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Cotton seedling plants adapted to cadmium stress by enhanced activities of protective enzymes

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ABSTRACT

Cotton (*Gossypium hirsutum* L.) is a global major crop with strong tolerance to abiotic stress, but its tolerance to cadmium (Cd) stress is unclear. The objective of this study was to determine the influence of Cd stress on the seedling growth and some physiological properties of cotton. Cotton seedlings with three fully expended leaves were treated with Cd at different concentrations (0, 25, 50 and 100 μmol/L), and seedling growth, chlorophyll (Chl) content, malonaldehyde (MDA) content, photosynthetic rate, superoxide dismutase (SOD) and peroxidase (POD) activity in the main-stem leaves were measured 5 days or 10 days after stress treatment. It was found that with the increase in the Cd concentration, the SOD and POD activity of the stressed seedlings displayed an increase first and then a decrease. The MDA content increased and the Chl decreased, which finally led to a decline in plant height and leaf area. The results suggest that cotton seedlings were adapted to low-concentration Cd stress by the increased protective enzyme activity, but over 50 μmol/L of Cd concentration would exert a significantly inhibitory effect on the photosynthetic properties and protective enzyme activity of the cotton leaves. Cotton plants can be adapted to low Cd stress by increasing the activity of the protective enzymes.

Keywords: toxic element; heavy metal; plant oxidative damage; reactive oxygen species (ROS)

Cadmium (Cd), one of the major heavy metal pollutants characterized by high toxicity and accumulation, can retard plant growth after being absorbed and enriched in plants. The rapid development of industrial and agricultural production has resulted in an increase in the three industrial wastes as well as urban waste and the increasing application of mineral fertilizers, which have caused the Cd content in the soil to increase continuously (Li et al. 2015). The total area of soil contaminated by Cd has increased to 280 000 ha, which accounts for 59.6% of heavy metal-polluted soils; this makes Cd one of the most severe sources of farmland pollution in China (Zhao and Zhao 2002). Many plants can accumulate relatively high levels of Cd without adverse effects on growth (Kuboi et al. 1986), which could be attributed to the alteration of the chlorophyll content, malonaldehyde (MDA) and antioxidant enzymes caused by the disruption of the intracellular membrane structure and the active centres of proteins and enzymes through a cascade of redox actions induced by Cd (Guo et al. 2015).

Cotton (*Gossypium hirsutum* L.) is considered to be a crop species suitable for soil remediation because of its high resistance to Cd stress as well as its strong ability to absorb, transfer and accumulate Cd (Chen et al. 2014). Currently, most research related to Cd stress in cotton has focused on the Cd accumulation and enrichment properties; microstructure of the root system; and fiber quality; etc. (Li et al. 2012, Liu et al. 2014), but they rarely touch upon the growth and protective enzyme system of cotton seedlings under Cd stress. To reveal the toxic effect of Cd on cotton seedling growth, this work analysed the influence of various concentrations of Cd on cotton seedling growth and the activity of its protective enzyme

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system, which is meaningful for both the planting of cotton in Cd-polluted areas and the breeding of Cd-resistant cotton cultivars.

MATERIAL AND METHODS

Plant materials and growing conditions. This study was carried out in an artificial climatic chamber in the College of Agronomy, Hebei Agricultural University in 2014. The insect-resistant cotton cultivars Guoxin #3 (GXM3) and Nongda #9 (NDM9) were used in this study. The selected cotton seeds were sterilized with 0.1% $HgCl_2$ for 20 min, rinsed with tap water three times and then with distilled water three times, in succession, and then germinated using Vermiculite nutritional culture. After the cotyledons were fully extended, seedlings with a uniform growth tendency were transferred and cultured in 1/4 Hoagland liquid culture bowls for 3 days, with one seedling in each bowl, and cultured in 1/2 Hoagland liquid culture with ventilation for 1 h each day. When three leaves were fully extended, seedlings with a uniform growth tendency were subject to Cd stress through a $CdCl_2$ treatment. A total of five different concentrations of Cd were used in this study: 0 μmol Cd/L (CK); 25 μmol Cd/L (T25); 50 μmol Cd/L (T50) and 100 μmol Cd/L (T100). Fifteen biological repeats of each treatment were provided in this study. The physiological indexes of the seedlings with a uniform growth tendency were measured 5 days and 10 days after Cd treatment, and the plant height and leaf area per plant were measured 10 days after the treatment.

