Australian Journal of Crop Science AJCS
AJCS 6(11):1527-1533 (2012) ISSN:1835-2707

Characterization of physiological traits, yield and fiber quality in three upland cotton cultivars grown under cadmium stress

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Abstract

Cadmium (Cd) by plants from contaminated soils induces various physiological and biochemical changes resulting reduction in the yield and the quality of the plant products. A pot experiment was performed in order to study Cd induced alterations in physiological characters, yield components and fiber quality of two transgenic cotton cultivars (ZD-90 and SGK3) and an upland cotton standard genotype (TM-1). ZD-90, derived from our laboratory, is a glyphosate-resistant cultivar with the *EPSPS-G6* gene, and SGK3 is an insect-resistant cultivar with the *Bt* gene. Cd stress significantly reduced the seed cotton yield, lint yield, boll numbers per plant, boll weight, net photosynthetic rate, and water use efficiency etc., while increased the activity of several antioxidant enzymes such as peroxidase (POD) and superoxide dismutase (SOD) in the three cotton cultivars. The Cd accumulation in different parts of cotton plant increased in the following order: fiber < seed < seed shell < root < leaf < shoot < boll shell < petiole. The least affected cultivar by Cd treatments in seed cotton yield and bolls per plant among the three cotton cultivars was SGK3, suggesting that it was less sensitive to Cd stress than the other two cultivars. From the present experiment, it can be well confirmed that the non-harvestable parts of cotton plant accumulated more Cd (BCF=1.854~2.449) than the fiber and seed (BCF=0.089~0.242), indicating that cotton is suitable for cultivation in industrially polluted regions and could be used as a potential crop for cleaning up contaminated soils by phytoremediation technology.

Keywords: Antioxidants; Cadmium stress; Cotton; Fiber quality; Photosynthesis; Yield.
Abbreviations: APX-ascorbate peroxidase; BCF-bioconcentration factors; Cd-cadmium; E*PESP*-5-enolpyruvylshikimate-3-phosphate synthase; Gs-stomatal conductance; MDA-malondialdehyde; Pn-net photosynthetic rate; POD-peroxidase; ROS-reactive oxygen species; SOD-superoxide dismutase; TBA-2-thiobarbituric acid; Tr-transpiration rate; WUE-water use efficiency.

Introduction

Cadmium (Cd) is one of the most toxic environmental pollutants, which has threatened our ecosystems and thus has been hazardous to men and animals (Wagner, 1993). It can be readily taken up by plant roots, translocated to their aerial parts and resultantly can easily enter the food chain (Satarug et al., 2003). Cd intake by human beings through plant foods is almost 70% (Wagner, 1993). In China, more than 1.3×10^5 km^2 of agricultural soils are contaminated by Cd, and 1.46×10^8 kg of agricultural products are polluted by Cd every year (Gu and Zhou, 2002). Cadmium is highly phytotoxic, resulting in growth retardation, leaf chlorosis or necrosis, damage to cell structures as well as disturbance in water balance, mineral nutrition, photosynthesis, respiration and plant development (Prasad, 1995). And thus it has significantly influenced the qualitative and quantitative traits of the crops and plant by-products (Myśliwa-Kurdziel and Strzałka, 2005). Due to its toxic nature, Cd can stimulate the formation of reactive oxygen species (ROS) either by direct electron transfer involving metal cations, or as a consequence of metal mediated inhibition of metabolic reactions (Halliwell and Gutteridge, 1984). To combat against these SOS, various antioxidants and antioxidative enzymes become active both at cellular and sub-cellular levels (Foyer et al., 1997). Regarding Cd tolerance, uptake and accumulation, wide differences among plant species and cultivars have been found in crops such as lettuce (*Lactuca sativa* L.; Crews and Davies, 1985) and wheat (*Triticum aestivum* L.; Zhang et al., 2002). Due to the advancements in the phytoremediation technology in recent years, more attention has been paid to look for hyperaccumulators being having the capability to reduce heavy metal content of the contaminated soils (Krämer, 2005). However, the application of phytoremediation technology is still very limited due to low biomass of hyperaccumulators. Thus, selective breeding for these hyperaccumulators is the need of the hour in order to select Cd-tolerant cultivars. Cotton (*Gossypium hirsutum* L.) is an important cash crop in the world, being used for natural fiber and edible oil. However, its productivity is adversely affected by various forms of biotic and abiotic stresses. How these stresses, particularly Cd directly and indirectly influence the biometric traits of the cotton plant need research. However, studies regarding Cd accumulation as well as its effects on fiber quality are very rare. In earlier research by Sengalevitch (1999) considered cotton as a suitable crop for industrially polluted regions. Keeping in view of the near unavailability of the related information about physiological and biochemical responses of the cotton crop under Cd stress, we designed the present research studies. The objectives of the study were: (1) to find out the possible toxic effects of Cd on cotton; (2) to determine the quantity and plant parts of Cd accumulation; (3) to study the mechanism of phytoremediation of Cd

contaminated soils using cotton cultivars.

