Bull Environ Contam Toxicol (2010) 85:256–263
DOI 10.1007/s00128-010-0067-0

Cadmium Accumulation and Tolerance of Two Safflower Cultivars in Relation to Photosynthesis and Antioxidantive Enzymes

Gangrong Shi · Caifeng Liu · Qingsheng Cai · Qingqing Liu · Cuiping Hou

Received: 13 April 2010 / Accepted: 8 July 2010 / Published online: 18 July 2010

Abstract To investigate the effects of cadmium (Cd) on photosynthetic and antioxidant activities of safflower (*Carthamus tinctorius* L.) plants, two cultivars (Yuming and New safflower No. 4) were used for long-term pot experiment, under 0, 25, 50 or 100 mg Cd kg^{-1} (DW) soil conditions. The results showed that there is a large amount of Cd (148.6–277.2 mg kg^{-1}) accumulated in the shoot of safflower, indicating this species might be a potential Cd accumulator. Exposure to 25–100 mg Cd kg^{-1} soil decreased the net photosynthetic rate by 25.6%–48.9% for New safflower No. 4, and 16.7%–57.3% for Yuming, respectively. The inhibition of photosynthesis might result from the limitation of stomatal conductance, reduction in photosynthetic pigment, and destruction of photosynthetic apparatus caused by Cd stress. Cd caused an enhancement of malondialdehyde (MDA), an increase in activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), and a decrease in catalase (CAT) activity for both cultivars. It seems that SOD and APX accounted for the scavenging of oxidant stress in safflower cultivars. The physiological response of safflower plants to Cd stress was cultivar- and dose- dependent. New safflower No. 4 exhibited high photosynthetic performance at high Cd stress, which may be contributed by high intercellular CO_2 concentration, APX activity and Car/Chl ratio. In contrast, Yuming is more tolerant to Cd toxicity at low Cd level, in which an efficient antioxidant system is involved.

Keywords Antioxidant activity · Cadmium · Photosynthesis · Safflower

Cadmium (Cd) is one of the most highly toxic environmental pollutants in the atmosphere, soil and water (Benavides et al. 2005). Although Cd is not an essential nutrient for plants, it can be accumulated to higher levels in the aerial organs (Pence et al. 2000), inducing phytotoxicity, e.g. leaf roll, chlorosis, growth reduction, and eventually death (Benavides et al. 2005).

Photosynthesis is sensitive to Cd because it directly disturbs the chloroplast function by inhibiting the activities of enzymes of chlorophyll biosynthesis and CO_2 fixation or the aggregation of pigment protein complexes of the photosystems (Mobin and Khan 2007). Cd exerts multiple effects on photosystem II (PS II). On the donor site, Cd affects the wateroxidizing system of PS II by replacing the Mn^{2+} ions, thereby inhibiting the oxygen evolving cycle and, consequently, oxygen evolution (Baszynski et al. 1980). On the acceptor site, it inhibits electron transfer from Q_A^- to Q_B^-, leading to the uncoupling of electron transport in the chloroplasts (Sigfridsson et al. 2004). With regard to the carboxylating phase, the main targets of Cd are two key enzymes of CO_2 fixation, ribulose 1, 5-bisphosphate carboxylase (RuBPC) and phosphoenol pyruvate carboxylase (PEPC). Cd ions damage structure of RuBPC by substituting for Mg^{2+} ions, and cause irreversible dissociation of the large and small subunits of RuBPC, thus leading to inhibition of the enzyme activity (Krantev et al. 2008).

G. Shi · C. Liu · Q. Liu · C. Hou
The Anhui Provincial Key Laboratory of the Resource Plant Biology in College of Life Sciences, Huaibei Normal University, 235000 Huaibei, People's Republic of China

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

Cadmium is a non-redox metal unable to participate in Fenton-type reactions, but it leads to the formation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{-\bullet}$), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (·OH) (Laspina et al. 2005). Plants have evolved protective enzymatic and non-enzymatic mechanisms to scavenge ROS and alleviate its deleterious effects. Protective enzymes include catalase (CAT, E.C. 1.11.1.6), peroxidases (POD, E.C. 1.11.1.7), ascorbate peroxidase (APX, EC 1.11.1.11)), superoxide dismutase (SOD, E.C. 1.15.1.1) and glutathione reductase (GR, EC 1.6.4.2), while several molecules such as glutathione, ascorbate, and carotenoids provide non-enzymatic protection (Tiryakioglu et al. 2006). Although extensively studies have showed that Cd can change the activities of antioxidant enzymes, the results varied in respect to different plant species or cultivars, and mostly the exposure of plants to Cd for short periods of time (Ekmekçi et al. 2008; Tiryakioglu et al. 2006). There are limited studies to date about antioxidative and photosynthetic responses of plant cultivars to long-term Cd stress.

Safflower (*Carthamus tinctorius* L.) is an oilseed crop cultivated throughout the semiarid region of the temperate climates in many areas of the world (Dordas and Sioulas 2008; Weiss 2000). The importance of safflower has increased in recent years, especially with the interest in the production of biofuels (Dordas and Sioulas 2008). Safflower is illustrated to be more drought resistant and salt tolerant than some other oil crops (Bassil and Kaffka 2002; Weiss 2000), however, little is known about the physiological response of safflower to heavy metal stress. A deeper understanding of this is necessary for screening safflower cultivars which are suitable to metal contaminated areas.

