakchoi and mustard using pot experiments under greenhouse conditions. Both shoot and root weight decreased progressively with increasing Cd concentration for pakchoi and mustard. Negative linear relationships were observed between total Chlorophyll contents and Cd concentrations in soils in pakchoi (r^2=-0.681, P<0.01) and mustard (r^2=-0.463, P<0.05). In comparison to the controls (Cd=0 $mg \cdot kg^{-1}$), significant reductions of chlorophyll-a and chlorophyll-b were both observed at 24 mg Cd kg^{-1}soil. The increase in cadmium concentration also caused a decline in the net rate of photosynthesis (P_n) and stomatal conductance (G_s). At lower concentrations of Cd (e.g. 6 $mg \cdot kg^{-1}$), a retarded development was observed only in mustard. However, a significant decrease of G_s for both plants was observed at concentrations above 12 mg Cd kg^{-1} soil. From this study, we found the photosynthetic activity of pakchoi more sensitive to Cd stress than that of mustard.

Keywords: Cadmium; Growth; Photosynthetic activities; Pigments.

INTRODUCTION

Heavy metals have been increasingly found in soils due to atmosphere deposition, sludge, sewage irrigation, utilization of metal-containing farmyard manures and fertilizers, and industry and mine residues, resulting in a potential risk for human health when thse metals are transferred from crops to the human diet. They play an important role in the environment not as a result of human activity but also as toxic species above certain concentrations (Ngayila et al., 2008; 2009). At high concentrations, a number of heavy metals have been reported to inhibit the growth and decrease the productivity of crops (Liu et al., 2003). Among them, cadmium (Cd) is well known as a highly toxic environmental element due to its great toxicity and high mobility from soil to plants and further down the food chain (Vig et al., 2003). It can be incorporated and accumulated by all organisms in large amounts and disturb physiological metabolisms in plants like transpiration, photosynthesis, respiration, and nitrogen assimilation (Chugh et al., 1999; Zhou et al., 2006; Wang et al., 2008). Additionally, Cd is a divalent heavy metal cation (Cd^{2+}) which is readily taken up and causes phytotoxicity. Cd excess in the environment decreases chlorophyll content (Shakya et al., 2008) and growth (Zhou and Qiu, 2005), affects chloroplast function or CO_2 fixation (Krupa and Baszynski, 1995; Seidlecka et al., 1997). Peter Faller et al. (2005) showed that Cd^{2+} inhibited photoactivation of photosystem II by competitive binding to the essential Ca^{2+} site. Earlier investigations have demonstrated that the net rate of photosynthesis can be conspicuously decreased with increasing concentrations of Cd (Lakshaman and Surinder, 1999). Studies on the dose-response of plants to Cd stress have shown that stomatal conductance varies with different Cd^{2+} concentrations (Chugh et al., 1999). Furthermore, the photosynthetic responses of monocotyledons to a stress factor have been observed as have those of dicotyledons (Drażkiewicz and Baszyński, 2005).

Thus, in our study, we chose to investigate pakchoi cabbage, which stood in for monocotyledons, and mustard, which represented dicotyledons. Both plants are very common in China, especially in the northeast, and are well known for their tolerance to heavy metals such as Cd (Fang et al., 2004; Xu et al., 2007). Cadmium, a major pollutant due to its great toxicity and high mobility in soil-plant-human (or animal) systems, was examined for its toxicological effects on chlorophyll content and on some photosynthetic parameters (e.g. photosynthetic rate and stomatal conductance) in leaf vegetables that are essential for human health. In addition, growth parameters were also determined. Therefore, the aim of this paper was to characterize the changes in growth parameters and photo-

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synthetic activity in two types of vegetables representing monocotyledons and dicotyledons and to relate them to defense strategies against Cd toxicity.

MATERIALS AND METHODS

Some abbreviations used in this article were as follows: TOC means total organic carbon; P_n means the net rate of photosynthesis; G_s means the stomatal conductance; Chl-a means chlorophyll-a contents; Chl-b means chlorophyll-b contents.