Results and discussion

Photosynthetic characteristics

Cadmium toxicity induces various physiological changes by inhibiting a number of photosynthetic parameters such as net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) in the three growth periods of cotton cultivars, were shown in Figure 1(A-L). Higher Pn was observed at the flowering and boll-forming stage than at the budding and boll-opening stages. In contrast with their controls, Pn was higher at 200 μM Cd, significantly for TM-1 and insignificantly for ZD-90, then declined to the lowest at 600 μM Cd level at the budding stage, although a significant difference compared with their check was found only in SGK3. There was a linear decrease in Gs as the growth progressed, but no significant differences were found in Gs values at all levels of Cd treatment in the three cultivars. Higher Tr was observed at the budding stage than at the other two stages. Compared to their controls, Tr increased gradually at 200 and 400 μM Cd for the three cultivars, and then decreased at 600 μM Cd at the flowering and boll-forming stage. A linear increase in Tr was observed with the increase of Cd in the three cultivars at the flowering and boll-opening stage. Water use efficiency (WUE) in Figure 1 showed changes similar to those of Pn but an inverse trend was observed with regards to Tr. The results from this study have clearly shown that Cd inhibited Pn and Gs at the three growth stages of the cotton cultivars, confirming the findings by other researchers during their studies on pea (*Pisum astivum* L.; Hattab et al., 2009) and mustard (*Brassica juncea* L.; Mobin and Khan, 2007). The reduction of Pn under Cd stress maybe due to arresting of the photosynthetic electron flow, inhibiting the water-splitting complex of the oxidizing site of PSII or competitively binding to the essential Ca^{2+} site in PSII during photo activation (Faller et al., 2005). There were no statistical differences for Tr and Gs among the Cd treatment in the three cultivars, although some variations on Tr and Gs were found among the different Cd treatments and different genotypes at the different plant development stages, shown in Figure 1(D-I).

Enzyme assay and estimation of lipid peroxides

Cadmium can increase the production of reactive oxygen species (ROS) in cells, which can be extremely harmful to plants at high concentrations (Sandalio et al., 2001). To protect themselves against ROS, plant cells have developed antioxidative mechanisms. In this experiment, the activities of antioxidant enzymes were assayed in order to evaluate the defensive capabilities against Cd stress, shown in Figure 2(A-L). Compared with their respective controls, the activity of peroxidase (POD) gradually increased with an increase in Cd levels in SGK3. Higher POD activity was observed in ZD-90 than other two cultivars, especially at the boll-opening stage. The activity of superoxide dismutase (SOD) increased at 200 and 400 μM Cd for TM-1 and SGK3, and then decreased at 600 μM Cd at all growth stages, while ZD-90 showed an inverse trend at the budding stage compared with its control. Superoxide dismutase is the first major antioxidant enzyme in the detoxifying process as it rapidly converts superoxide radicals into H_2O_2 (Tanyolaç et al., 2007). The results from this study were in agreement with the findings of Ahmad et al. (2011), who reported an increase in SOD activity when mustard plants were exposed to toxic Cd concentrations. Compared with the controls, the activity of ascorbate peroxidase (APX) increased at lower Cd levels, and then decreased at 600 μM Cd for ZD-90 at three growth stages. A decrease in APX activity was found for TM-1 at the flowering and boll-forming stage and SGK3 at the budding stage at all Cd levels. Higher APX activity was observed at the budding stage than at the other two stages. A significant increase in lipid peroxidation was observed at the boll-opening stage as compared to other two stages at all Cd levels, shown in Figure 2(A-C). The malondialdehyde (MDA) content increased up to the highest at 400 μM Cd compared with their controls, following a minor decrease at 600 μM Cd in both ZD-90 and SGK3, while there was a linear increase with elevated Cd levels in TM-1 at boll-forming stage. The content of MDA was higher at 600 μM Cd than the control at all growth stages in the three cultivars. A linear increase in MDA concentration was found in the three cotton cultivars with the increase in Cd up to moderate level as well as with the extension of the growth stage in this experiment. Similar results have been found in rice (*Oryza sativa* L.; Shah et al., 2001) and mung bean (*Phaseolus vulgaris* L.; Somashekaraiah et al., 1992) under Cd stress.

