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# Cadmium-induced ultramorphological and physiological changes in leaves of two transgenic cotton cultivars and their wild relative

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ABSTRACT

The present study describes cadmium-induced alterations in the leaves as well as at the whole plant level in two transgenic cotton cultivars (BR001 and GK30) and their wild relative (Coker 312) using both ultramorphological and physiological indices. With elevated levels of Cd (i.e. 10, 100, 1000 μM), the mean lengths of root, stem and leaf and leaf width as well as their fresh and dry biomasses linearly decreased over their respective controls. Moreover, root, stem and leaf water absorption capacities progressively stimulated, which were high in leaves followed by roots and stems. BR001 accumulated more cadmium followed by GK30 and Coker 312. Root and shoot cadmium uptakes were significantly and directly correlated with each other as well as with leaf, stem and root water absorption capacities. The ultrastructural modifications in leaf mesophyll cells were triggered with increase in Cd stress regime. They were more obvious in BR001 followed by GK30 and Coker 312. Changes in morphology of chloroplast, increase in number and size of starch grains as well as increase in number of plastoglobuli were the noticed qualitative effects of Cd on photosynthetic organ. Cd in the form of electron dense granules could be seen inside the vacuoles and attached to the cell walls in all these cultivars. From the present experiment, it can be well established that both apoplastic and symplastic bindings are involved in Cd detoxification in these cultivars. Absence of tonoplast invagination reveals that Cd toxic levels did not cause water stress in any cultivars. Additionally, these cultivars possess differential capabilities towards Cd accumulation and its sequestration.



## 1. Introduction

Cadmium (Cd) is probably the most significant pollutant due to its high toxicity and large solubility in water [1]. It is the outcome of various anthropogenic activities like mining, fertilization, industrialization, etc. [2,3]. Both animals and plants are directly and indirectly being affected by Cd, howsoever, plants are more prone to the attack of Cd because it has been accumulated in different soils for many decades. Resultantly, it can enter into a plant very rapidly accumulating in roots [4,5] with a variable amount being translocated to the upper parts of the plant [6,7].

The phytotoxic effects of Cd could probably be a consequence of its interference with a number of metabolic processes associated with normal development of plant [8]. However, plants can develop tolerance mechanisms to reduce the concentration of free $Cd^{2+}$ in the cytosol. These worth mentioning tolerance strategies are detoxification, accumulation due to plant chelators and compartmentalization of $Cd^{2+}$ ions in the vacuoles [9]. Literature reports are available about the interaction of Cd with diverse biochemical processes in plants; however, very few studies describe structural and ultrastructural modifications induced by Cd, which may be either consequences or causes of physiological disfunction [10]. An increase in number of nucleoli and vacuoles, condensation of cytoplasm, reduction of mitochondrial cristae, severe plasmolysis, highly condensed chromatin materials, enlargement of vacuoles, disorganization of chloroplast structure, and disruption of nuclear envelope, plasmalemma and mitochondrial membranes are some of the most concernable effects of Cd at ultrastructural levels [11–14].

Among its cytotoxic activities, Cd causes oxidative stress [15–17], which can contribute to aging of chloroplasts and cells [18]. Moreover, it stimulates the synthesis of ethylene, whose involvement in

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events of plant cell senescence is well known [19]. Baszyński et al. [20] in tomato, Reese et al. [21] in tobacco, Barceló et al. [10] in bush bean and Rascio et al. [22] in maize have documented that disorganization of grana and an increase in the number and size of plastoglobuli in chloroplasts as well as increased cell and vacuole size and induced vesiculation was the main consequence of Cd stress. Ouzounidou et al. [23] found wavy appearance of grana and stroma thylakoids and dilation of thylakoid membranes in chloroplast of wheat under Cd stress.

