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# Effects of cadmium (Cd) on seedling growth traits and photosynthesis parameters in cotton (*Gossypium hirsutum* L.)

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#### Abstract

Cadmium (Cd) is a widespread toxic heavy metal that usually causes deleterious effects in living organisms. In this study, the effects of Cd on seedling growth traits and photosyntheses parameters in cotton were investigated. The seedlings at three-leaf stage cultured in four nutrient solutions containing different Cd concentrations (0, 25, 50, and 100  $\mu$ M) were subjected to assay the growth traits and the photosyntheses parameters. The growth traits analyzed included plant height, leaf area, biomasses of leaf, root, and stem, whereas the photosynthetic parameters measured included chlorophyll (Chl) content, gas exchange, and chlorophyll fluorescence. The results indicated that after 3 and 7 d treatments of Cd, the growth of cotton seedlings was significantly inhibited, showing that the plant height, biomass, and leaf area were all decreased. The Cd treatments also affected the photosyntheses parameters of the cotton seedlings. Under treatments of 50 and 100  $\mu$ M concentrations of Cd, the seedlings exhibited significant decreases on Chl *a* and Chl *b*, photosynthetic rate (P<sub>n</sub>), stomotal conductance (G<sub>s</sub>), transpiration rate (E), maximal photochemical efficiency (F<sub>v</sub>/F<sub>m</sub>), quantum yield of electron transport ( $\Phi_{PSII}$ ), photochemical quenching (qP), and electronic transport rate (ETR) (P≤0.05). However, the Chl *a/b* and C<sub>i</sub> were increased after the Cd treatments. The dramatic variations of chlorophyll fluorescence values under Cd treatments in comparison with the control (CK) suggested that the photosystem II (PS II) activity in cotton seedlings was much more sensitive to Cd toxicity. Taken together, our results confirm that Cd significantly inhibits the growth traits as well as the photosynthetic parameters in cotton seedling such and cotton seedling that the photosynthetic photosynthetic photosynthetic photosynthetic photosynthetic photosynthetic photosynthetic photosynthetic to cotton seedlings. The non-stomata limitation, Chl reduction, and PSII system damage act as main limiting factors for affe

Keywords: Cotton seedling; Cadmium (Cd); Seedling growth; Gas exchange; Chlorophyll fluorescence.

**Abbreviations:** Cd\_Cadmium; Chl a\_Chlorophyll a; Chl b\_Chlorophyll b; Pn\_Net photosynthetic rate; C<sub>i</sub>\_Intercellular CO<sub>2</sub> concentration; G<sub>s</sub>\_Stomatal conductance; E\_Transpiration rate; PS II\_Photosystem II;  $F_v/F_m$ \_Maximal photochemical efficiency;  $F_0$ \_Initial chlorophyll fluorescence yield;  $F_m$ \_Maximum chlorophyll fluorescence yield;  $F_s$ \_Steady-state fluorescence;  $F'_m$ \_Maximum chlorophyll fluorescence at actinic light;  $\Phi_{PSII}$ \_Quantum yield of electron transport; qP\_Photochemical quenching; ETR\_Electronic transport rate; PPFD\_Photosynthetic photon flux density.

#### Introduction

has been continuously exacerbating Pollution the environmental deterioration at a global scale. For instance, the negative effects of heavy metal ions, such as cadmium (Cd), copper, mercury, zinc, and lead on plant growth have become increasingly in past decade (Wang, et al., 2007). Cd, a widely distributed heavy metal pollutant along with mining, fertilization, and industrialization, has been paied more attention to because of its increasing environmental burden (Wagner, 1993). Cd is also a non-essential element and extremely toxic to plants. The Cd in the soil is taken up by roots and then transported across plant tissues, and finally accumulated in roots, shoots, fruits, and grain (Qian et al., 2009). Excess Cd accumulation in plants generally causes various symptoms of phytotoxicity, results in inhibititon of plant growth and development (di Cagno et al., 1999; Milone et al., 2003). These negative effects of Cd on plants may be associated with that Cd interferes with several metabolic processes (Liu et al., 2011). Numerous studies reported that excessive amount of Cd in plants can cause plant growth retardation, chlorosis, leaf rolls, and necrosis (Xue et al., 2013). At physiological level, excess Cd can result in the inhibition of photosynthesis and Chl fluorescence efficiency (Azevedo et al.,