Fiber quality

The fiber quality in the three cotton cultivars with different Cd treatments is shown in Figure 3(A-E). In comparison to their controls, SGK3 possessed better fiber at lower Cd level and more elongation under various Cd levels than TM-1. ZD-90 showed lesser changes than other cultivars based on fiber length and strength exposed to Cd. The rise in Ca^{2+} concentration in plant cells, which may have been caused by environmental stress. Qin and Zhu (2011) reported that Ca^{2+} influx to the fiber tips was required for sustaining fast cell elongation. The promotion of fiber elongation exposed to lower amounts of Cd in this study might be due to an elevation in Ca^{2+} level, which is required for fiber tips growth. Calcium-mediated signal transduction plays a crucial role in the growth of fiber tips (Qin and Zhu, 2011). Therefore, it can be deduced that Cd^{2+} might have been entered into the cells through calcium channels competitively. Compared to their controls, fiber uniformity was found to be the highest at 200 μM Cd and then decreased at higher Cd level in the three cultivars. There were no significant differences in fiber quality in the three cultivars exposed to Cd.

Yield and its components

Reduction in yield and yield-related traits is a typical index of plant sensitivity to various stresses. In various crop species (e.g. pea, wheat, fodder vetch, rapeseed and maize), yield's reduction under multiple heavy metal stresses has been observed (Sharma and Agrawal, 2005). The effect of Cd on yield and its components is presented in Figure 4 (A-F). The deleterious effect of Cd on cotton yield was doze-dependent. With the increase in Cd levels, changes in yield and its components also became evident. As compared to control plants, seed cotton yield and boll weight first increased up to the highest at 200 μM Cd, and then significantly declined at 600 μM Cd for SGK3, while a continuously decreasing trend was observed for ZD-90 and TM-1. Mean values of lint yield and the number of bolls significantly declined in the three cultivars. Compared to their controls, the mean values of fuzz percent progressively declined with the increase in Cd levels only for SGK3. There were no significant differences in lint index at all Cd treatment levels in the three cultivars. Moreover, the deleterious effect of Cd on cotton yield was also

Table 1. Cd bio-concentration factors from soil to different plant parts or organs in the three cotton cultivars (TM-1, ZD-90, SGK3).

Plant parts	TM-1			ZD-90			SGK3		
	200μM	400μM	600μM	200μM	400μM	600μM	200μM	400μM	600μM
Seed kernel	0.099	0.081	0.072	0.117	0.123	0.109	0.209	0.159	0.176
Fiber	0.021	0.022	0.016	0.021	0.022	0.024	0.033	0.021	0.020
Leaf	0.331	0.334	0.256	0.437	0.311	0.224	0.428	0.310	0.268
Petiole	0.729	0.491	0.424	0.677	0.464	0.473	0.490	0.601	0.529
Boll shell	0.309	0.382	0.394	0.343	0.477	0.453	0.416	0.364	0.398
Seed shell	0.244	0.296	0.215	0.219	0.183	0.134	0.228	0.194	0.163
Shoot	0.216	0.348	0.326	0.521	0.355	0.419	0.410	0.412	0.385
Root	0.143	0.254	0.239	0.253	0.135	0.306	0.190	0.298	0.251
Reproductive organs	0.120	0.103	0.089	0.138	0.145	0.133	0.242	0.180	0.196
Vegetable organs	1.972	2.105	1.854	2.449	1.925	2.009	2.162	2.179	1.994
Aboveground parts	1.949	1.955	1.704	2.334	1.935	1.836	2.213	2.061	1.939
The whole plants	2.092	2.208	1.943	2.587	2.070	2.142	2.404	2.359	2.191