Physiological index determination. Chlorophyll content was determined by the method of Zou (2000). The photosynthetic gas exchange parameters were measured using an open-flow infrared gas analyzer LI-COR 6400 (LI-COR, Lincoln, USA) and an LED light source (Di Cagno et al. 1999). superoxide dismutase (SOD) activity was estimated based on the method of Read (Zhao et al. 1998) using the photochemical nitro blue tetrazolium chloride (NBT) method. Peroxidase (POD) activity was determined by the method of Zhao et al. (1998), and the malonaldehyde (MDA) content was determined by the method of Zou (2000).

Statistical analysis. The data shown in this paper are reported as the mean ± standard deviation (SD). We performed three independent experiments for each condition. The statistical significance was considered to be significant when the *P* value was less than 0.05 when using a one way analysis of variance.

RESULTS AND DISCUSSION

After Cd treatment for 10 days, the heights of both cotton cultivars displayed a reduction as the Cd concentration increased, and significant differences between the treatment groups and the control group ($P < 0.05$) were observed (Figure 1). After the treatment with 100 μmol Cd/L, the heights of cv. NDM9 and cv. GXM3 were reduced by 45.2% and 40.8%, respectively, when compared with the control group, and both reached the extremely significant level ($P < 0.01$). In addition, different levels of difference were observed between the two

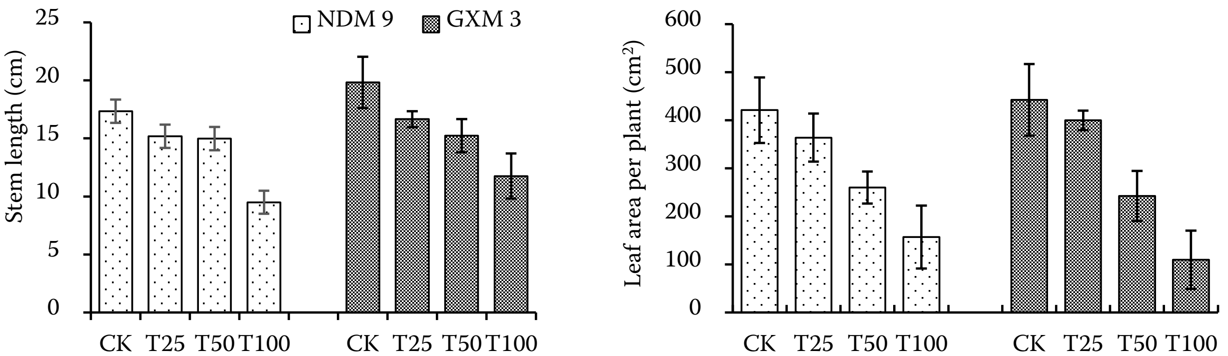

Figure 1. Effects of cadmium (Cd) treatment on stem length and leaf area per plant in cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences ($P < 0.05$) among four treatments and within two cultivars. CK – 0, T25 – 25, T50 – 50, T100 – 100 μmol Cd/L

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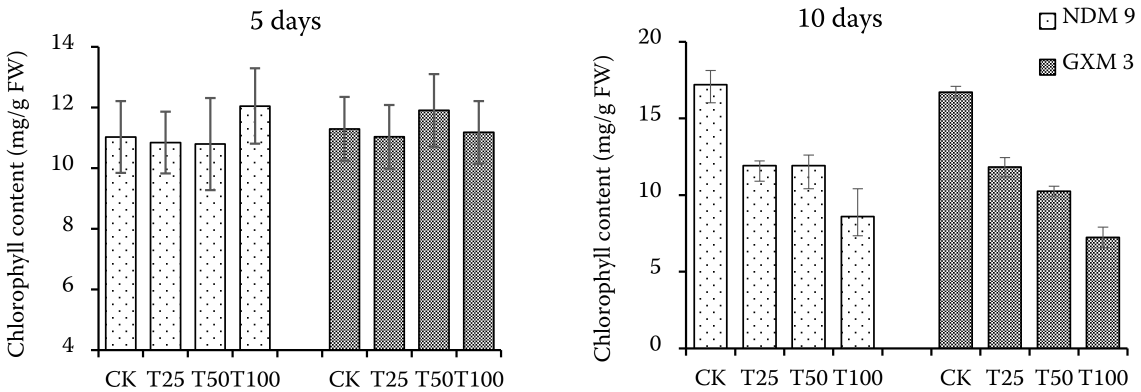

Figure 2. Effects of cadmium (Cd) treatment on chlorophyll content in cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences ($P < 0.05$) among four treatments and within two cultivars. FW – fresh weight; CK – 0, T25 – 25, T50 – 50, T100 – 100 μmol Cd/L

cotton cultivars. The leaf area per plant displayed a tendency to decrease with an increase in the Cd concentration (Figure 1). Significant differences ($P < 0.05$) were caused by both middle and high concentrations (50 μmol/L and 100 μmol/L) of the Cd treatment. After treatment with 100 μmol Cd/L, the heights of cvs. NDM9 and GXM3 increased by 62.7% and 75.2%, respectively, when compared with the control group, and both reached the extremely significant level ($P < 0.01$). This indicated that a high-concentration Cd stress could exert a severe inhibitory effect on the leaf area per plant. The research results in this study revealed that the cotton height and leaf area were significantly inhibited by increases in the Cd concentration, which was consistent with the results of previous research on *Oryza sativa* (Zhang et al. 2012) and *Glycine max* (Liu et al. 2010).