The objectives of the present study were, (1) to establish and verify Cd accumulation in two safflower cultivars; (2) to evaluate their capacity of Cd tolerance on the basis of photosynthetic performance and antioxidant enzymes, and (3) to determine whether the physiological response to long-term Cd stress is varied in cultivars.

Materials and Methods

Two non-thorn safflower cultivars, Yuming (YM) and new safflower No. 4 (NS-4), that were widely cultivated in the northwest of China, were used for pot experiment. The experiment was carried out in a greenhouse, of which the average temperature (day/night) was 25.8 ± 2.7°C/23.1 ± 3.6°C, and the relative humidity (day/night) was 62.7 ± 1.3%/68.6 ± 1.8%. The soil type was the gravel blackland (sand:clay; 23:77, by dry weight), which is characterized as: pH, 7.24; N, 68.4 mg kg^{-1}; P_2O_5, 9.36 mg kg^{-1}; K_2O, 75.3 mg kg^{-1}; organic matter, 1.12%; electrical conductivity, 23.6 μs cm^{-1}; Cd, 0.126 mg kg^{-1}. The soil characteristics were determined according to methods detailed in Page et al. (1982).

After incubating in water for 12 h, seeds were sown in pot (16 cm × 18 cm) filled with 2 kg of soil, Soils were additionally supplemented with 1.0 g KH_2PO_4 kg^{-1} soil (DW), 1.5 g NH_4NO_3 kg^{-1}. Four levels of Cd (0, 25, 50, or 100 mg kg^{-1}) were added in soil as $CdCl_2{\cdot}2.5H_2O$. All the supplementary (i. e. $CdCl_2{\cdot}2.5H_2O$, KH_2PO_4 and NH_4NO_3) were included in soil by spraying the solutions and thoroughly mixed. Each of the Cd treatment had three replications (pots). The pots were watered daily to 60% of water holding capacity. Since the seedlings were very small, to meet the experimental requirement, 10 uniform plants were allowed to grow in each pot, at uniform spacing. After 50 days of growing, when plants of the control have seven to eight leaves, four seedlings from each pot, including the senescing leaves, were sampled for evaluation of biomass and Cd accumulation. The second leaves of the other six plants were used for physiological analyses.

After harvesting, the plants were washed with running tap water and rinsed with de-ionized water to remove any soil particles attached to the plant surfaces. The shoot and root materials were oven-dried for 30 min at 105°C, and then at 70°C to a constant weight. The dried tissues were weighed and digested with mixed acid [HNO_3 + $HClO_4$ (3:1, v/v)]. Cd concentration was determined by flame atomic absorbance spectrometry.

The bioconcentration factor (*BCF*), translocation factor (*TF*) and total Cd in shoot were calculated as follows (Ait Ali et al. 2002; Monni et al. 2000).

$$BCF = [\mathrm{Cd}]_{\text{shoot or root}}/[\text{total Cd}]_{\text{soil}}$$

$$TF = 100 \times [\mathrm{Cd}]_{\text{shoot}}/[\mathrm{Cd}]_{\text{root}}$$

$$\text{Total Cd in shoot} = \mathrm{DW}_{\text{shoot}} \times [\mathrm{Cd}]_{\text{shoot}}$$

Pigments were extracted from 0.2 g leaf materials with 5 ml 80% acetone. Light absorbance at 663, 645 and 470 nm was determined by spectrophotometry. The contents of chlorophyll (a, b) and carotenoids were calculated according to Lichtenthaler (1987).

The chlorophyll fluorescence parameters were measured using the Mini PAM (Walz, Effeltrich, Germany). After dark-adapted for 30 min, the leaves were used to determine the minimum fluorescence (F_0), maximum fluorescence (F_m) and the quantum yield of PS II photochemistry (F_v/F_m). The effective quantum yield of PS II (ϕPS II) was monitored under ambient irradiation (500 μmol quanta m^{-2} s^{-1}).

Net photosynthetic rate (P_n), transpiration rate (E), stomatal conductance (G_s), and intercellular CO_2 concentration (C_i) were determined with a portable photosynthesis

system (LiCor-6400; LiCor Inc. Lincoln, Nebraska, USA) and a LED light source (6400-02). Light intensity, leaf temperature and CO_2 concentration inside the leaf chamber were kept constant at 1,000 μmol m^{-2} s^{-1}, 25 ± 0.8°C and 400 ± 5 μmol CO_2 mol^{-1}, respectively.

The content of MDA was determined according to Li (2000). Fresh leaf material (0.2 g) was homogenized in 5 ml of 10% TCA. The homogenate was centrifuged at 10, 000×*g* for 20 min, and then 2 ml supernatant was reacted with 2 ml of 0.6% 2-thiobarbituric acid. The absorbance was monitored at 600, 532 and 450 nm, respectively. MDA content was calculated by the following formula: C (μmol L^{-1}) = $6.45(A_{532} - A_{600}) - 0.56\ A_{450}$.

A 0.2 g of leaf tissue was crushed into powder in a mortar and pestle under ice cold condition. Soluble protein was extracted by homogenizing the powder 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×*g* at 4°C for 30 min. 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 SOD 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 NBT reduction. Guaiacol POD activity was based on the determination of guaiacol oxidation ($\varepsilon = 26.6$ mM cm^{-1}) at 470 nm by H_2O_2 (Putter 1974). APX activity was determined according to the method of Nakano and Asada (1981). The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate ($\varepsilon = 2.8$ mM cm^{-1}) at 290 nm. Catalase (CAT) activity was determined by the comsumption of H_2O_2 conversion at 240 nm ($\varepsilon = 39.4$ mM cm^{-1}) (Aebi 1984).