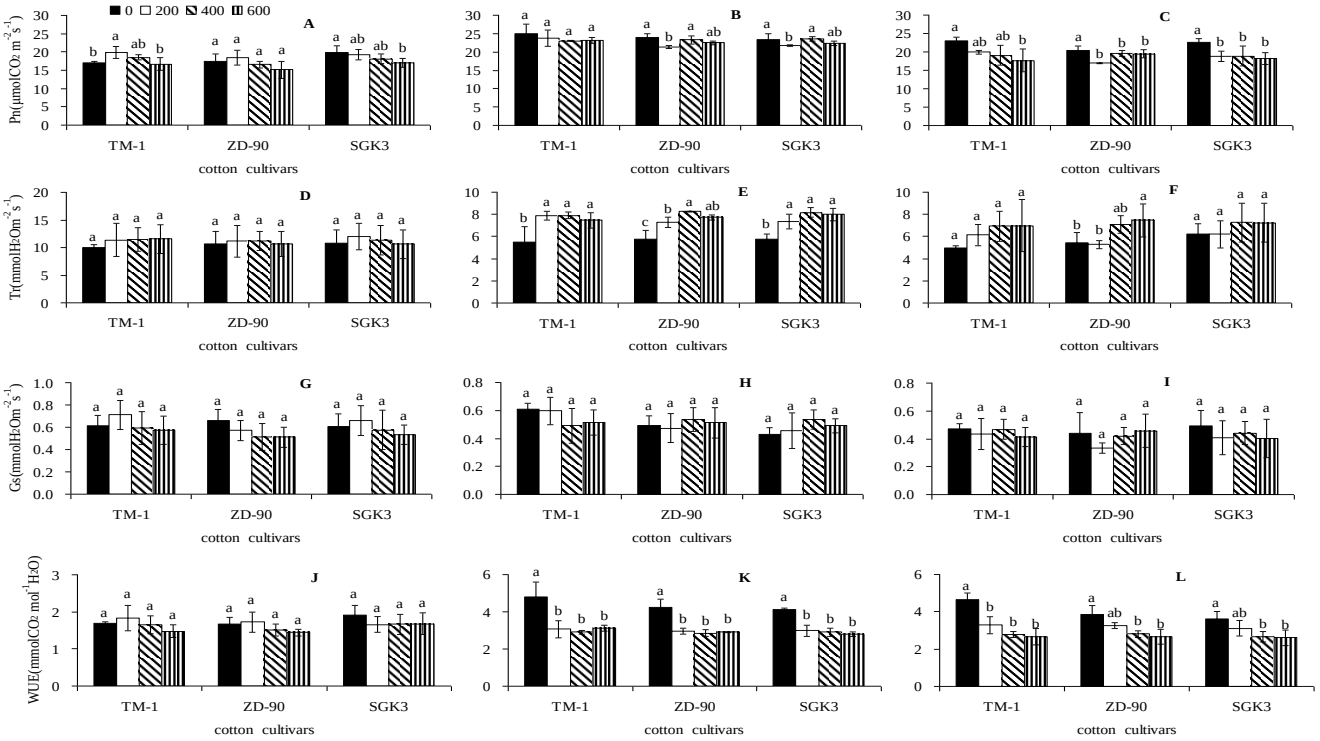

Fig 1. Effects of Cd stress on the physiological parameters during different stages in the three cotton cultivars (TM-1, ZD-90, SGK3). A: net photosynthesis (Pn) at the budding stage; B: Pn at the flowering and boll-forming stage; C: Pn at the boll-opening stage; D: transpiration rate (Tr) at the budding stage; E: Tr at the flowering and boll-forming stage; F: Tr at the boll-opening stage; G: stomatal conductance (Gs) at the budding stage; H: Gs at the flowering and boll-forming stage; I: Gs at the boll-opening stage; J: water use efficiency (WUE) at the budding stage; K: WUE at the flowering and boll-forming stage; L: WUE at the boll-opening stage. Each value is the mean of three replications and the vertical bars give the standard error (SE) of the mean. In all figures, the different letters of the same cultivar indicate significant difference among the treatments and control at the 5% level.

significantly different among genotypes. It was found that SGK3 was the least, while TM-1 was the most affected among the three cultivars. Compared to the controls and other Cd treatments, yield decrease was more prominent at 600 μM Cd in all the three cultivars. These results were in agreement with those of Zhang et al. (2002), who found a significant reduction of wheat grain weight per plant under Cd treatment.

Cd accumulation

Cadmium accumulation in different parts of the experimental cultivars closely correlated with Cd concentration in the soils shown in Figure 5 (A-H). The results clearly demonstrated that the Cd accumulation enhanced with the increase in Cd concentration. There was a linear increase in Cd uptake in different plant parts. Its accumulation was in order of fiber < seed < seed shell < root < leaf < shoot < boll shell < petiole. In general, roots are the primary sites through which cadmium gains access into the plant. Cadmium is absorbed by the plant root and transported to the other parts via xylem. In most cases, a large fraction of Cd is retained in the roots, and only comparatively small amounts are transported to the shoots and the seedlings. The results obtained in this experiment were not in agreement with Lozano-Rodríguez et al. (1997) who indicated that Cd accumulated more in roots than in shoots of maize and pea. These diverged differences might be due to