modification of gene expressions (Herbette et al., 2006; Liu et al., 2011). Of these, one of the most evident effects of Cd toxicity is Chl loss, i.e., chlorosis (Baryla et al., 2001; Schützendübel et al., 2001). Under high concentration of Cd (such as 100µM), the photosynthetic rate, content of Chl and Rubisco, activities of Rubisco and PSII system, and Calvin cycle circulation are all significantly reduced in comparison with the control (Pietrini et al., 2003; di Cagno et al., 1999; Krupa et al., 1993). Although Cd limits the CO<sub>2</sub> fixation rate, it does not affect the rates of electron transport in photosystem I or II (PSI or PSII) or the rate of dark reaction (Greger and Ögren, 1991). Although a lot of studies have demonstrated that excess Cd limits plant growth, morphological traits and photosynthesis. However, only a few studies have focused on the effects of Cd on the growth in cotton have been performed (Khan et al., 2013; Li et al., 2012). The effects of Cd on the growth traits of cotton seedlings as well as on the photosynthetic parameters are still to be determined. In this study, using the seedlings cultured in nutrient solutions containing different Cd, we systematically investigated the

2005), imbalance of mineral nutrients (Gouia et al., 2000),

variation of enzyme activities (Hasan et al., 2009), and

effect of Cd on growth traits and photosynthetic parameters in cotton. Our results could deepen the understanding of Cd toxic mechanism on cotton from the scope of the relations between plant growth and photosynthetic parameters.

#### **Results and Discussion**

#### Plant growth

The seedling growth was dramatically limited by Cd treatments (Fig. 1). Along with the increase of Cd concentrations, plant height and leaf area were decreased. Of the Cd treatments, the stress symptoms were much more evident at Cd concentrations of 50 and 100  $\mu$ M. At 100  $\mu$ M of Cd, plant height and leaf area decreased 43.0% and 34.1% after 3 and 7 d treatment, respectively, in comparison with that of the control (CK).

The inhibitory effect of Cd was also observed in plant biomass. The plant biomass under different Cd concentration treatments are shown in Fig. 2. Similarly, the plant biomasses were gradually decreased along with the increase of Cd concentrations. High concentrations of Cd, such as 50 and 100 µM, resulted in a much more loss of plant biomass. For instance, 100 µM of Cd resulted in biomass decreases of 45.8%, 33.8%, and 50.7% in root, stem, and leaf after 7 d of Cd treatment, respectively, in comparison with the CK. Among aforementioned tissues, the biomass of leaf decreased more than those of root and stem reduced. In addition, the effects of Cd at a low concentration (25  $\mu$ M) on plant biomass were investigated in two cultivars, including GXM9 and NDM9. It was observed that although the plant biomass of NDM9 was lower than that of the control group, the plant biomass of GXM9 did not show difference between the Cd treatment and the control group. Therefore, there are different response capacity to Cd among the cotton genotypes. The plant height and leaf area of cotton seedlings were also affected by Cd treatments. These results are consistent with those previous reported in other studies (di Cagno et al., 1999; Liu et al., 2011; Ouariti at al., 1997; Zhou and Qiu, 2005). In this study, we also found that the treatment with the highest Cd concentration (100 µM) together with prolonged treatment resulted in most genitive effects. These results confirm the Cd toxicity in cotton exhibiting interaction effects of the concentration and the treatment duration. Similar results have already been reported in other plant species, such as cucumber (Zhang et al., 2002) and sunflower (Azevedo et al., 2005).

