ORIGINAL PAPER

Salicylic acid-mediated alleviation of cadmium toxicity in hemp plants in relation to cadmium uptake, photosynthesis, and antioxidant enzymes

G. R. Shi · Q. S. Cai · Q. Q. Liu · L. Wu

Abstract To assess the role of salicylic acid (SA) in alleviating cadmium (Cd) toxicity in hemp (*Cannabis sativa* L.) plants, the growth parameters, Cd accumulation, photosynthetic performance and activities of major antioxidant enzymes were investigated in hemp seedlings treated with 500 μM SA, under 0, 25, 50, and 100 mg Cd kg^{-1} sands (DW) conditions, respectively. Cd exposure resulted in a small reduction in biomass (12.0–26.9% for root, and 8.7–29.4% for shoot, respectively), indicating hemp plants have innate capacity to tolerant Cd stress. This was illustrated by little inhibition in photosynthetic performance, unchanged malondialdehyde content, and enhancement of superoxide dismutase (SOD) and peroxidases (POD) activities in hemp plants. Cd content in root is 25.0–29.5 times' higher than that in shoot, suggesting the plant can be classified as a Cd excluder. It is concluded that SA pretreatment counteracted the Cd-induced inhibition in plant growth. The beneficial effects of SA in alleviating Cd toxicity can be attributed to the SA-induced reduction of Cd uptake, improvement of photosynthetic capacity, and enhancement of SOD and POD activities.

Keywords Antioxidant enzyme · Cadmium · *Cannabis sativa* · Photosynthesis · Salicylic acid

Communicated by G. Klobus.

G. R. Shi · Q. S. Cai (✉)
College of Life Sciences, Nanjing Agricultural University, 210095 Nanjing, People's Republic of China
e-mail: qscai@njau.edu.cn; plant_sci@njau.edu.cn

G. R. Shi · Q. Q. Liu · L. Wu
The Anui Provincial Key Laboratory of the Resource Plant Biology, Department of Biology, Huaibei Coal Industry Teachers College, 235000 Huaibei, People's Republic of China

Abbreviations

Car	Carotenoids
CAT	Catalase
Chl	Chlorophyll
C_i	Intercellular CO_2 concentration
E	Transpiration rate
F	Steady-state chlorophyll fluorescence yield
F_o	The minimal fluorescence
F_m	The maximal fluorescence
F_m'	The maximal fluorescence of light-adapted leaf
F_v	The variable fluorescence
F_v/F_m	Maximal quantum yield of PS II
G_s	Stomatal conductance
MDA	Malondialdehyde
PS II	Photosystem II
PS I	Photosystem I
P_n	Net-photosynthetic rate
POD	Peroxidases
ΦPS II	The effective quantum yield of PS II
R_d	Dark respiration
ROS	Reactive-oxygen species
SOD	Superoxide dismutase
TF	Translocation factor

Introduction

Cadmium (Cd) is a highly toxic trace element that enters the environment mainly from industrial processes and phosphate fertilizers. It can reach high levels in agricultural soils and is easily accumulated in plants. Excess Cd induces complex changes in plants at genetical, biochemical and physiological levels, leading to phytotoxicity. The most obvious symptoms are (1) depression of plant growth

and even resulting in plant death by disturbing the uptake of nutrients (Sandalio et al. 2001; Drazkiewicz et al. 2003); (2) destruction of photosynthesis via degradation of chlorophyll (Somashekaraiah et al. 1992; Sandalio et al. 2001) and inactivation of enzymes involved in CO_2 fixation (Greger and Ogren 1991; De Filippis and Ziegler 1993) or the aggregation of pigment protein complexes of the photosystems (Horvath et al. 1996); (3) interaction with the water balance of the plant, and inhibiting stomatal opening (Barcelo and Poschenrieder 1990), (4) alteration of proline and polyamine contents (Sharma and Dietz 2006), and (5) changes in the content of reactive oxygen species (ROS) and activity of antioxidant enzymes (Sandalio et al. 2001; Schützendübel and Polle 2002).