Data of two cultivars under different Cd concentrations were subjected to a two-way ANOVA using the SPSS Version 11.5 software (SPSS Inc.). Multiple comparisons of means among different Cd treatments within a cultivar were performed using Duncan's test at the 0.05 significance level.

Results and Discussion

Increasing Cd concentrations decreased shoot and root biomass in safflower cultivars (Table 1). The decrease of shoot and root biomass was less in YM than that in NS-4. For example, at 25 mg Cd kg^{-1} treatment, shoot biomass decreased by 42.3% for NS-4, but increased by 3% for YM, in comparison with the control. Similarly, root biomass under 25, 50 and 100 mg Cd kg^{-1} diminished by 49.2%, 67.1%, 84.9% for NS-4, and 12.3%, 46.5%, 70.2% for YM, respectively. Two-way ANOVA indicated that both the shoot and root biomass were significantly affected by Cd concentrations ($p < 0.001$), but not by cultivars. A Cd × cultivar interaction was also observed in shoot biomass ($p < 0.05$).

Table 1 Effects of cadmium on biomass, Cd content, bioconcentration factor (*BCF*), total Cd in shoot, and translocation factor (*TF*) of two safflower cultivars

Cd treatments (mg kg^{-1})	Biomass (g $plant^{-1}$)		Cd content (mg kg^{-1} DW)		*BCF*		Total Cd in shoot (μg $plant^{-1}$)	*TF* (%)
	Shoot	Root	Shoot	Root	Shoot	Root		
NS- 4								
0	0.99^{a}	0.41^{a}	0.8^{c}	1.7^{d}	6.1^{a}	13.1^{a}	0.8^{c}	46.9^{a}
25	0.57^{b}	0.21^{b}	148.6^{b}	392.2^{c}	5.9^{a}	15.7^{a}	84.5^{a}	38.7^{a}
50	0.39^{bc}	0.14^{c}	179.8^{b}	729.3^{b}	3.6^{b}	14.6^{a}	69.1^{ab}	24.7^{b}
100	0.21^{c}	0.06^{d}	247.6^{a}	879.4^{a}	2.5^{c}	8.8^{b}	52.2^{b}	28.3^{b}
YM								
0	0.74^{ab}	0.35^{a}	0.8^{c}	1.7^{d}	6.2^{a}	13.5^{b}	0.6^{c}	46.0^{a}
25	0.77^{a}	0.31^{a}	150.0^{b}	433.4^{c}	6.0^{a}	17.3^{a}	113.4^{a}	34.7^{b}
50	0.49^{bc}	0.19^{b}	206.8^{b}	736.4^{b}	4.1^{b}	14.7^{b}	100.3^{a}	28.1^{c}
100	0.28^{c}	0.10^{b}	277.2^{a}	892.6^{a}	2.8^{c}	8.9^{c}	75.9^{b}	30.9^{bc}
ANOVA								
Cd	31.2***	41.3**	102.2***	349.4***	46.4***	25.4***	72.4***	28.7***
Cultivar	0.46	2.37	1.76	0.54	0.93	0.80	16.8**	0.02
Cd × Cultivar	3.94*	2.90	0.54	0.19	0.20	0.31	1.99	1.04

Mean values (n = 3) with different letters in the same column for each cultivar are significantly different according to the Duncan's test ($p < 0.05$)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 2 Effects of Cd on photosynthetic pigments in leaves of two safflower cultivars

Cd treatments (mg kg^{-1})	Chl a (mg g^{-1} DW)	Chl b (mg g^{-1} DW)	Chl a + b (mg g^{-1} DW)	Car (mg g^{-1} DW)	Chl a/b	Car/Chl
NS- 4						
0	10.6^{a}	1.9^{a}	12.5^{a}	2.6^{ab}	5.7^{a}	0.21^{b}
25	10.6^{a}	1.9^{a}	12.5^{a}	2.8^{a}	5.7^{a}	0.22^{b}
50	9.0^{ab}	1.6^{a}	10.6^{ab}	2.3^{ab}	5.5^{a}	0.22^{b}
100	7.1^{b}	1.2^{b}	8.3^{b}	2.0^{b}	5.7^{a}	0.25^{a}
YM						
0	10.6^{a}	1.8^{a}	12.4^{a}	2.9^{a}	6.0^{a}	0.24^{a}
25	10.3^{a}	1.8^{a}	12.0^{a}	2.7^{a}	5.8^{a}	0.23^{a}
50	8.2^{b}	1.3^{a}	9.5^{b}	2.2^{b}	6.1^{a}	0.23^{a}
100	7.6^{b}	1.4^{a}	9.0^{b}	2.1^{b}	5.5^{a}	0.23^{a}
ANOVA						
Cd	11.04**	7.86**	10.86**	8.22**	0.49	2.46
Cultivar	0.10	0.74	0.16	0.05	1.42	2.33
Cd × Cultivar	0.38	1.19	0.49	0.58	1.08	6.65**

Means (n = 3) followed by different letters in the same columns for each cultivar are different significantly according to the Duncan's test