#### **Pigment content**

Chlorophyll (Chl), an important pigment to maintain plant growth, is mainly composed of Chl a and Chl b. The effects of Cd on Chl a, Chl b, and Chl (a + b) in cotton seedlings after 7 d treatment of Cd are shown in Figure 3. The Chl content of the cotton seedlings was significantly inhibited by low concentration treatment of Cd (25 µM), and the inhibitory effects were enhanced as the Cd concentration increased. For the two components of Chl, Chl a was strongly reduced by 7 d treatment of 100 µM Cd (decreased by 45.5% compared with that of the control group) (Fig. 3B). In comparison with Chla, aforementioned Cd treatment caused relative higher decrease of Chlb (Fig. 3C). Therefore, higher Cd concentrations promoted the degradation of Chl b and thus increased the Chl a/b ratio (Fig. 3D). The yellowish of leaves is one of the most commonly observed sympotom under Cd toxicity. Thus, Cd is a potent inhibitor of Chl biosynthesis (Somashekaraiah et al., 1992; Hsu and Kao, 2003). In the present study, the Chl contents were significantly reduced even in low concentration of Cd (25  $\mu$ M), and remarkably decreased as Cd concentrations increased. In addition, Cd treatments caused much more reduction of Chl *b* than of Chla, resulting in increased Chl a/b ratio. These results suggested that Cd caused a decrease in PSII light harvesting complexes (LHCII) that is related to reaction centers, which contains higher Chl *b* than PSII chlorophyll-binding proteins (Hikosaka and Terashima, 1995).

#### Gas exchange

Photosynthesis is the main source of biomass production. As Cd concentration increased (at 100 µM Cd), P<sub>n</sub> decreased by 28.1% and 57.0% at treatments of 3 and 7 d, respectively, in comparison with the control group (Fig. 4A). Similarly, stomotal conductance (G<sub>s</sub>) was also significantly decreased as the Cd concentration increased (Fig. 4B). At treatment of 3 and 7d under 100µM Cd, Gs was both significantly decreased. Cd treatments also resulted in  $C_{\rm i}$  decreased (by 9.8% at 7 d treatment under 100µM Cd) (Fig. 4C). However, there were significant genetic variations of C<sub>i</sub> in responding to Cd. GXM3 exhibited a relative higher value than NDM9 under high concentration of Cd treatment. E also decreased by Cd treatments, showing decrease by 50.4% and 35.4% at 3 and 7 d treatment under 100 µM Cd concentration, respectively, in comparison with the control group (Fig. 4D). Gas exchange factors affect stomatal and non-stomatal conductances of CO<sub>2</sub> that further determine P<sub>n</sub> behavior (Farquhar and Sharkey, 1982). In this study, gas exchange parameters were assayed and it was found that these parameters were also affected by Cd treatments, similar to previous reports (Krantev et al., 2008; Liu et al., 2011; Mobin and Khan, 2007; Shi et al., 2010). In contrast with Pn, Gs, and E, the Ci was increased at 7 d treatments of Cd. Thus, the decrease in photosynthetic rate under Cd treatment conditions may be related to the stomatal conductance limitation resulted from the reduction of CO<sub>2</sub> supply (Panković et al., 2000; Xu et al., 2005). Moreover, the decreased rates of CO<sub>2</sub> assimilation can also be partly attributed to the corresponding decrease of the photosynthetic unit density, i.e., the reduction in Chl content regulated by Cd (di Cagno et al., 1999; Chaneva et al., 2010).

#### Chlorophyll fluorescence

Chlorophyll fluorescence efficiency was also affected by Cd treatments. F<sub>v</sub>/F<sub>m</sub> was decreased by Cd treatments (Fig. 5A). Similarly,  $\Phi_{PSII}$ , a parameter reflecting the light conditions of PSII photochemical efficiency (Fig. 5B), qP and ETR (Fig. 5C and 5D), were all decreased after Cd treatments, and exhibited much more reduction extent along with the increase of Cd concentration and prolonged treatment duration. Previous studies have confirmed that associated with the decrease of Chl content by Cd, the chlorophyll fluorescence efficiency was also negatively affected, but the Chl content is inhibited by Cd to a greater extent than PSII efficiency (Chugh and Sawhney, 1999; Liu et al., 2011). In the present study, the fluorescence parameters were also affected by Cd treatments. The F<sub>v</sub>/F<sub>m</sub>,  $\Phi_{PSII}$ , qP, and ETR were all significantly decreased after 7 d of Cd treatments. Similar results have been reported in other plants, such as pakchoi (Sun et al., 2005), castor (Liu et al., 2011), maize (Ekmekçi et al., 2008), and safflower (Shi et al., 2010).  $F_v/F_m$  represents the maximum efficiency of PSII primary photochemistry capacity. This ratio decreased as Cd concentration increased, suggesting that Cd resulted in a decrease in the PSII maximum quantum efficiency, by which the electron transport in photosystem was blocked. Similar results have been observed in other plants (Drążkiewicz et al., 2003). We also observed a decrease in  $\Phi_{PSII}$ , the proportion of light absorbed by chlorophyll in PSII (a variable used in photo-



**Fig 1.** Plant height (A) and leaf area (B) of cotton seedlings (two cotton cultivars, namely, NDM 9 and GXM 3) treated with CdCl<sub>2</sub> at 7 d of treatment. Data (mean  $\pm$  S.E., n = 5) with different letters are significantly different according to Duncan's multiple range test (P $\leq 0.05$ ).