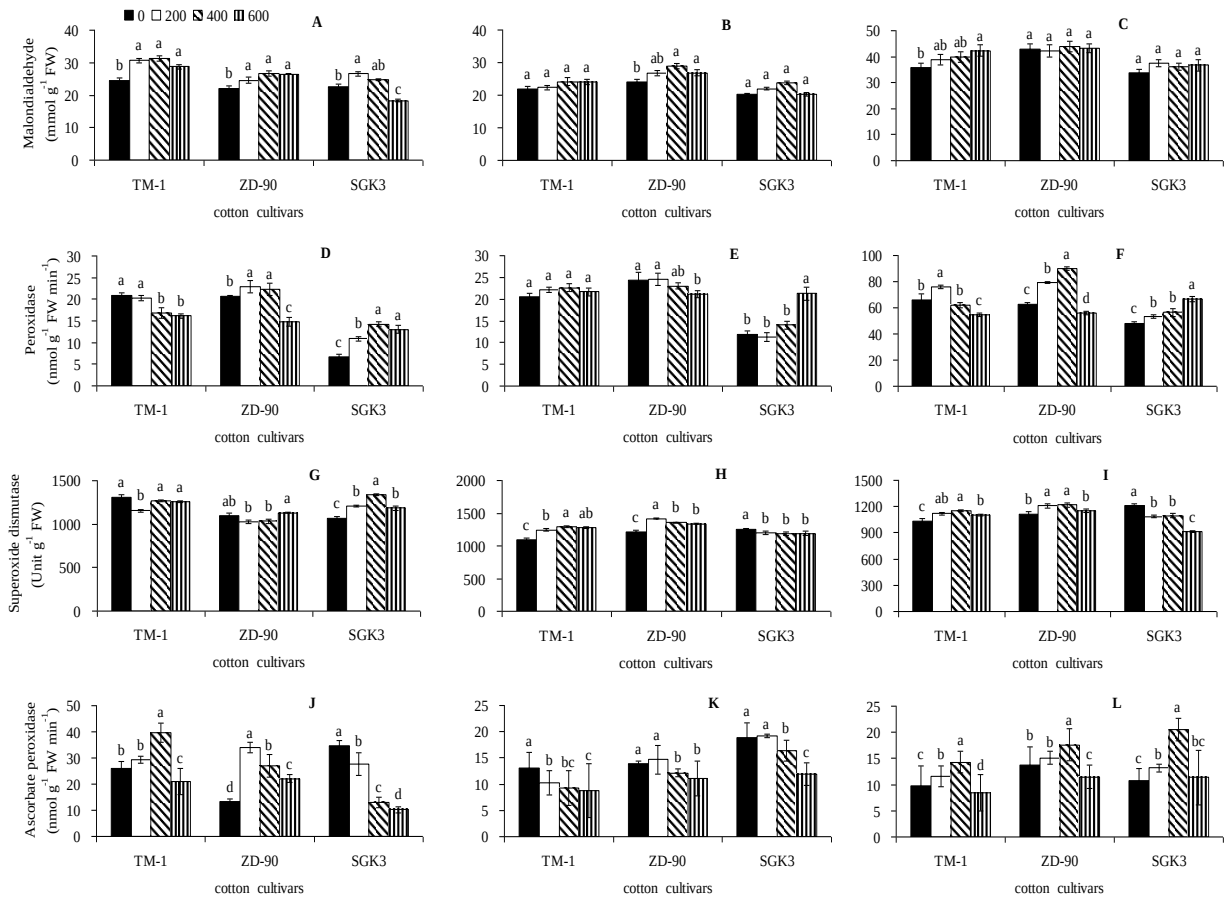

Fig 2. Effect of Cd stress on the activity of antioxidant systems (SOD, POD, APX) and malondialdehyde content during different stages in the three cotton cultivars (TM-1, ZD-90, SGK3). A: malondialdehyde (MDA) at the budding stage; B: MDA at the flowering and boll-forming stage; C: MDA at the boll-opening stage; D: peroxidase (POD) at the budding stage; E: POD at the flowering and boll-forming stage; F: POD at the boll-opening stage; G: superoxide dismutase (SOD) at the budding stage; H: SOD at the flowering and boll-forming stage; I: SOD at the boll-opening stage; J: ascorbate peroxidase (APX) at the budding stage; K: APX at the flowering and boll-forming stage; L: APX at the boll-opening stage. Each value is the means of three replications and the vertical bars give the standard error (SE) of the mean. In all figures, the different letters of the same cultivar indicate significant difference among the treatments and control at the 5% level.