** $p < 0.01$, *** $p < 0.001$

Excess Cd in soil resulted in an increase of Cd content in shoot and root (Table 1). Both safflower cultivars have high capacity of Cd accumulation in the shoot. Under 25–100 mg kg^{-1} Cd soil condition, the shoot Cd content reached up to 148.6–277.2 mg kg^{-1} (DW), and the BCF is ranged from 2.5 to 6.0 (Table 1). Although both the shoot Cd content and BCF exceeded the critical level for a Cd-hyperaccumulator (Baker 1981), the TF of two safflower cultivars, ranged from 28.7% to 38.7% (Table 1), were greatly lower than the critical level (TF > 1). Moreover, In contrast to Cd-hyperaccumulators, such as *Thlaspi caerulescens* (Boominathan and Doran 2003) and *Solanum nigrum* (Sun et al. 2008), a shortcoming of safflower is less tolerance to high Cd concentrations. Therefore, this species may be considered as a potential Cd accumulator.

Due to safflower is a deep-rooted oilseed crop with high annual biomass yield (Weiss 2000), and it is also considered to be an important energy crop for producing biodiesel from seed oil (Sims et al. 2006). Considering the agricultural plants are typically exposed to much lower Cd concentrations (Sanita di Toppi and Gabrielli 1999). The concentration of soil Cd in the Zhangshi sewage irrigation area in the west Shenyang suburb was 7–25 mg kg^{-1} (Sun et al. 2008). We suggest that safflower may be a good candidate for practical application in combining phytoremediation of Cd contaminated soils with energy crop cultivation.

Although no significant difference was observed in shoot Cd content and BCF between the two cultivars, the total Cd in shoot was significantly higher in YM than in NS-4 ($p < 0.01$), especially in 25 mg kg^{-1} Cd soil condition (Table 1). High total Cd in shoot of YM may be contributed by the high shoot biomass. Exposed YM seedlings to 25 mg kg^{-1} Cd soil did not change shoot biomass (Table 1), photosynthetic pigment (Table 2), chlorophyll fluorescence (Fig. 2), as well as the MDA content (Fig. 3), but enhanced the activity of SOD, POD and APX (Fig. 4), suggested they can grow healthily in soil containing 25 mg Cd kg^{-1} soil. Hence, the cultivar YM is more efficient for cultivation in Cd contaminated arable soils.

As seen in Table 2, 25 mg Cd kg^{-1} soil did not inhibit photosynthetic pigment for two safflower cultivars, whereas 100 mg Cd kg^{-1} soil treatments significantly diminished the chlorophyll a (Chl a), total chlorophyll (Chl a + b) and carotenoids (Car) content by 33.5%, 33.5%, 22.8% for NS-4, and 28.3%, 27.2%, 29.2% for YM, respectively. At 50 mg Cd kg^{-1} soil treatments, no difference was observed in Chl a, Chl a + b, and Car for NS-4, while the reduction of these parameters was significant statistically in YM. Chlorophyll b (Chl b) was significantly decreased at 100 mg Cd kg^{-1} soil in NS-4, but in YM the changes in Chl b was non-significant. The Chl a/b and Car/Chl ratios were similar in different Cd treatments and cultivars, however, an interaction of Cd and cultivar on Car/Chl was observed according to ANOVA ($p < 0.01$) (Table 2).

The P_n and G_s in both cultivars decreased gradually with the increase of Cd concentrations (Fig. 1a, b). Reduction in P_n was 25.6%, 35.6% and 48.9% in NS-4, while 16.7%, 45.2% and 57.3% in YM with 25, 50 and 100 mg Cd kg^{-1} soil, respectively, compared to the control. The inhibition in G_s was 7.0%, 37.6% and 39.8% in NS-4 and 16.0%, 52.5% and 55.3% in YM as the Cd concentration increased