Chlorophyll content reduction is an early response of plants to Cd stress. According to Figure 2, after 10 days of Cd treatment, the chlorophyll content in the cotton seedling leaves showed a significant decrease, and also an extremely significant difference between the treatment groups and the control group ($P < 0.01$). Under the treatment of 100 μmol Cd/L, the chlorophyll contents of cvs. NDM9 and GXM3 decreased by 50.1% and 56.7%, respectively, when compared with the control group.

According to Figure 3, after 5 days of Cd treatment, the P_n in the cotton seedling leaves showed a significant decrease. Under the 100 μmol/L Cd treatment, the photosynthetic rate (P_n) values of cvs. NDM9 and GXM3 decreased by 22.2% and 34.3%, respectively, when compared with the control group. After the treatment for 10 days, the

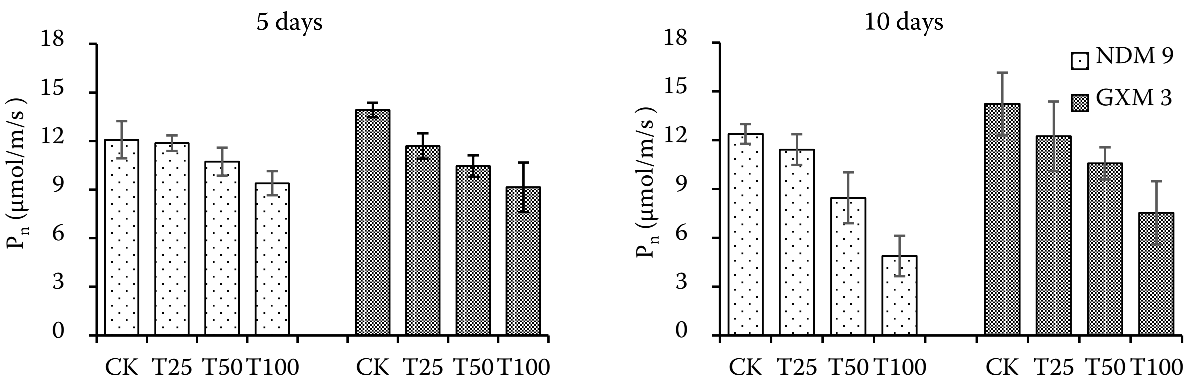

Figure 3. Effects of cadmium (Cd) treatment on photosynthetic rate (P_n) values of cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences ($P < 0.05$) among four treatments and within two cultivars. CK – 0, T25 – 25, T50 – 50, T100 – 100 μmol Cd/L

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P_n values reduced significantly under the middle and high concentration Cd treatments ($P < 0.05$). Under the 50 μmol Cd/L treatment, the P_n values of cvs. NDM9 and GXM3 decreased by 31.7% and 25.7%, respectively, when compared with the control group. Under the 100 μmol Cd/L treatment, the P_n values of cvs. NDM9 and GXM3 decreased by 60.6% and 47.0%, respectively, when compared with the control group ($P < 0.05$). It was also shown in this study that Cd presented an inhibitory effect on the plant leaf photosynthesis (Singh and Prasad 2015), and that the photosynthetic rate decreased with an increase in the treatment duration and Cd concentration. The decrease in the photosynthetic rate was caused by a disruption in the photosynthesis system in the leaf or an inhibition of the dark reaction by Cd, which further repressed the photosynthesis reaction (Aravind and Prasad 2003).