**Fig 2.** Plant biomass of cotton seedlings (two cotton cultivars, namely, NDM9 and GXM3) treated with CdCl<sub>2</sub>at 7 d of treatment: (A) root; (B) stem; and (C) leaf. Data (means  $\pm$  S.E., n = 5) with different letters are significantly different according to Duncan's multiple range test (P $\leq$ 0.05).



**Fig 3.** Chl of cotton seedlings (two cotton cultivars, NDM9 and GXM3) treated with CdCl<sub>2</sub>at 7 d of treatment: (A) Chl (a+b), (B) Chla, (C) Chlb, and (D) Chla/b. Data (means ± S.E., n = 5) with different letters are significantly different according to Duncan's multiple range test (P≤0.05).



**Fig 4.** Effects of Cd on  $P_n$  (A),  $G_s$  (B),  $C_i$  (C), and E (D) in the leaves of cotton seedlings (two cotton cultivars, NDM9 and GXM3) at 3 and 7 d of treatment. Data (means ± S.E., n = 5) with different letters are significantly different according to Duncan's multiple range test (P $\leq 0.05$ ).

chemistry), which was in consistent with the results of Maxwell and Johnson (2000). This decrease can be attributed to the decreased capacity of carbon metabolism or to the low utilization of ATP and NADPH in the dark phase of photosynthesis (Liu et al., 2011; Subrahmanyam and Rathore, 2001). qP indicates the proportion of open PSII reaction centers. In this study, it was observed that this parameter was decreased by Cd treatment, indicating that high percentage of PSII reaction centers was closed by Cd and further reduced the capacity of PSII to re-oxidize QA. The balance between electron transfer and excitation rates changed and thus reduced the state of the PSII reaction centers, i.e., captured light cannot be effectively used (Huang et al., 2013). ETR was significantly affected under serious stress conditions, suggesting that photosynthetic electron transport via PSII in higher Cd concentration treatments was inhibited. . A similar result has been reported by Vassilev et al. (2004) in Cd-treated barley

plant. Taken together above results, we suggest that Cd could inhibit the potential activity and the photochemical efficiency of PSII system, which plays an important role in affecting the plant growth traits, such as the plant height, leaf area, and biomass.

#### Materials and methods

#### Plant material and growth conditions

The seeds of two transgenic cotton cultivars, namely, NDM9 and GXM3, were immersed in 0.1% HgCl<sub>2</sub> for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dishes at 25°C in the dark. After 14 h of incubation, uniformly germinated seeds were selected and cultivated in a pot containing quarter-strength Hoagland solution (600 ml) with following macro- and micro-elements:



**Fig 5.** Effects of Cd on  $F_v/F_m$  (A),  $\Phi_{PSII}$  (B), qP (C), and ETR (D) in the leaves of cotton seedlings (two cotton cultivars, NDM9 and GXM3) at 3 and 7 d of treatment. Data (means ± S.E., n = 5) with different letters are significantly different according to Duncan's multiple range test (P≤0.05).

5.0 mMCa(NO<sub>3</sub>)<sub>2</sub>; 5.0 mM KNO<sub>3</sub>; 2.5 mM MgSO<sub>4</sub>; 2.0 mMKH<sub>2</sub>PO<sub>4</sub>; 2.0 mM NaH<sub>2</sub>PO<sub>4</sub>; 5.0 mM NaNO<sub>3</sub>; 2.5 mM MgCl<sub>2</sub>; 20  $\mu$ MFeEDTA; 6.722  $\mu$ MMnSO<sub>4</sub>; 0.316  $\mu$ MCuSO<sub>4</sub>; 0.765  $\mu$ MZnSO<sub>4</sub>; 46.25  $\mu$ MH<sub>3</sub>BO<sub>3</sub>; and 0.5  $\mu$ MH<sub>2</sub>MoO<sub>4</sub>. The nutrient solution was adjusted to pH 6.0 by 1 M of HCl/NaOH. The applied nutrient solution for cultivation of cotton seedlings was renewed twice a week. The hydroponically cultivated seedlings were grown in a phytotron under following condition: 25°C/20°C temperature (day/night), 60% relative air humidity, 14 h/10 h photoperiod (day/night) and of 400  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup> of PPFD which provided by high-pressure sodium lamps (General Photonics, USA).