Salicylic acid (SA) is considered as a hormone-like substance, which plays a key role in plant disease resistance (Klessig and Malamy 1994; Durner et al. 1997; Alvarez 2000). SA has been reported to induce a number of defense responses to abiotic stress, such as low temperature (Janda et al. 1999; Kang and Saltveit 2002; Tasgin et al. 2003), thermo-stress (Chen et al. 1997; Dat et al. 1998a, b), salt and osmotic stresses (Borsani et al. 2001), ozone or UV light (Sharma et al. 1996), and drought stress (Senaratna et al. 2000). In addition, SA is also known to be involved in plant protection against heavy metals (Mishra and Choudhuri 1999).

Although several studies have showed that SA could alleviate Cd toxicity (Metwally et al. 2003; Drazic and Mihailovic 2005; Dražić et al. 2006; Zawoznik et al. 2007), the mechanism is varied according to different species. Metwally et al. (2003) concluded that SA alleviated Cd toxicity to barley roots not at the level of antioxidant defense, but by affecting other mechanisms of Cd detoxification. Drazic and Mihailovic (2005) and Dražić et al. (2006) reported that the influence of SA on the alleviation of toxic effects of Cd is probably indirect, through maintenance of ionic homeostasis. More recently, Zawoznik et al. (2007) and Guo et al. (2007, 2009) suggested that SA may function as a signaling molecule necessary to generate, to sustain or to amplify Cd-induced oxidative stress. Krantev et al. (2008) suggested that SA may protect cells against oxidative damage and photosynthesis against Cd toxicity. Thus, a deeper understanding of the roles of SA in alleviating Cd toxicity is necessary.

Hemp (*Cannabis sativa* L.), a fast growing and high-biomass producing plant, has been traditionally grown as fiber crop and recently considered as an energy crops (Venendaal et al. 1997). Previous studies demonstrated that hemp is a metal-tolerant organism, and the amount of Cd accumulated in its shoot was not negligible (Linger et al. 2002, 2005; Citterio et al. 2003). However, little is known about the mechanism of Cd tolerance.

This study was undertaken (1) to assess Cd tolerance capacity of hemp plants according to their growth and physiological changes; (2) determine the effects of SA pretreatment on growth, Cd uptake, and physiological changes in hemp plants during Cd-induced stress; and (3) investigate whether this regulator is involved in the induction of defense responses.

Materials and methods

Plant growth and treatment with Cd

Seeds of hemp were sterilized and divided into two groups. One group of the seeds was soaked in 500 μM SA solution for 6 h, while the other group was soaked in distilled water (control). Subsequently, seeds were sown in pot (16 × 18 cm) filled with acid washed sands, additionally supplemented with Cd as $CdCl_2 \cdot 2.5 H_2O$ at levels of 0, 25, 50, and 100 mg Cd kg^{-1} sands (DW) (three replications). After germinating, five uniform plants were allowed to grow in each pot, at uniform spacing. Pot cultivation was carried out in greenhouse, the average temperature was between $25.4 \pm 0.5°C$ (day) and $22.6 \pm 0.4°C$ (night), and the relative humidity was $60.5 \pm 1.2\%$ (day) and $68.4 \pm 1.7\%$ (night). The pots were watered daily to 60% water holding capacity of the sand, and fertilized twice a week with 100 mL of Hoagland's nutrient solution. The 40-day seedlings were first used to measure gas exchange and chlorophyll fluorescence, and then harvested to determine the other morphological and physiological parameters.

Evaluation of plant growth and Cd accumulation in plants

At harvest, the plants were washed with running tap water to remove any sand particles attached to the plant surfaces. The roots were soaked in 20 mM Na_2-EDTA for 15 min to remove the metal ions adhering to root surfaces (Yang et al. 2004). The roots and shoots were separated and oven-dried for 30 min at 105°C, then at 70°C till the materials reach their constant weights. The dried tissues were weighed and grinded into powder for the determination of Cd concentration, which was measured by flame-atomic absorbance spectrometry after digested with mixed acid [$HNO_3 + HClO_4$ (3:1, v/v)]. The translocation factors (TF) were calculated as: TF = $[Cd]_{shoot}/[Cd]_{root}$.