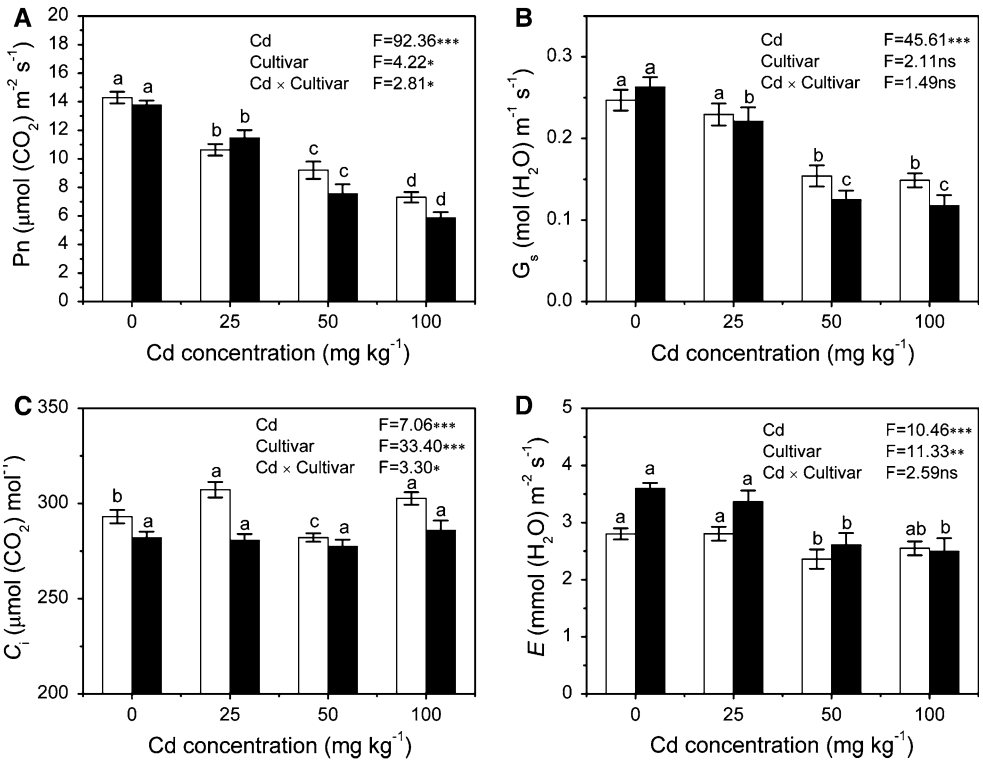

Fig. 1 Effects of Cd on **a** net photosynthetic rate (P_n), **b** stomatal conductance (G_s), **c** intercellular CO_2 concentration (C_i) and **d** transpiration rate (E) of safflower cultivars (*white square* New safflower No. 4; *black square* Yuming). Mean values ± SE are shown (n = 27). The different letters above the *bars* in the same cultivar are significantly different according to the Duncan's test ($p < 0.05$); *ns*: not significantly, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

from 25 to 100 mg Cd kg^{-1} soil in comparison to the control. Two-way ANOVA revealed that P_n was different between two cultivars ($p < 0.05$), and it was also affected by a Cd × cultivar interaction ($p < 0.05$). Under high Cd conditions, NS-4 showed higher P_n than YM.

A significant effect of Cd ($p < 0.001$) and cultivar ($p < 0.001$), as well as Cd × cultivar interaction ($p < 0.05$) was detected in the C_i. It was observed that C_i unchanged in YM, but fluctuant in NS-4 at different Cd levels (Fig. 1c). The E declined at 50 and 100 mg kg^{-1} Cd treatments by 15.7%, 9.0% for NS-4 and 27.5%, 30.6% for YM, respectively, but unaltered at 25 mg kg^{-1} Cd treatments for both cultivars, compared with the control. When exposed to the control and 25 mg kg^{-1} Cd soil, YM exhibited higher E than NS-4 ($p < 0.05$) (Fig. 1d).

The chlorophyll fluorescence parameters, F_v/F_m and ΦPS II, gradually decreased with the increasing of Cd concentrations (Fig. 2). Exposing seedlings to high level of Cd (100 mg kg^{-1}) caused decrease of 3.5% and 2.6% in F_v/F_m ratio, and 6.2% and 5.1% in ΦPS II for NS-4 and YM, respectively.

Effects of Cd on MDA varied in respect to Cd concentrations and cultivars (Fig. 3). Increasing of Cd concentrations enhanced MDA content by 31.6%, 40.2% and 41.7% in NS-4 under 25, 50 and 100 mg Cd kg^{-1}, respectively. Similar trend was found in YM in Cd treatments, except for 25 mg kg^{-1} Cd treatment, in which MDA unchanged compared with the control. Two-way ANOVA indicated a significant main effect of both Cd ($p < 0.001$) and cultivar ($p < 0.01$) in MDA. There was also a Cd × cultivar interaction ($p < 0.001$) in MDA. In contrast with NS-4, MDA was significantly low in YM at 25 mg kg^{-1} Cd treatment, as well as the control.

Cadmium treatment altered antioxidant enzyme activity for both safflower cultivars compared with controls (Fig. 4). In contrast to the controls, moderate Cd treatments enhanced SOD activity in both cultivars, while high level of Cd led to a marked decline in it (Fig. 4a). The POD activity did not differ in different Cd treatments for both cultivars, however, a high POD activity was observed in YM compared with NS-4 (Fig. 4b). Moderate Cd treatments induced an increase in APX activity in both cultivars compared with those of controls, but it is declined as Cd reached high toxic level. The increase of APX activity was higher in NS-4 than in YM (Fig. 4c). CAT activity decreased in both cultivars with the increase of Cd concentration in soil, YM showed a higher CAT activity compared with NS-4 (Fig. 4d). Two-way ANOVA indicated a significant main effect of both Cd and cultivar on POD and CAT activity, and there was also a Cd by cultivar interaction ($p < 0.01$) in APX activity.

The obtained results showed that physiological response of safflower plants to Cd stress is found to be cultivar- and dose- dependent. By contrast, the cultivar NS-4 exhibited high photosynthetic performance at high Cd treatments (50–100 mg Cd kg^{-1} soil), while cultivar YM had a high activity of antioxidant enzymes (POD, APX and CAT) under low Cd stress (25–50 mg Cd kg^{-1} soil).