MDA, an important product of membrane lipid peroxidation, can crosslink with protein, nucleic acid, amino acid and other active materials to form insoluble chemical precipitates and interrupt the normal biological activities of cells (Wang et al. 2011). From Figure 4 it can be seen that the MDA content increased with an increase in the Cd concentration, and that, after the treatment for 5 days, the MDA content in cv. GXM3 under the 100 μmol Cd/L treatment reached its peak, i.e., 132.9% higher than the control group; while, the MDA content in cv. NDM9 was 33.2% higher than the control group. After treatment for 10 days, the MDA contents of the two cultivars showed slight differences, but presented similar overall tendencies. The middle and high level Cd treatments resulted in severe differences from the control group, which indicated that the middle and high Cd levels promoted the MDA accumulation and aggravated membrane lipid peroxidation. The results from this study indicated that the MDA content in the leaf increased as the Cd concentration and treatment duration increased, which suggests that the Cd induced the accumulation of reactive oxygen species (ROS) and the aggravation of the membrane lipid peroxidation in the plant, and this further impaired the integrity and coordination of the cells (He et al. 2011).

The activity of SOD, which can scavenge free radicals in plant tissues and protect the plants, is an indicator of the plants' antioxidant ability. From Figure 5 it can be seen that the consistent tendencies in the two measuring periods, both of which showed a single-peak curve tendency with an increase in Cd concentration, were that the low-concentration Cd stress could enhance the antioxidant ability, promote a protective effect on the plants and maintain the integrity and coordination of the plant cells, but only to a limited extent. When the Cd concentration exceeded 50 μmol/L, the self-protecting ability could no longer resist the adverse conditions. POD, an important protective enzyme in plants, can effectively scavenge the free radicals produced in cells under adverse circumstances, and it plays a critical role in responding to stresses. In Figure 6, the POD activity displays a single-peak curve as the Cd concentration increases. After Cd treatment for 5 days, the climax for the POD activity in both cultivars occurred at T25. Then after Cd treatment for 10 days, the climax for the POD ac-

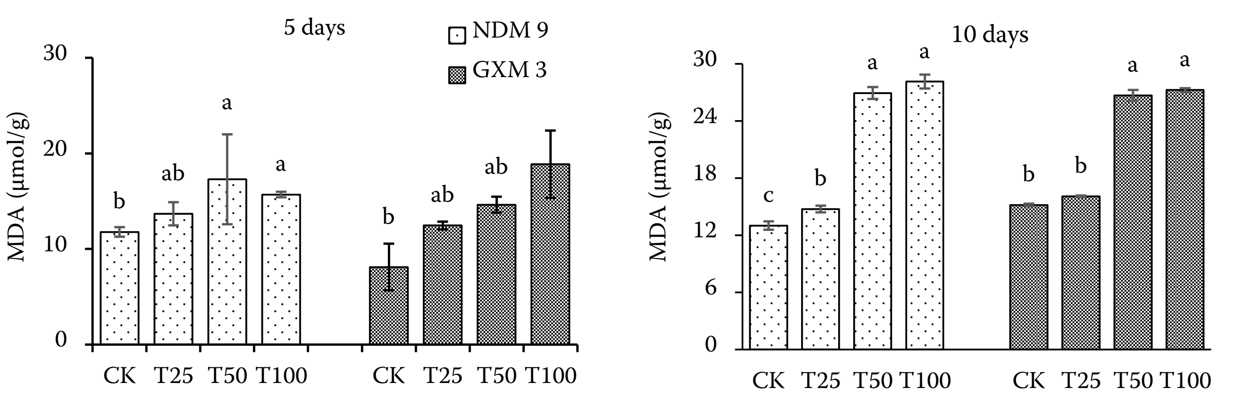

Figure 4. Effects of cadmium (Cd) treatment on malonaldehyde (MDA) of cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences ($P < 0.05$) among four treatments and within two cultivars. CK – 0, T25 – 25, T50 – 50, T100 – 100 μmol Cd/L

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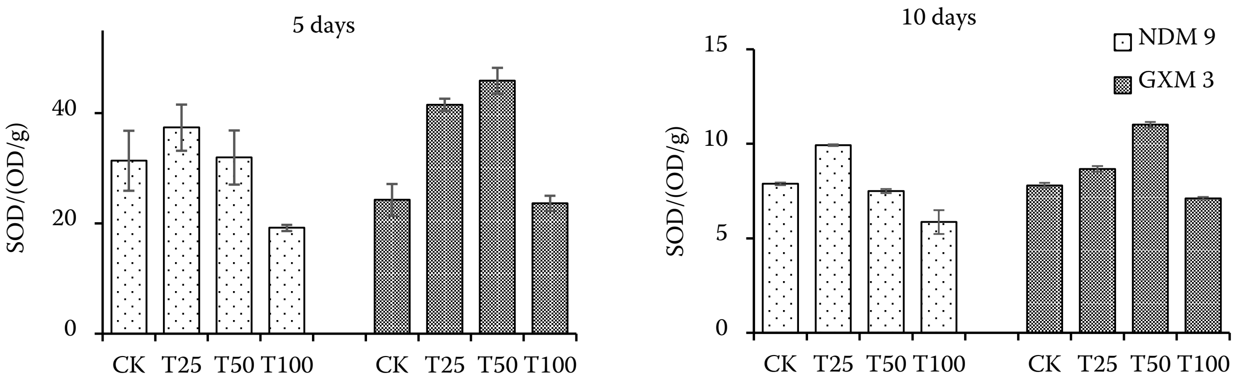