Chlorophyll concentration analysis

Mature leaves (0.2 g) were extracted in dark at 4°C with 5 ml mixture of acetone and ethanol (v/v = 1:1) until color disappeared completely. Light absorbance at 663, 645,

and 470 nm was determined using spectrophotometry. The concentrations of chlorophyll (a, b) and carotenoids were calculated using adjusted extinction coefficients (Lichtenthaler 1987).

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence parameters were performed using the Mini PAM (Walz, Effeltrich, Germany). After dark-adapted for 30 min, leaves were used to determine the F_o (the minimal fluorescence after the dark adaptation), F_m (the maximal fluorescence after the pulse of red light), and F_v/F_m (the ratio of variable to maximal fluorescence). Steady-state chlorophyll a fluorescence yield (F) was monitored under ambient irradiation (500 µmol quanta m^{-2} s^{-1}). A saturating light pulse (5,000 µmol quanta m^{-2} s^{-1}; 1 s) was applied to measure the maximal fluorescence of light-adapted leaf (F_m'). The effective quantum yield of PS II (ΦPS II) was calculated as follows: ΦPS II $= \Delta F/F_m = (F_m' - F)/F_m'$.

Gas-exchange measurements

The net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (G_s), intercellular CO_2 concentration (C_i) and dark respiration (R_d) were determined with a portable photosynthesis system (Li-6400; LiCor Inc. Lincoln, Nebraska, USA) and an LED light source (6400–02). This was conducted at light intensity of 1,000 µmol m^{-2} s^{-1} (for dark respiration, the light intensity is 0 µmol m^{-2} s^{-1}), leaf temperature of 25°C, and constant CO_2 of 380 ± 5 µmol CO_2 mol^{-1} in the sample chamber provided with buffer volume.

Malondialdehyde (MDA) determination

The MDA determination was followed the method described by Li (2000). Fresh leaf material (0.2 g) from different plants was homogenized in 5 ml of 10% trichloroacetic acid. The homogenate was centrifuged at $10,000 \times g$ for 20 min, and then 2 ml supernatant of sample was reacted with 2 ml of 0.6% 2-thiobarbituric acid. The absorbance was monitored at 600, 532, and 450 nm, respectively. Calculation of MDA was based on the following formula: C (µM) $= 6.45(A_{532} - A_{600}) - 0.56\,A_{450}$.

Enzyme extraction and enzyme assays

A 0.2 g of leaf tissue was used to extract soluble protein by homogenizing in 5 ml of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA and 1% polyvinylpolypyrrolidone. The homogenate was centrifuged at $10,000 \times g$ at 4°C for 20 min, and the supernatant was used for the following enzyme assays. Protein content was determined according to the method of Bradford (1976) with bovine serum albumin as standard.

The superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed according to Beauchamp and Fridovich (1971). One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibited nitroblue tetrazolium chloride reduction. Peroxidases (POD, EC 1.11.1.7) activity was based on the determination of guaiacol oxidation ($\varepsilon = 26.6$ mM cm^{-1}) at 470 nm by H_2O_2 (Putter 1974). Catalase (CAT, EC1.11.1.6) activity was determined by the consumption of H_2O_2 conversion at 240 nm ($\varepsilon = 39.4$ mM cm^{-1}) (Aebi 1984).

Statistical analysis

Data were subjected to a two-way ANOVA using the SPSS Version 11.5 software (SPSS Inc.). Multiple comparisons of means among different Cd treatments with or without SA pretreatment were performed using Duncan's test at the 0.05 significance level.

Results

Growth parameters

Elevated Cd reduced root and shoot growth in hemp plants. Shoot biomass decreased proportionally with increasing Cd concentration, and the reduction in the values of this parameter under 25, 50, and 100 mg Cd kg^{-1} sand was 8.7, 18.7, and 29.4%, respectively, compared with the control. For root biomass, the reduction was 12.0, 26.3, and 26.9%, respectively (Table 1).

In the absence of Cd, SA pretreatment decreased the biomass of shoots and roots by 8.6 and 8.1%, respectively. However, when plants were exposed to Cd stress, SA pretreatment alleviated Cd toxicity; this was illustrated by the enhancement of biomass in SA pretreated plants, in comparison with the non-pretreated one at the same Cd level. The beneficial effect of SA was statistically significant in shoot biomass, but less pronounced on root biomass.