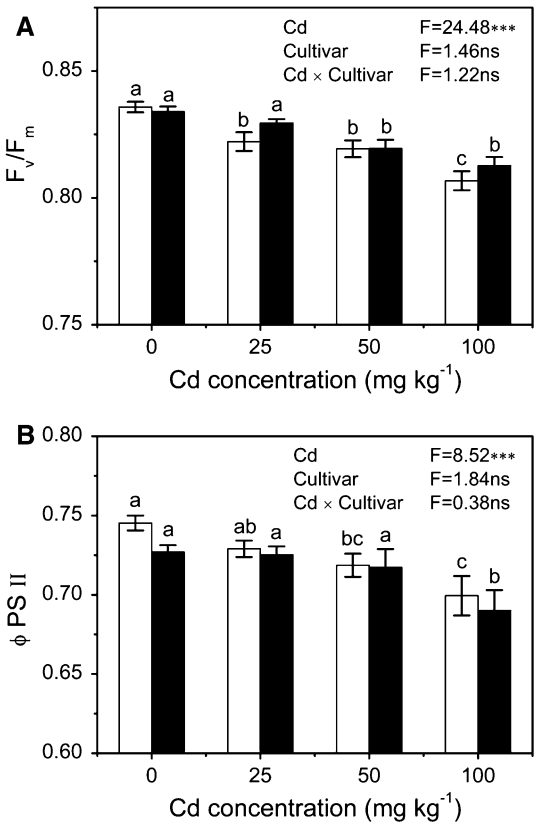

Fig. 2 Effect of Cd on **a** maximum quantum efficiency of PS II (F_v/F_m) and **b** quantum efficiency of PS II (ΦPSII) of safflower cultivars (*white square* New safflower No. 4; *black square* Yuming). Mean values ± SE are shown (n = 18). The different letters above the bars in the same cultivar are significantly different according to the Duncan's test ($p < 0.05$); *ns*: not significantly, *** $p < 0.001$

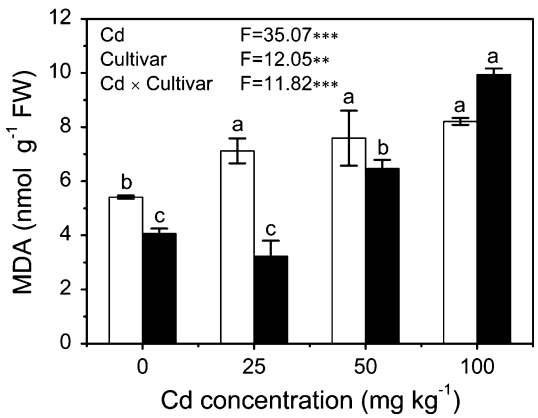

Fig. 3 Effects of Cd on MDA content in leaves of safflower cultivars (*white square* New safflower No. 4; *black square* Yuming). Mean values ± SE are shown (n = 3). The different letters above the *bars* in the same cultivar are significantly different according to the Duncan's test ($p < 0.05$); ** $p < 0.01$, *** $p < 0.001$

Cadmium toxicity may decrease stomatal conductance to CO_2, thus inhibiting photosynthesis (Baryla et al. 2001). Cd-induced inhibition of photosynthesis is also attributed to an inhibition of the activities of key enzymes of the Calvin cycle and the photosynthetic electron transport chain (Nwugo and Huerta 2008). Our research has clearly illustrated that elevated Cd decreased P_n for both safflower cultivars, this was accompanied with a decreasing of E and G_s. The parallel changes of P_n and G_s in two safflower cultivars reinforce hypothesis that the decrease of the photosynthetic rate could be mainly attributed to the stomatal limitation (Chartzoulakis et al. 2002). However, the unchanged or even high C_i in Cd-exposed seedlings indicated that the Cd-induced inhibition of photosynthesis was also due to an inhibition of Calvin cycle enzymes and/or an inhibition of the photosynthetic electron transport chain (Nwugo and Huerta 2008).

Our results also revealed that Cd exposure resulted in a decrease of chlorophyll (a and a + b) and carotenoids contents, as well as Fv/Fm and ΦPS II for both safflower cultivars. This may be partly responsible for the Cd-induced decrease of the photosynthetic rate. The reduction in chlorophyll contents suggest that the chlorophyll synthesizing system and chlorophyllase activity were affected at higher Cd concentrations (Van Assche and Clijsters 1990). The decrease in Fv/Fm and ΦPS II indicated that the photoactivation of PS II was inhibited by Cd toxicity, which result from the destruction of antennae pigments, and the limitation of Q_A reoxidation by the decrease or partial block of electron transport from PS II to PS I (Mallick and Mohn 2003).

Since the two cultivars were similar in photosynthetic pigments and chlorophyll fluorescence parameters under Cd stress, it is inferred that the high P_n in the seedlings of NS-4 may be contributed by high level of C_i and G_s. This was clearly demonstrated by the two-way ANOVA, which revealed a significant main effect of Cd and cultivar, and a Cd by cultivar interaction on P_n and C_i. Likewise, significant Cd × cultivar interactions were also found in APX activity, MDA content and Car/Chl ratio. APX plays a most important role in removing H_2O_2, which result from the decomposition of $O_2^{-\bullet}$ and and is toxic as it acts both as an oxidant as well as a reductant (Hsu and Kao 2007). The increase in Car/Chl ratio for NS-4 at 100 mg kg^{-1} treatment reveals relative more carotenoids in leaves. High activity of APX and more carotenoids suggests a promotion in the defense potential against reactive oxygen species (Bhargava et al. 2005). Therefore, the high photosynthetic performance of cultivar NS-4 under high Cd level may also benefit from a relative high activity of Calvin cycle enzymes, for which the high APX activity and Car/Chl ratio accounted.