different materials used in our experiment, which indicated that the Cd accumulation in cotton is different to other plant greatly. For the plant parts investigated, petiole had the highest Cd accumulation in all the three cultivars. The Cd content in petiole was almost 20 times more than that in fiber. This might be due to the presence of carrier proteins in vascular tissues (Prasad, 1995), and the formation of chelation and metallothioneins in these vascular tissues. Moreover, there was low Cd content in cotton fiber, suggesting that these cotton cultivars could be grown in Cd polluted areas. Like other fiber crops, cotton plants extract heavy metals from the soil through their root system and accumulate them in the above ground parts. These findings were in line with those of Angelova et al. (2004) who stated that Pb was comparatively evenly distributed in cotton roots and stems, and offered a high resistance to pollution with the presence of protective mechanisms in cotton. In this study, Cd absorption was retained in aboveground plant parts and the accumulation was higher than 100 μg g^{-1} at 600 μM Cd, and the bioconcentration factors (BCF) of Cd was more than 1.0 (Table 1) in the three cultivars. It was suggested that high concentrations of Cd could be translocated by cotton from roots to aboveground parts. The greater Cd uptake in different plant parts was observed in SGK3 and ZD-90. It indicated that SGK3 had lower damage in yield and fiber quality than the other cotton cultivars, and had higher potential for absorbing and removing Cd from moderately contaminated soil. It could thus be used for the phytoremediation of Cd polluted soils.

Materials and methods

Plant materials, growth conditions and Cd-treatment levels

Three cotton cultivars (TM-1, ZD-90 and SGK3) were used in the present experiment. ZD-90 is a transgenic glyphosate tolerant cotton cultivar derived from our laboratory with the *EPESP-G6* gene cloned by Zhejiang University. SGK3 is an insect-resistant cultivar with *Bt* obtained from the Biotechnology Center of the Chinese Academy of Agricultural Sciences. TM-1 is the upland cotton genetic standard line obtained from USDA, ARS, College Station, Texas, USA.

The experiment was carried out at the experimental station of Zhejiang University. The seeds of each cotton genotype were sown in pots (35 cm diameter × 40 cm depth) on 5 May 2009 and 2010. One plant in each pot was grown outdoors in a large and rainproof net house till maturity. Each pot was filled with 15 kg uncontaminated soil with pH=6.2, 2.99% organic matter, 1.35% total N (162.58 mg/kg available N), 0.13% total P (23.82 mg/kg available P), and 0.83% total K (112.79 mg/kg available K). Cadmium in form of $CdCl_2 \cdot 2.5H_2O$ was added,