### Experimental design

At the three-leaf stage, the seedlings were treated by Cd with different concentrations of CdCl<sub>2</sub>, including 0 (CK), 25, 50, and  $100\mu$ M CdCl<sub>2</sub>. Each treatment was replicated by 20 times (pots), with one seedling planted in each pot. These pots were randomly arranged in growth room during the treatment period. The plant growth, biomass, photosynthetic parameters, and fluorescence parameters were dassayed after 3 and 7 d of Cd treatment.

Plant heights, stem diameters, and leaf areas were measured by conventional method. In which, the leaf area was calculated by following equation: leaf area= length  $\times$  width  $\times$  0.73. Each seedling was grouped into follow three parts: roots, stem, and leaves. Plant biomass was obtained after drying at 105°C for 30 min and followed at 80°C for 24 h.

#### Chl content

0.1 g of leaves was extracted by 5 ml of 95% (v/v) alcohol. Chl a and Chl b were obtained by calculating the light absorbance at 665, 649, and 470 nm calculated according to Zou (2000).

#### Gas exchange

Net photosynthetic rate (P<sub>n</sub>), stomatal conductance (G<sub>s</sub>), intercellular CO<sub>2</sub> concentration (C<sub>i</sub>), and transpiration rate (E) in the third-leaf stage were measured using an open-flow infrared gas analyzer LI-COR 6400 (LI-COR, Lincoln, NE, USA) and an LED light source. The light intensity, leaf temperature, and CO<sub>2</sub> concentration inside the leaf chamber were kept constant by follows: 400 ± 2 µmol m<sup>-2</sup> s<sup>-1</sup>, 25 ± 0.3°C, and 400 ± 5 µmol CO<sub>2</sub> mol<sup>-1</sup>, respectively (di Cagno et al.,1999).

#### Chlorophyll fluorescence

Chlorophyll fluorescence parameters were measured by a pulse-modulated fluorimeter (FMS2, Hansatech, UK).  $F_v/F_m$  was determined in the dark-adapted (20 min) leaves. For that,  $F_0$  was determined in low-modulated measuring light, a 0.7 s pulse of saturating white light (>3,000 µmol m<sup>-2</sup> s<sup>-1</sup>) was applied to obtain  $F_m$ .  $F_s$  and  $F'_m$  were measured as the seedlings were exposed to 400 µmol m<sup>-2</sup> s<sup>-1</sup>. Other four indices were calculated as follows: (1)  $F_v/F_m = (F_m - F_0)/F_m$ ); (2)  $\Phi_{PSII} = (F'_m - F_s)/F'_m$ ; (3)  $qP = (F'_m - F_s)/(F'_m - F_0)$ ; and (4) ETR = PSII × PPFD × 0.5 × 0.84 (Maxwell and Johnson, 2000).

#### Statistical analysis

The results were the means of at least five replicates for plant growth, Chl content, gas exchange, and chlorophyll fluorescence and three replicates for biomass in each treatment. The significance of differences between the control group and each treatment group across the assayed growth traits and photothesis parameters was determined using Duncan's multiple range test at the 5% and 1% probability level (SPSS software). Data were expressed as mean  $\pm$  SD.

#### Conclusion

We investigated the effects of Cd treatment on growth traits and photosynthesis parameters in cotton, such as plant height, leaf area, and plant biomass as well as Chl content, gas exchange, and chlorophyll fluorescence in cotton seedlings. The results indicate that Cd significantly inhibits plant growth, reduces the contents of Chl, and most photosynthesis parameters of the cotton seedlings. It is suggested that Cd acts as the main limiting factors for photosynthesis via non-stomata limitation, Chl reduction, and PSII damage, which results largely in plant growth inhibition in cotton.

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