Although Cd does not generate ROS directly, it generates oxidative stress via interference with the antioxidant defense system (Hsu and Kao 2007). In the present study, Cd-induced oxidative stress is indicated by the increased

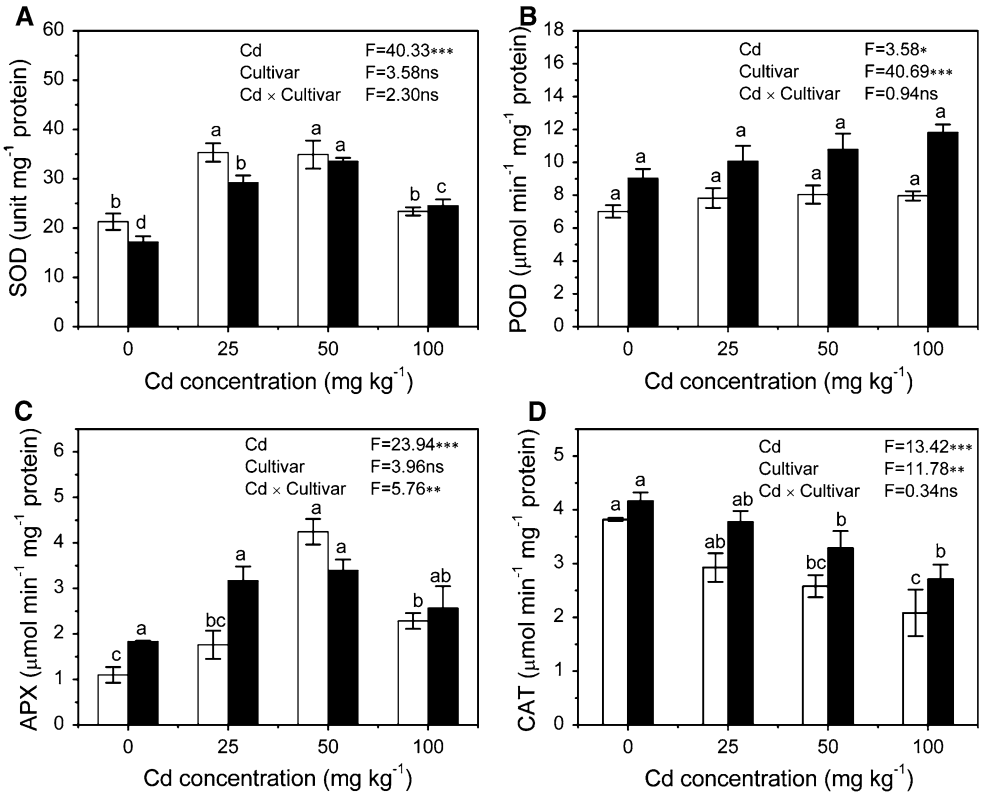

Fig. 4 Changes in activities of **a** SOD, **b** POD, **c** APX and **d** CAT in leaves of safflower cultivars (*white square* New safflower No. 4; *black square* Yuming) exposed to different levels of Cd. Mean values ± SE are shown (n = 3). The different letters above the *bars* in the same cultivar are significantly different according to the Duncan's test ($p < 0.05$); *ns*: not significantly, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

MDA contents, which suggest that both safflower cultivars suffered oxidative stress under high Cd conditions. Similar results were reported for many plant species, including bean (Chaoui et al. 1997), pea (Metwally et al. 2005), rice (Chien et al. 2001), sunflower (Gallego et al. 1996), mustard (Mobin and Khan 2007) and maize (Krantev et al. 2008).

ROS scavenging can be achieved by antioxidant enzymes such as SOD, POD, CAT and APX. SOD catalyzes the conversion of superoxide anion to O_2 and H_2O_2. The increased SOD activity suggest plants have greater O_2 radical-scavenging ability (de Azevedo Neto et al. 2006). APX appeared to play an essential protective role in the scavenging process when co-ordinated with SOD. In the present study, we found that moderate Cd exposure enhanced SOD and APX, but decreased CAT activity for both safflower cultivars, whereas the POD activity remains unaffected. It seems that SOD and APX accounted for the elimination of AOS in safflower cultivars.

As for cultivar YM, the small reduction in shoot biomass, low MDA, and high activity of antioxidant enzymes (POD, APX and CAT) at 25 mg kg^{-1} Cd soil, indicated that the plants had high capacity to resist Cd toxicity at low Cd level. According to the two-way ANOVA, there were significant cultivar × Cd interactions in APX activity, MDA content and biomass, suggesting the high biomass of YM under low Cd level was mainly contributed by a high APX activity, which protect plants against Cd-induced oxidative stress.

In conclusion, when exposed to Cd contaminated soil, the seedlings of safflower can accumulate large amount of Cd in the shoots, thus the species can be considered as a potential Cd accumulator. The physiological response of safflower plants to long-term Cd stress is found to be cultivar- and dose- dependent. The cultivar NS-4 exhibited high photosynthetic performance at high level of Cd, which may be contributed by high level of C_i, APX activity and Car/Chl ratio. In contrast, YM is more tolerant to Cd toxicity at low Cd level, in which an efficient antioxidant system is involved. Cd-induced inhibition of photosynthesis in safflower plants can be attributed to: (1) stomatal limitation; (2) photosynthetic pigment reduction, and (3) photosynthetic apparatus destruction.

Acknowledgments Financial support from the National Natural Science Foundation of China (No. 40971296) and the Natural Science Foundation for College of Anhui Province (KJ2009B073) is gratefully acknowledged.