Cd accumulation in plants

Cd accumulation in shoots and roots was high, and it was found to be dose-dependent (Table 1). Cd content ranged from 44.6 to 79.7 mg kg^{-1} (DW) in shoot, and from 1,115 to 2,148 mg kg^{-1} (DW) in root, respectively. Cd content in roots exposed to 25, 50, and 100 mg Cd kg^{-1} sand, were about 25.0-, 29.5, and 27.0-fold higher than that in shoots, respectively. SA pretreatment decreased Cd accumulation by 9.0, 8.6, and

Table 1 The effect of SA and Cd on biomass, Cd content, and TF in hemp plants exposed to different Cd concentrations

Cd treatments (mg kg^{-1})	Biomass (g plant^{-1})		Cd content (mg kg^{-1} DW)		TF
	Shoot	Root	Shoot	Root	
SA−					
0	1.17 a	0.17 a	n.d.	n.d.	–
25	1.07 b	0.15 ab	44.6 b	1,115 b	0.040 a
50	0.92 c	0.12 b	52.4 b	1,546 ab	0.035 a
100	0.83 c	0.12 b	79.7 a	2,148 a	0.038 a
SA+					
0	1.07 ab	0.15 a	n.d.	n.d.	–
25	1.13 a	0.16 a	40.6 b	921 a	0.045 a
50	1.02 bc	0.15 a	47.9 b	1,276 a	0.042 a
100	0.95 c	0.13 a	64.5 a	1,310 a	0.053 a
ANOVA					
Cd	28.1***	5.7**	29.8***	6.9***	0.5 n.s.
SA	4.7**	1.0 n.s.	6.0*	7.7*	2.1 n.s.
Cd × SA	6.0**	1.4 n.s.	1.3 n.s.	1.7 n.s.	0.3 n.s.

Mean values ($n = 3$) with different letters in the same column for control (SA−) or SA pretreatment (SA+) are significantly different according to the Duncan's test ($P < 0.05$)

n.d. not determined, *n.s.* not significant

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 2 Effect of Cd and SA on photosynthetic pigments in hemp seedlings subjected to Cd stress

Cd treatment (mg kg^{-1})	Chl a (mg g^{-1}FW)	Chl b (mg g^{-1}FW)	Chl $a + b$ (mg g^{-1}FW)	Car (mg g^{-1}FW)	Chl a/b	Car/ Chl
SA−						
0	1.09 a	0.21 a	1.29 a	0.28 a	5.25 a	0.22 a
25	1.03 a	0.18 a	1.21 a	0.29 a	5.68 a	0.24 a
50	1.16 a	0.21 a	1.38 a	0.32 a	5.60 a	0.23 a
100	1.04 a	0.19 a	1.23 a	0.28 a	5.45 a	0.23 a
SA+						
0	1.07 b	0.20 a	1.27 b	0.30 bc	5.28 a	0.24 a
25	1.22 a	0.23 a	1.45 a	0.33 a	5.30 a	0.23 a
50	1.14 ab	0.22 a	1.36 ab	0.32 ab	5.18 a	0.23 a
100	1.06 b	0.19 a	1.25 b	0.28 c	5.50 a	0.22 a
ANOVA						
Cd	3.0 n.s.	1.5 n.s.	2.8 n.s.	6.6**	0.7 n.s.	0.6 n.s.
SA	2.6 n.s.	3.3 n.s.	2.9 n.s.	6.8*	2.2 n.s.	0.2 n.s.
Cd × SA	3.5*	2.0 n.s.	3.4*	2.6 n.s.	1.1 n.s.	3.9*

Mean values ($n = 3$) with different letters in the same column for control (SA−) or SA pretreatment (SA+) are significantly different according to the Duncan's test ($P < 0.05$)

n.s. not significant

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

19.1% in shoots, and by 17.4, 17.4, and 39.0% in roots under 25, 50, and 100 mg Cd kg^{-1} sand conditions, respectively. TF was unaffected neither by Cd nor by SA treatments (Table 1).