The tested soil was collected from an agricultural field in the suburb of Shenyang, Liaoning Province, China. This soil is grey meadow soil, and chemical analysis showed that its soil bulk density, total organic carbon (TOC), total N, total P, and pH were 1.33±0.09 $g \cdot cm^{-3}$, 1.67±0.13%, 0.91±0.11 $g \cdot kg^{-1}$, 0.47±0.24 $g \cdot kg^{-1}$, and 7.63±0.57, respectively. Surface soil (0-20 cm) samples, which were ground to pass through a 4-mm nylon sieve, were used in the pot-culture experiment. 5.5 kilograms of soils were mixed with $CdCl_2 \cdot 5H_2O$ solutions for a series of soil Cd levels of 3 (T1), 6 (T2), 9 (T3), 12 (T4), 24 (T5) $mg \cdot kg^{-1}$ dry soil, and treatment without adding Cd was regarded as control (T0). Soluble fertilizers including 150 mg N, 200 mg P and 300 mg K kg^{-1} dry soil were applied at the same time. Soils were mixed sufficiently and then placed into plastic pots (20 cm×20 cm×20 cm), incubated for two weeks. Each treatment was replicated five times. This experiment was carried out in a greenhouse.

Leaf vegetables used in this investigation were pakchoi (*Brassica campestris* ssp.) and mustard (*Brassica juncea* Czernajew), which were cultivated until 4-5 cm height in seedbeds, and then transplanted into each pot. Each pot had three plants. All the plastic pots were buried in the earth randomly in the greenhouse situated in the Shenyang Station of Experimental Ecology, Chinese Academy of Sciences (123°41' N and 41°31' E), and watered daily to maintain around 75% of the field water holding capacity during the whole test period. The tested vegetables were harvested after 60 days when they reached their physiological maturity. They were washed thoroughly first with running tap water followed by distilled water and then dried at 105ºC for 10 min. and thereafter at 80ºC until completely dry.

Pigments were extracted by grinding 0.2 g freshly sampled leaves in 80% (v/v) acetone/water at room temperature for 24 h in the dark according to Arnon (1949) and Wellburn (1994) with some modifications. Photosynthetic pigments of all the samples were extracted in triplicate to minimize experimental errors. Chlorophyll and carotenoid contents were measured by using absorbance recorded at 647 nm, 663 nm and 470 nm for maximum absorption of chlorophyll-a, chlorophyll-b and carotenoid, respectively. The extinction coefficients were determined by a UV-Vis spectrophotometer (SPECORD50; Analytik Jena AG; Germany). Pigment contents were calculated in $mg \cdot g^{-1}$ fresh weight by applying the absorption coefficient equations described by Lichtenthaler (1987).

Analysis of photosynthetic parameters, the net rate of photosynthesis (P_n) and stomatal conductance (G_s) were carried out on the third (fully expanding) leaves of each entire plant just prior the harvest, using an open gas exchange system (Li-6400; Li-Cor Inc., NE, USA). All the measurements were made between 9:30 and 11:30 am. These determinations were recorded on five plants in a treatment.

Based on the data obtained from the experiment, the results presented are the mean ± standard deviation (SD) gained from at least three replicate samples using Microsoft Office Excel 2003. Statistical analysis by the least significant difference (LSD) for multiple comparisons, taking $P<0.05$ as significant, was calculated by SPSS 13.0.

RESULTS

Effect of Cadmium on plant growth

Exposing leaf vegetables to different levels of Cd resulted in reductions of growth as shown in Table 1. A retarded development in Cd-treated plants compared to the controls was observed. In the presence of 12 mg Cd kg^{-1} soil, significant reduction was found in shoot weight for pakchoi ($P<0.05$), and a marked decrease in root weight was observed at 6 mg Cd kg^{-1} soil. Compared with the non-treated mustard, shoot weight in mustard was significantly suppressed at 9 mg Cd kg^{-1}soil, and root weight was noticably decreased at 12 mg Cd kg^{-1} soil. Furthermore, the Cd tolerance index in root was less than in shoot.