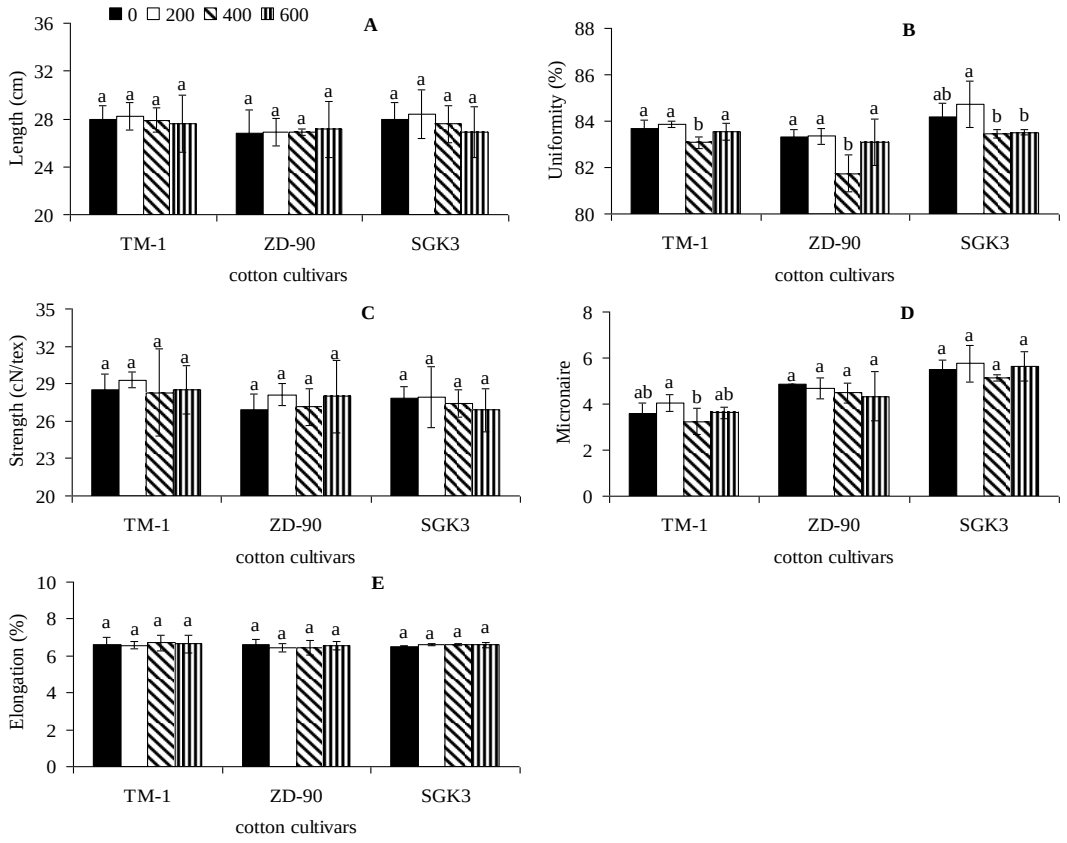

Fig 3. Effects of Cd stress on fiber length (A), uniformity (B), strength (C), micronaire (D) and elongation (E) in the three cotton cultivars (TM-1, ZD-90, SGK3). Each value is the mean of three replicates and the vertical bars give the standard error (SE) of the mean. In all figures, the different letters of the same cultivar indicate significant difference among the treatments and control at the 5% level.

keeping a relatively stable concentration at 0 (control), 200, 400 and 600 μM for the whole growing period. There were three replications per treatment with 10 pots for each treatment, which were arranged in completely randomized experimental design.

Determination of physiological parameters

Physiological parameters such as net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs) were measured on the fourth leaf three times from 9:30 to 11:00 at each growing stage by a portable photosynthesis system Li-6400 (Li-Cor, Lincoln, USA). These parameters were determined at a CO_2 flow of 500 L min^{-1} and a photon flux density of 1000 $\mu mols^{-1}m^{-2}$. The water use efficiency (WUE) was calculated as the ratio of net photosynthetic rate and transpiration rate.

Antioxidative enzyme assay and estimation of lipid peroxides

Total superoxide dismutase (SOD) activity was measured spectrophotometrically at 560 nm (Beyer and Fridovich, 1987). Peroxidase (POD) activity was based on the determination of guaiacol oxidation at 470 nm by H_2O_2 (Pütter, 1974). Ascorbate peroxidase (APX) activity was measured according to the procedure of Chen and Asada (1989). The level of lipid peroxidation products in leaf samples was expressed as malondialdehyde (MDA) content and was determined as 2-thiobarbituric acid (TBA) reactive metabolites (Heath and Packer, 1968).

Measurements of yield parameters, yield and fiber quality

For each treatment, yield and its components were calculated after harvesting. Fiber properties (staple length, uniformity, strength, elongation, and micronaire) were determined by the Supervision, Inspection and Testing Center for Cotton Quality in China.

Determination of Cd content

For the quantification of Cd in cotton plant parts, each sample was dried to constant weight at 80 ℃ and ground into the fine powders. They were then wet digested for 4-5 hours after adding a mixture of 4-5 ml HNO_3 and 0.5 ml H_2O_2. Each digested sample was transferred at constant volume to a 50 ml in flask for Cd determination. Cadmium was quantified using an ICP-Mass Spectrograph (7500a, Agilent).
Moreover, the bioconcentration factors (BCF) in different parts at various Cd levels were calculated according to Ait et al. (2002) using the following formula;

$$BCF = [Cd]_{shootor\,root} / [Cd]_{soil}$$

Statistical analysis

One-way ANOVA was performed using SAS v.9 software and Microsoft Office Excel 2003 for statistical significance at