Photosynthetic pigment contents

Cd exposure caused little inhibition in photosynthetic pigment in hemp plants. No pronounced changes were found in chlorophyll (a, b, $a + b$) and carotenoids (Car) content, as well as the Chl a/b and Car/Chl ratios (Table 2). SA pretreatment did not affect the Chl a, Chl b, Chl $a + b$, Chl a/b, and Car/Chl, but enhanced Car in the control and 25 mg kg^{-1} Cd treatment. There were Cd × SA interactions on Chl a, Chl $a + b$, and Car/Chl (Table 2).

Chlorophyll fluorescence

As shown in Fig. 1a, the F_v/F_m ratio, ranged from 0.80 to 0.81, did not differ in all Cd and SA treatments. The ΦPS II was the same in different Cd treatments, but pretreatment with SA resulted in an increase in ΦPS II under Cd stress. Two-way ANOVA of ΦPS II indicated a significant main effect of Cd ($P < 0.05$) and SA ($P < 0.01$), as well as Cd by SA interaction ($P < 0.01$) (Fig. 1b).

Gas exchange

No significant reductions were detected in photosynthetic parameters of plants treated with Cd (Table 3). Furthermore, low Cd treatment (25 mg kg^{-1}) result in a significant
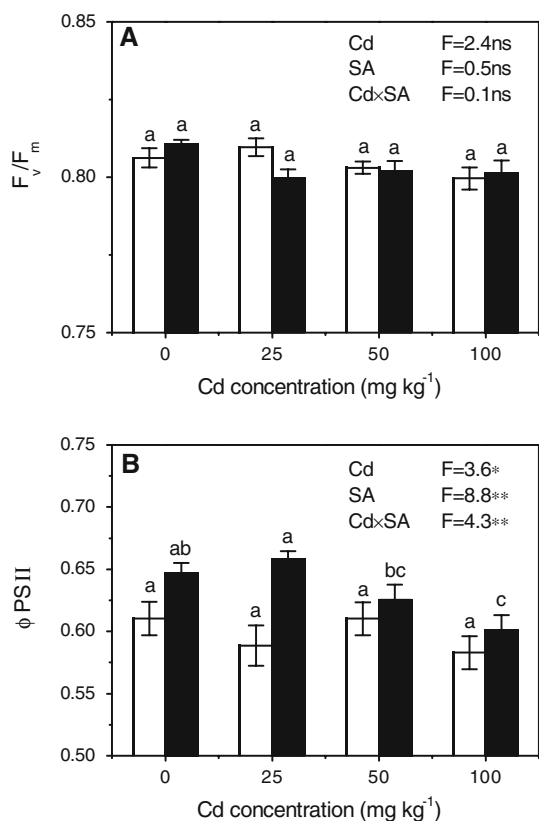
Fig. 1 The effect of SA and Cd on chlorophyll fluorescence in hemp seedlings subjected to Cd stress. The *different letters above the bars* (*open square* SA − or *filled square* SA+) of the same SA treatment are significantly different according to the Duncan's test ($P < 0.05$). *n.s.* not significant, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

27.1, 20.2, and 78.8%, respectively, under 25, 50, and 100 mg Cd kg^{-1} sand conditions. SA pretreatment decreased C_i was also observed in the 25 and 50 mg kg^{-1} Cd treatments. There were significant main effects of SA, as well as Cd × SA interactions for P_n, E, G_s, and C_i, Cd had a significant main effect on P_n and E, but not on G_s and C_i. The R_d did not change in different Cd and SA treatments (Table 3).

MDA content and SOD, CAT, and POD activities

Figure 2 compares MDA contents and activity of antioxidant enzymes of leaves from hemp plants subjected to Cd with or without presoaking in SA. The presence of Cd in sands did not lead to disturbances in the MDA level, however, a significant reduction of MDA was observed in SA-treated plants (Fig. 2a). The POD activity was increased with Cd levels in both the control and SA-treated plants, and it was significantly high in plants subjected to Cd with SA presoaking, compared with the control (Fig. 2b). Similar pattern was also observed in the changes of SOD activity, and it was significantly affected by Cd and SA, as well as Cd by SA interaction (Fig. 2c). No major changes were found in CAT activity in both Cd and SA treatments (Fig. 2d).