Effect of Cadmium on photosynthetic pigments

The expanding leaves of mustard had higher total chlorophyll than those of pakchoi (Figure 1). Chlorophyll-b concentration was significantly lower than that of chlorophyll-a in both tested plants ($P<0.01$). The pigment contents in plants showed an almost linear decrease in response to Cd concentration increases in the soil, which ranged from 0 $mg \cdot kg^{-1}$ (the control) to 24 $mg \cdot kg^{-1}$. Nega-

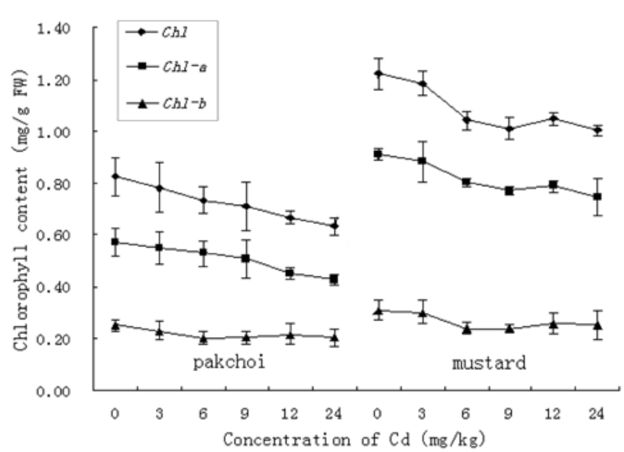

Figure 1. Impacts of Cd on the chlorophyll contents of pakchoi and mustard. Values are average of five replicates.

Table 1. Shoot and root weight in plants exposed to different Cd concentrations.

Plants	Cd concentration ($mg\cdot kg^{-1}$)	Shoot		Root	
		Dry Weight ($g\cdot plant^{-1}$)	Cd tolerance index (%)	Dry Weight ($g\cdot plant^{-1}$)	Cd tolerance index (%)
Packchoi	0 (Control)	8.99±0.66a	100.0	0.90±0.10a	100.0
	3	8.35±0.29ab	92.9	0.81±0.05ab	89.7
	6	8.09±0.24ab	90.0	0.74±0.09bc	82.6
	9	7.68±0.84ab	85.4	0.72±0.05bc	80.6
	12	6.71±1.68b	74.6	0.64±0.05c	70.8
	24	6.66±1.53b	74.0	0.47±0.02c	52.8
Mustard	0 (Control)	7.31±0.09a	100.0	1.20±0.09a	100.0
	3	7.12±0.13ab	97.4	1.15±0.08ab	95.9
	6	6.98±0.62ab	95.5	0.94±0.06b	78.2
	9	6.54±0.40b	89.5	0.79±0.10b	65.7
	12	6.41±0.54b	87.7	0.62±0.14c	51.2
	24	5.61±0.45c	76.8	0.51±0.03c	42.4

Cadmium tolerance index was calculated as the ratio of shoot or root dry weight at Cd supply to that of control. Means ± SD with different letters are significantly different from each other ($P\leq 0.05$) according to LSD (n=5).

tive linear relationships were observed between total Chl contents and Cd concentrations in soils in pakchoi (r^2=-0.681, $P<0.01$) and mustard (r^2=-0.463, $P<0.05$). Chlorophyll-a content was conspicuously greater than that of chlorophyll-b in all test plants. Additionally, parallel decreases in Chl-a and Chl-b due to Cd stress have also been observed. Significant reductions of Chl-a and Chl-b were also observed at 24 mg Cd kg^{-1} soil. In comparison to the control (Cd=0 $mg\cdot kg^{-1}$), the Chl-a in pakchoi and mustard decreased by 32.29% and 20.56% at 24 mg Cd kg^{-1} soil. However, the decreases in the Chl-b in pakchoi and mustard were 21.54% and 21.55% with 24 mg Cd kg^{-1}soil in comparison to the control. The highest carotenoid content was measured in the control plants of test vegetables, and these contents decreased with increasing Cd concentration (Figure 2). The content of mustard was higher than that of all Cd treatments.

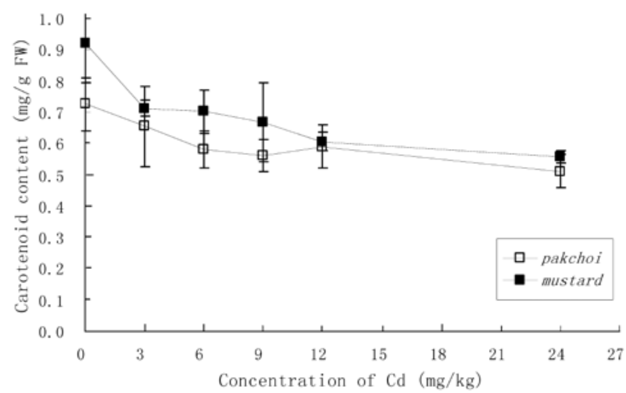

Figure 2. Impacts of Cd on the carotenoid content of pakchoi and mustard. Values are average of five replicates.