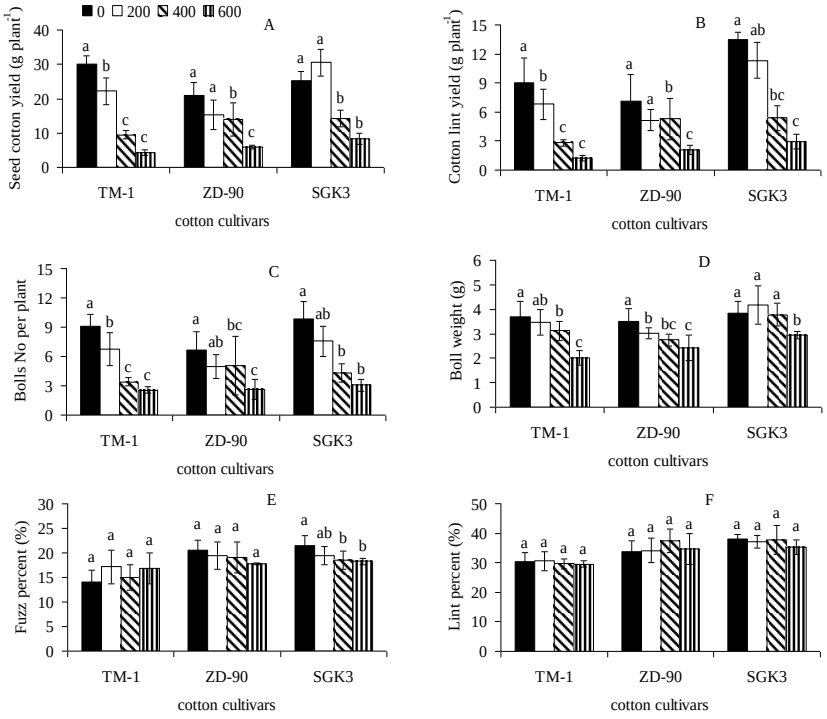

Fig 4. Yield characters of the three cotton cultivars (TM-1, ZD-90, SGK3) at harvest subjected to increasing Cd concentrations. A: seed cotton yield; B: cotton lint yield; C: bolls number per plant; D: boll weight; E: fuzz percent; F: lint percent. Each value is the mean of three replicates and the vertical bars give the standard error (SE) of the mean. In all figures, the different letters of the same cultivar indicate significant difference among the treatments and control at the 5% level.

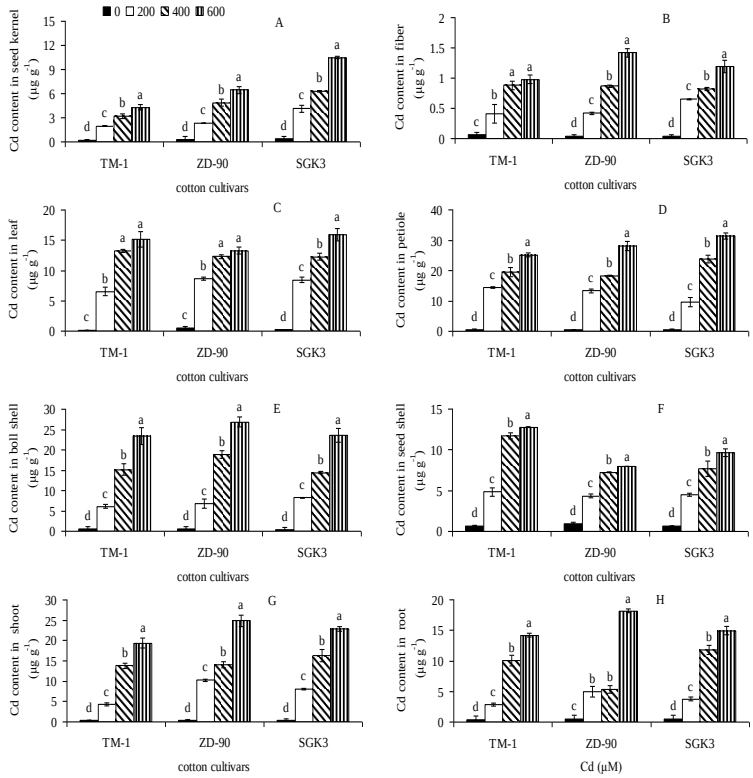

Fig 5. Cadmium content in different plant parts of the three cotton cultivars (TM-1, ZD-90, SGK3) at harvest under various Cd treatments. A: Cd content in cottonseed kernel; B: Cd content in fiber; C: Cd content in leaf; D: Cd content in petiole; E: Cd content in boll shell; F: Cd content in seed shell; G: Cd content in shoot; H: Cd content in root. Each value is the mean of three replicates and the vertical bars give the standard error (SE) of the mean. In all figures, the different letters of the same cultivar indicate significant difference among the treatments and control at the 5% level.

P=0.05. All the results were expressed as mean ± SE for three replications. The mean values were separated by Least Significant Difference (LSD) test at 5% level of significance.

Conclusion

The phytotoxic effect of Cd on cotton yield was genotype as well as concentration-dependent. The effect became more notable at the highest Cd concentration (600μM) than at other Cd levels. SGK3 was the least affected among the three cultivars, while TM-1 was the most sensitive one. For fiber quality, ZD-90 showed greater tolerance to Cd in fiber length and strength, and SGK3 was more tolerant to Cd in fiber elongation. Less Cd accumulation occurred in fiber. Therefore, it is suitable for growing in industrially polluted regions, since it can remove considerable quantities of Cd from the soil with its non-harvested parts.

Acknowledgements

The project was financially supported by 973 Project of National Natural Science Foundation of China (2010CB126006) and the Important Program for Transgenic Biological New Cultivar Breeding (2008ZX08005-005).

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