Discussion

Cd tolerance and accumulation in hemp plants

In this study, we demonstrated that hemp plants have innate resistance to Cd stress. This was in consistent to the results reported by Citterio et al. (2003) and Linger et al.

enhancement in P_n and G_s. SA pretreatment caused a significant increase in P_n, E, and G_s under Cd stress. In comparison with the control, SA presoaking increased P_n by 61.9, 24.3, and 46.0%, G_s by 23.1, 2.4, and 62.0%, and E by

Table 3 Effect of Cd and SA on net photosynthetic rate (P_n), stomatal conductance (G_s), intercellular CO$_2$ concentration (C_i), transpiration rate (E) and dark respiration (R_d) in hemp seedlings exposed to different Cd levels

Mean values ($n = 18$) with different letters in the same column for control (SA−) or SA pretreatment (SA+) are significantly different according to the Duncan's test ($P < 0.05$)

n.s. not significant

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Cd treatments (mg kg^{-1})	P_n (µmol m^{-2} s^{-1})	G_s (mol m^{-2} s^{-1})	C_i (µmol mol^{-1})	E (mmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})
SA−					
0	11.0 b	0.40 b	310 b	7.8 a	2.9 a
25	12.7 a	0.49 a	315 b	9.0 a	3.1 a
50	10.7 b	0.48 ab	326 a	8.9 a	3.2 a
100	10.9 b	0.40 b	314 b	8.2 a	3.0 a
SA+					
0	11.9 b	0.51 b	320 a	9.5 c	2.9 a
25	17.3 a	0.59 ab	299 b	11.3 b	3.3 a
50	15.8 a	0.50 b	309 ab	10.9 bc	3.0 a
100	15.8 a	0.65 a	315 a	14.6 a	3.0 a
ANOVA					
Cd	10.9***	2.5 n.s.	0.4 n.s.	12.1***	3.2 n.s.
SA	99.3***	30.1***	5.3*	94.8***	0.2 n.s.
Cd × SA	10.2***	4.9**	10.9***	12.4***	0.6 n.s.

Fig. 2 The effect of SA pretreatment on MDA content, and POD, SOD, and CAT activity in hemp seedlings subjected to Cd stress. The *different letters above the bars* (*open square* SA − or *filled square* SA+) of the same SA treatment are significantly different according to the Duncan's test ($P < 0.05$). *n.s.* not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$
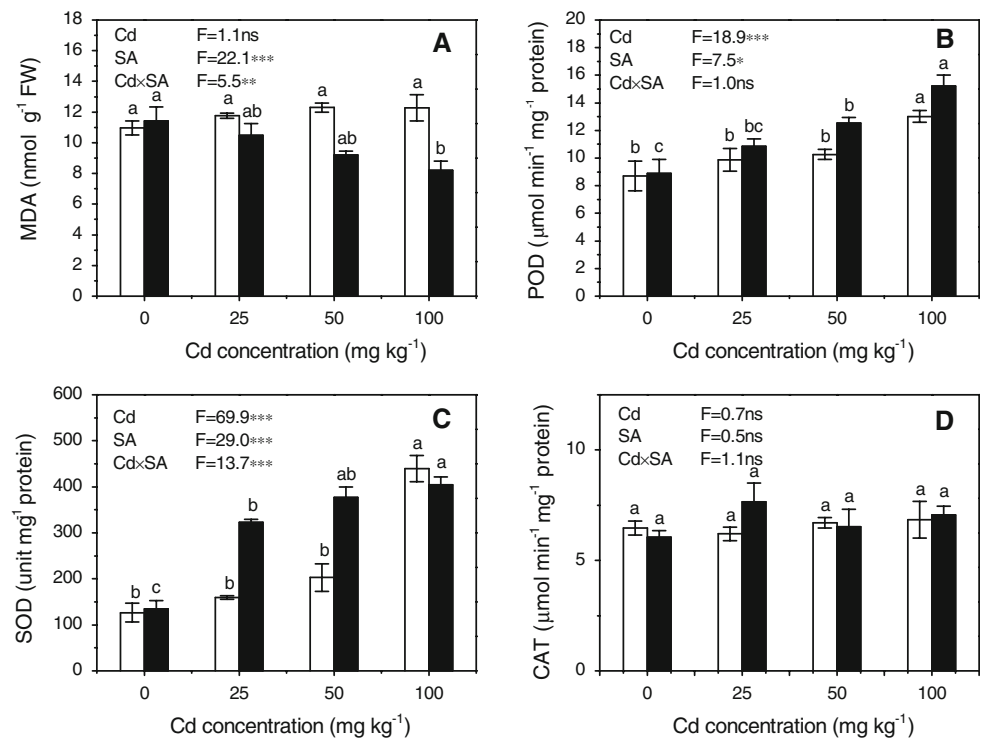
(2005). The high tolerance of hemp plants to 25–100 mg kg^{-1} Cd stress was illustrated by the small reduction of roots and shoots biomass, little inhibition in photosynthesis, and less oxidative stress maintained by POD and SOD.