Effect of Cadmium on photosynthesis

Exposing plants to different levels of Cd resulted in changes to the photosynthetic parameters, P_n and G_s in leaves, as shown in Figure 3. The effect of Cd on these parameters declined progressively with the increasing concentration of applied cadmium. In comparison to control, the P_n of pakchoi at 12 mg Cd kg^{-1} soil decreased ($P<0.05$) by 9.33% and reached the bottom at 24 mg Cd kg^{-1} soil (Figure 3a). Mustard supplied with 6 mg Cd kg^{-1} soil showed a significant decline in the rate of photosynthesis, with a decline of 9.02%. Cadmium also exerted a profoundly deleterious effect on stomatal conductance (G_s) (Figure 3b). Compared with theG_s of non-treated (control) plants, the G_s of pakchoi was suppressed by 12.26% at the highest Cd concentration (Cd=24 $mg\cdot kg^{-1}$). However, the mustard G_s was depressed by 15.95% at 12 mg Cd kg^{-1} soil.

DISCUSSION

Parameters of plant growth like biomass have been shown to be very sensitive to heavy metals in higher plants (Arun et al., 2005). Our research has clearly illustrated that cadmium inhibited plant growth. Both shoot and root weight decreased progressively with increasing Cd concentration for both vegetables, and significantly higher reductions of weight were observed at lower Cd concentration in root than in shoot (Table 1). Low concentrations of Cd retard root growth without toxic effects in leaves, and moderately higher concentrations severely inhibit root growth and lead to Cd accumulation in leaves (Prasad, 1995). Cadmium affected root more than shoot, leading to a lower tolerance index for root. The phenomenon can be attributed to the fact that roots are the first organs receiving cadmium ions in soils via apoplastic transport, resulting in a higher Cd accumulation there (Drażkiewicz et al.,

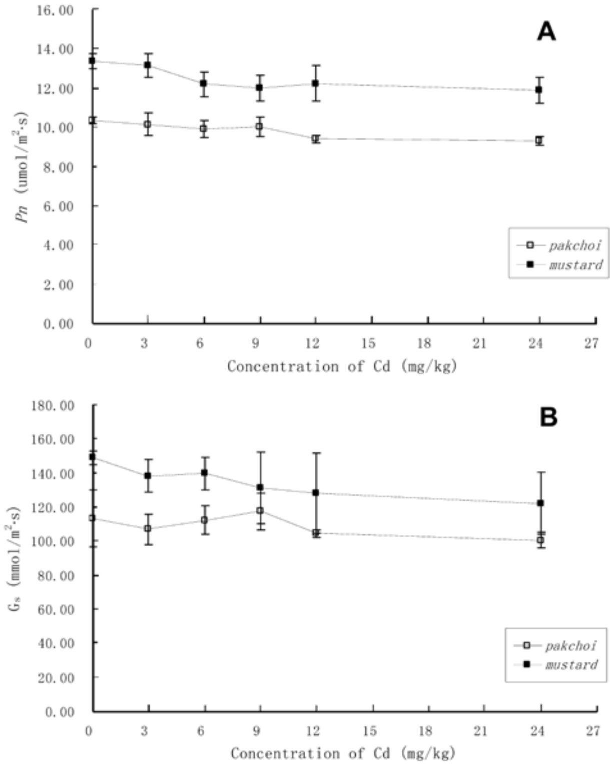

Figure 3. Impacts of Cd on the net rate of photosynthesis (P_n) and stomatal conductance (G_s) of pakchoi and mustard. Values are average of five replicates.

2003). Heavy metals such as Cd can efficiently inhibit the synthesis of proteins, such as phosphoenolpyruvate carboxylase (Stiborová et al., 1986).