Figure 5. Effects of cadmium (Cd) treatment on superoxide dismutase (SOD) of cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences (P < 0.05) among four treatments and within two cultivars. CK – 0, T25 – 25, T50 – 50, T100 – 100 µmol Cd/L

tivity in both cultivars occurred at 50 µmol/L. This suggests that the toxicity of the Cd stress on the cotton increases as the stress duration increases.

The antioxidant enzymes involved in scavenging the ROS, such as SOD and POD, function synergistically under stress conditions to enhance the ROS-scavenging ability of plants. The strongly oxidative OH can be formed by H_2O_2 produced by SOD dismutation, so H_2O_2 must be scavenged immediately due to the lack of a specific OH-scavenging enzyme in plant cells (Jiang et al. 2007). In plants, the POD enzyme is responsible for the scavenging of H_2O_2, and it has dual functions (Tian et al. 2001): on the one hand, POD can catalyze the production of O_2 from NADH or NADPH, and engage in the formation of ROS; while, on the other hand, it can metabolize the accumulated H_2O_2 to exert its protective effects (Zhang et al. 2015). In this study, a tendency of first ascending and then descending SOD and POD activities was observed with increases in the Cd concentration, which was consistent with the results reported by other studies. The increase in the enzymatic activity under the low-concentration Cd treatment indicated the initiation and enhancement of the protective enzyme system, which was consistent with the research results of Chen et al. (2014). Under the high-concentration Cd (100 µmol/L) treatment, the decrease in the protective enzyme system might have been caused by the overproduction of ROS under Cd stress, which surpassed the scavenging ability of the protective enzyme system (Li et al. 2012).

In conclusion, cotton seedlings exhibit obvious stress reactions under Cd stress and show increased protective enzyme system activity and certain stress resistance mechanisms to relieve the stress. However, such physiological responses oc-

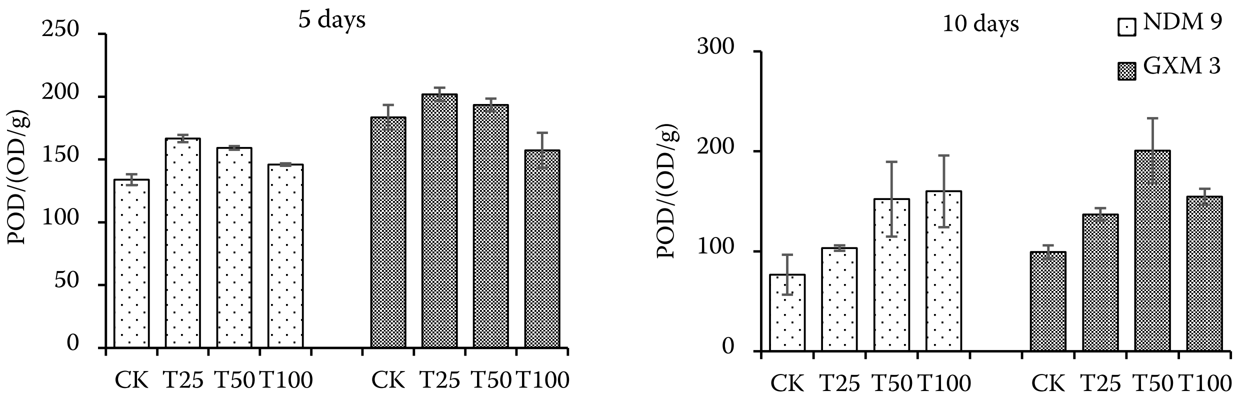

Figure 6. Effects of cadmium (Cd) treatment on peroxidase (POD) of cotton seedlings. The errors bars represent standard deviation values (n = 4), different letters indicate significant differences (P < 0.05) among four treatments and within two cultivars. CK – 0, T25 – 25, T50 – 50, T100 – 100 µmol Cd/L

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cur only when the Cd stress is at a low level. When the Cd concentration exceeded 50 μmol/L under this experimental setting, the Cd stress exerted a significant inhibitory effect on the photosynthetic properties of the cotton seedling leaves and their protective enzyme system, which could further induce damage to the plant cells and inhibit the growth of the plants.

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