Generally, Cd caused the destruction of photosynthesis resulting from severe alterations in the chlorophyll level, chloroplast structure, photochemistry, and carboxylating enzyme activities, and the high extent of lipid peroxidation (Krantev et al. 2008). In the present study, excess Cd caused little inhibition in photosynthetic pigment contents (Chl *a*, Chl *b*, Chl, Chl *a/b*, Car, and Car/Chl), the chlorophyll fluorescence parameter (the F_v/F_m ratio and ΦPS II), and gas exchange parameters (P_n, G_s, E, and C_i), these results suggest hemp plant had a stability in the PS II/PS I ratio (Varadi et al. 2003) and in the antennae composition (Durnford et al. 2003). Thus, the plants did not suffer Cd stress in their photosynthetic apparatus.

Although Cd does not generate ROS directly, it generates oxidative stress via interference with the antioxidant defense system (Foyer and Noctor 2005). MDA content is an index of the membrane integrity and lipid peroxidation. Cd-induced increase in MDA content were reported for many plant species, including bean (Chaoui et al. 1997), pea (Lozano-Rodriguez et al. 1997; Metwally et al. 2005), rice (Chien et al. 2001), sunflower (Gallego et al. 1996), mustard (Mobin and Khan 2007), and maize (Pál et al. 2007; Krantev et al. 2008). In the present study, however,

the MDA was similar in different Cd treatments, indicating the plants not suffering a Cd-induced oxidative damage.

The obtained results showed that the ROS scavenging in hemp plants may be contributed by the dramatic enhancement of SOD and POD activity (Fig. 2b, c). SOD catalyzes the conversion of superoxide anion to O_2 and H_2O_2 (Sudhakar et al. 2001), its activity reflects the O_2 radical-eliminating ability of plants (Azevedo Neto et al. 2006; Ekmekçi et al. 2008). POD catalyzes H_2O_2-dependent oxidation of substrate, while CAT eliminates H_2O_2 by breaking it down directly to form water and oxygen. CAT is less efficient than POD in H_2O_2 scavenging because of its low-substrate affinity, so as long as the stress is not too strong for the plant defense capacity, the main response to heavy metal is an increase in SOD and POD activities along with a decrease in CAT (Siedlecka and Krupa 2002).

The mechanism of metal tolerance in plants includes metal exclusion and accumulation (Baker 1987). Metal exclusion is the avoidance of absorption and restriction of translocation to shoot. Metal accumulation is an extreme type of physiological response that plants absorb and accrete high concentration of metal (Dahmani-Muller et al. 2000). As showed in Table 1, most of Cd absorbed in hemp plant was retained in the root, reaching 25–29.5 times higher values than that in shoots. The results obtained in this study suggest that hemp plant strongly limited translocation of Cd to shoot. Such metal immobilisation in root cells is related to an exclusion strategy.

Effects of SA on Cd tolerance of hemp plant

Our results showed that, although treated with SA or Cd alone both caused an inhibition in plant growth, when applied simultaneously, they act antagonistically on shoot and root biomass. Such SA-mitigated Cd toxicity has been shown in other plant species, including barley (*Hordeum vulgare*) (Metwally et al. 2003), soybean (*Glycine max*) (Drazic and Mihailovic 2005), alfalfa (Dražić et al. 2006), maize (Krantev et al. 2008), and rice (Choudhury and Panda 2004; Guo et al. 2007, 2009).