In agreement with earlier reports (Papazpglou et al., 2005; Sun et al., 2008), we find that photosynthetic activity is suppressed by heavy metals. This can be attributed to the disruptive action of metals on chlorophyll synthesis (Vajpayee et al., 2000), on photosystem efficiency (Chugh et al., 1997), on the activity of photosynthetic enzymes (Mobin and Nafees, 2007), and on plant water balance (Zhou and Qiu, 2005). It is also attributable to chloroplast damage (Baszyński et al., 1988). The levels of pigment content were affected negatively by the presence of cadmium. The chlorophyll contents of vegetables decreased with increasing Cd concentrations (Figure 1). Similar results have been obtained in other laboratory studies (Jiang et al., 2007). Continuous metabolization of chlorophyll in plants is adapted to different physiological processes. In the process of chlorophyll synthesis, chlorophyll-a can be synthesized earlier, and transformed to Chlorophyll-b (Guo et al., 2006). Therefore, the similar trend of Chl-a and Chl-b was noted as shown in Figure 1. Chlorophyll-a content exceeded that of chlorophyll-b in all test plants, which has been proven by other researchers (Mobin and Nafees, 2007; Yasemin et al., 2008). In addition, high concentrations of heavy metals can degrade the activities of photosynthetic enzymes and block the photosynthetic electron transport chain, resulting in reduction of chlorophyll content (Thapar et al., 2008). It was reported that cadmium caused a decline in carotenoid content (Thapar et al., 2008). In this study, the carotenoid content of both vegetables fell as cadmium concentrations increased (Figure 2). In our experiment, the highest net rate of photosynthesis (P_n) was observed in the control plants of test vegetables, and it declined with increasing Cd concentration (Figure 3a). Compared with the controls, significant decreases of photosynthetic rate and shoot weight for pakchoi were observed after 12 mg Cd kg^{-1} soil. However, in mustard the net rate of photosynthesis showed sensitivity to lower Cd concentrations than did plant growth. We conclude that mustard is more sensitive to net rate of photosynthesis changes than is pakchoi under Cd stress. Therefore, it is deduced that P_n can be used as a bioindicator of photosynthesis in plants undergoing metal stress. Cadmium had no significant effects on stomatal conductance from 3 to 9 mg Cd kg^{-1} soil in tested vegetables, but a significant decrease of G_s was observed after 12 mg Cd kg^{-1} soil (Figure 3b). The decrease in stomatal conductance in response to Cd treatment was accompanied by a decline in the net rate of photosynthesis. Similar results have been reported by other researchers (Dunand et al., 2002). According to our results, the physiological responses of tested plants to cadmium exposure were much more conspicuous than was growth. Furthermore, Cd affects the photosynthetic activity of mustard via inhibiting chlorophyll biosynthesis and photosynthetic rate while stomatal conductance was not sensitive to Cd at moderately higher concentrations. In pakchoi, chlorophyll content, the net rate of photosynthesis, and stomatal conductance all can be inhibited by cadmium, which results in a significant reduction of shoot weight. Based on the results, we can conclude that the resistance of pakchoi to Cd is greater than that of mustard in respect to photosynthetic activity and growth.

From this study, it can be concluded that certain concentrations of Cd inhibit plant growth, cause chlorophyll loss, and affect photosynthetic activities. The photosynthesis of pakchoi seems to be more sensitive to Cd stress probably due to more rapid damage in the cell membrane and to the biosynthesis of the photosynthetic enzymes of this species. Therefore, further investigation on a cellular or molecular level is necessary to understand the mechanism behind the differences in Cd reaction between the monocotyledon pakchoi and the dicotyledon mustard.

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鎘脅迫對小白菜、油菜生長和光合活性的影響

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本文研究了溫室盆栽條件下，不同濃度鎘對小白菜和油菜生長及光合作用的影響。結果表明：小白菜和芥菜地上、地下幹重隨 Cd 濃度升高而減少，葉綠素含量與土壤 Cd 呈顯著負相關，相關係數分別為 -0.681 和 -0.463。Cd 濃度達到 24 mg · kg^{-1} 時，葉綠素 a 和葉綠素 b 含量顯著低於對照處理。Cd 濃度升高會降低淨光合速率（P_n）和氣孔導度（G_s）。在較低 Cd 濃度（6 mg · kg^{-1}）時，只有芥菜的淨光合效率受到了顯著抑制，而當 Cd 濃度達到 12 mg/kg，小白菜和芥菜的氣孔導度均顯著低於對照處理。研究發現，小白菜光合活性對鎘脅迫較芥菜敏感。

關鍵詞：鎘；生長；光合活性。