References

Aebi H (1984) Catalase in vitro. Meth Enzymol 105:121–126

Ait Ali N, Bernal MP, Ater M (2002) Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. Plant Soil 239:103–111

Baker AJM (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. J Plant Nutr 3:643–654

Baryla A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M (2001) Leaf chlorosis in oilseed rape plants (*Brassica napus)* grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta 212:696–709

Bassil E, Kaffka S (2002) Response of safflower (*Carthamus tinctorius* L.) to saline soils and irrigation I. Consumptive water use. Agr Water Manage 54:67–80

Baszynski T, Wajda L, Krol M, Wolinska D, Drupa Z, Tukendorf A (1980) Photosynthetic activities of cadmium-treated tomato plants. Physiol Plant 48:365–370

Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287

Benavides M, Gallego MS, Tomaro ML (2005) Cadmium toxicity in plants. Braz J Plant Physiol 17:21–34

Bhargava P, Kumar Srivastava A, Urmil S, Chand Rai L (2005) Phytochelatin plays a role in UV-B tolerance in N_2-fixing cyanobacterium *Anabaena doliolum*. J Plant Physiol 162: 1220–1225

Boominathan R, Doran PM (2003) Cadmium tolerance and antioxidative defenses in hairy roots of the cadmium hyperaccumulator, *Thlaspi caerulescens*. Biotechnol Bioeng 83:158–167

Bradford M (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Chaoui A, Mazhoudi S, Ghorbal M, El Ferjani E (1997) Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). Plant Sci 127:139–147

Chartzoulakis K, Loupassaki M, Bertaki M, Androulakis I (2002) Effects of NaCl salinity on growth, ion content and CO_2 assimilation rate of six olive cultivars. Sci Hortic 96:235–247

Chien H, Wang J, Lin C, Kao C (2001) Cadmium toxicity of rice leaves is mediated through lipid peroxidation. Plant Growth Regul 33:205–213

de Azevedo Neto A, Prisco J, Enéas-Filho J, Abreu C, Gomes-Filho E (2006) Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot 56:87–94

Dordas CA, Sioulas C (2008) Safflower yield, chlorophyll content, photosynthesis, and water use efficiency response to nitrogen fertilization under rainfed conditions. Ind Crop Prod 27:75–85

Ekmekçi Y, Tanyolaç D, Ayhan B (2008) Effects of cadmium on antioxidant enzyme and photosynthetic activities in leaves of two maize cultivars. J Plant Physiol 165:600–611

Gallego S, Benavides M, Tomaro M (1996) Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. Plant Sci 121:151–159

Hsu Y, Kao C (2007) Toxicity in leaves of rice exposed to cadmium is due to hydrogen peroxide accumulation. Plant Soil 298: 231–241

Krantev A, Yordanova R, Janda T, Szalai G, Popova L (2008) Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. J Plant Physiol 165:920–931

Laspina NV, Groppa MD, Tomaro ML, Benavides MP (2005) Nitric oxide protects sun flower leaves against Cd-induced oxidative stress. Plant Sci 169:323–330

Li HS (2000) Principles and techniques of plant physiological biochemical experiment. Higher Education Press, Beijing, pp 260–263

Lichtenthaler H (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382

Mallick N, Mohn FH (2003) Use of chlorophyll fluorescence in metal-stress research: a case study with the green microalga *Scenedesmus*. Ecotox Environ Safe 55:64–69

Metwally A, Safronova VI, Belimov AA, Dietz KJ (2005) Genotypic variation of the response to cadmium toxicity in *Pisum sativum* L. J Exp Bot 56:167–178

Mobin M, Khan NA (2007) Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J Plant Physiol 164:601–610

Monni S, Salemaa M, Millar N (2000) The tolerance of *Empetrum nigrum* to copper and nickel. Environ Pollut 109:221–229

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880

Nwugo C, Huerta A (2008) Effects of silicon nutrition on cadmium uptake, growth and photosynthesis of rice plants exposed to low-level cadmium. Plant Soil 311:73–86

Page A, Miller R, Keeney D (1982) Methods of soil analysis. American Society of Agronomy, Inc, Madison

Pence NS, Larsen PB, Ebbs SD, Letham DL, Lasat MM, Garvin DF, Eide D, Kochian LV (2000) The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. Proc Natl Acad Sci USA 97:4956–4960

Putter J (1974) Peroxidases. In: Bergmeyer HU (ed) Methods of enzymatic analysis: II. Academic Press, New York., pp 685–690

Sanita di Toppi L, Gabrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Sigfridsson KG, Bernat G, Mamedov F, Styring S (2004) Molecular interference of Cd (2+) with photosystem II. Biochim Biophys Acta 1659:19–31

Sims R, Hastings A, Schlamadinger B, Taylor G, Smith P (2006) Energy crops: current status and future prospects. Global Change Biol 12:2054–2076

Sun Y, Zhou Q, Diao C (2008) Effects of cadmium and arsenic on growth and metal accumulation of Cd-hyperaccumulator *Solanum nigrum* L. Bioresour Technol 99:1103–1110

Tiryakioglu M, Eker S, Ozkutlu F, Husted S, Cakmak I (2006) Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. J Trace Elem Med Biol 20:181–189

Van Assche F, Clijsters H (1990) Effects of heavy metals on enzyme activity in plants. Plant Cell Environ 13:195–206

Weiss E (2000) Oilseed crops. Second Addition. Blackwell Science, London