A moderate resistance to heavy metals can be realized by selective Cd exclusion, lowered uptake, or active efflux from the roots, i.e. by mechanisms leading to lower cytoplasmic Cd contents (Hall 2002). Our results showed that presoaking hemp seeds for 6 h with SA before exposure to Cd diminishes the Cd accumulation, This may be one of the important causes for SA-induced Cd tolerance in hemp plants. Similar results was also found in rice (Choudhury and Panda 2004; Guo et al. 2009) and maize (Pál et al. 2002; Krantev et al. 2008). However, in barley seedlings, Cd contents in tissue were unaltered (Metwally et al. 2003). Furthermore, the cases of SA increased Cd accumulation were observed in soybean (Drazic and Mihailovic 2005) and alfalfa (Dražić et al. 2006). Hence, the effects of SA on Cd accumulation depend on its concentration, treatment duration, plant species, age at treatment, and plant organ examined.

One of the important results in this study is that SA pretreatment significantly enhanced P_n in hemp plants subjected to Cd stress. This was accompanied with an increase in G_s and E. The parallel changes of P_n and G_s suggested a SA-induced stomatal effect existed in SA-treated plants (Chartzoulakisa et al. 2002). Previous studies suggested that SA induce a reversal of inhibited transpiration rate in zucchini yellow mosaic virus-infected leaves (Radwan et al. 2006) and a reversal of ABA-induced stomatal closure (Rai et al. 1986), hence, the positive effect of SA pretreatment on G_s and E in hemp plant might be accounted for by the SA-reversed stomatal closure. In addition, SA pretreatment caused a decrease of C_i at low Cd concentrations (25 and 50 mg kg^{-1}), indicating that enzymatic dark reaction of photosynthesis may be affected (Vaillant et al. 2005; Hura et al. 2007). This was strengthened by Krantev et al. (2008), who found that SA pretreatment alleviate the inhibitory effect of Cd on the activity of RuBPC and PEPC in maize seedlings.

Generally, transpiration is the most important mechanism for the uptake of nutrient elements by its gradient of water potential, which can drive them to move into roots and upward to shoots (Novák and Vidovič 2003; Tani and Barrington 2005; Liao et al. 2006; Guo et al. 2009). A previous study with Indian mustard showed that Cd accumulation in shoots was strongly influenced by transpiration (Salt et al. 1995). However, in the present study, a high transpiration along with lower Cd accumulation in shoot and root was observed in SA-treated plants. Moreover, it was also observed that the TF was similar in control and SA-treated plants. Therefore, low Cd uptake in SA-treated plants may be result from the decreasing of uptake area or/and inhibition of metal transporters.

Chlorophyll content and chlorophyll fluorescence are considered as indicators of damages to photosynthetic system induced by environmental stressors (Lichtenthaler and Miehe 1997; Maxwell and Johnson 2000). The present study showed that SA pretreatment increased Car and ΦPS II in absent of Cd or present of low Cd level (25 mg kg^{-1}), indicating SA takes a positive role in protecting photosynthetic pigment and apparatus.

It has been recognized that SA acts as a signaling molecule necessary to generate, to sustain or to amplify Cd-induced oxidative stress (Zawoznik et al. 2007). In the present study, SA pretreatment alleviated Cd-induced oxidative stress as evidenced by a decrease in MDA content, and a sustain increase in SOD and POD activity. Our result was in consistent with those reported by Guo et al. (2007, 2009) and Krantev et al. (2008), but disagreed with Metwally et al. (2003).

In conclusion, hemp has innate capacity of Cd tolerance. Cd exposure caused little inhibition in photosynthesis, but result in an increase in activities of SOD and POD. Most of the Cd uptake by plants was located in roots, which indicated that the plant as a Cd excluder. SA pretreatment alleviate Cd toxicity in hemp seedlings, which result from the reduction of Cd uptake, the improvement of photosynthesis, and the enhancement of SOD and POD activities.

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