Transgenic crops have almost occupied a significant portion of agricultural land in most parts of the world. Cotton, globally an important economic crop mainly utilized for fiber and oil production, has been exploited in a number of ways by using new plant biotechnology techniques. Now we have a number of transgenic cotton cultivars possessing foreign genes, which are being grown on a wide acreage throughout the world. In view of the growing importance of transgenic cotton in both developed and developing world, it is utmost important to exploit them against various environmental stresses. For the above-stated reasons and the near unavailability of studies regarding their possible roles against cadmium stress, we considered two transgenic cotton cultivars namely BR001 (herbicide resistant), GK30 (insect resistant) as well as their wild relative cotton genotype (Coker 312).

The main objectives of our present study were to explore the effect of Cd at the cellular level of leaf mesophyll cells and the possible cellular mechanism being involved in the Cd sequestration in these cells. Moreover, to find out the possible toxic effects of Cd on morphology and physiology at the whole plant level as well as on leaf. In addition, the potential of two diverse nature transgenic cotton cultivars for Cd detoxification in comparison to their wild relative cotton genotype has been investigated.

## 2. Materials and methods

### 2.1. Seed surface sterilization and treatment process

Mature seeds of two transgenic cotton cultivars (i.e. BR001 and GK30) and their wild relative cotton genotype (i.e. Coker 312), having uniform grade were first immersed in 70% ethanol for 3 min and then in 0.1% $HgCl_2$ for 8–10 min. They were washed first with $ddH_2O$ for three times and finally with distilled water. Subsequently, they were directly treated with tested solution for approximately 2 h. The treated seeds were spread over sterilized petri dishes (90 mm) lined with double-layered filter papers. In each petri plates, ten seeds were placed. The tested solution was comprised of four treatments of Cd including 0, 10, 100 and 1000 μM. There were three replications per treatment, which were arranged in a completely random manner. To each petri plates, a 2–3 ml of the tested solution was applied for first 3 days. Then on day 4, the germinated seedlings were transferred to another set of sterilized petri dishes with double-layered filter papers and 7–9 ml tested solution was applied to each petri dish.

Independent experiments were run for measurements of qualitative and quantitative traits of whole plant and leaves as well as for microscopic studies. The petri plates were sealed with parafilm and placed in dark for 48 h followed by 4-day exposure to a 16 h photoperiod of 50 μmol $m^{-2}$ $s^{-1}$ under white fluorescent light with 28 ± 2 °C culture temperature. Cadmium as $CdCl_2{\cdot}2.5H_2O$ of analytical grade was used. Control was provided with distilled water without Cd.

### 2.2. Plant growth parameters and Water Absorption Capacity (WAC)

A number of qualitative and quantitative plant growth parameters, namely, root–shoot lengths, leaf length and diameter, fresh and dry biomasses were determined in two independent experiments.

The Water Absorption Capacity (WAC) of roots, stems and leaves was calculated in another of set of experiment according to Kim et al. [24] using the following formula;

$$\text{Water Absorption Capacity}\,(\%) = \left[\frac{(\text{FB} - \text{DB})}{\text{FB}}\right] \times 100$$

where FB and DB are fresh biomass and dry biomass of the plant materials, respectively.

Moreover, the relative increase or decrease (%) in different qualitative and quantitative traits at various Cd levels over their respective controls was calculated as follows;

$$\frac{\text{Relative Increase}}{\text{Decrease}}(\%) = \left[\frac{(\text{Mean value in treatment} - \text{Mean value in control})}{\text{Mean value in control}}\right] \times 100$$

### 2.3. Determination of Cd content

To determine the bioavailability of Cd, the seedlings were thoroughly washed first with distilled water and then with 20 mM $Na_2$-EDTA for about 15 min in order to remove excess Cd adhering to the surfaces. After three times washing with distilled water, the plants were finally washed with $ddH_2O$. For quantification of Cd, the seedlings were separated into roots and shoots, and dried at 70 °C for 48 h. The samples were ground to fine powder and wet digested

**Table 1**
Mean length of root, stem and leaf of two transgenic cotton cultivars (BR001, GK30) and their wild relative (Coker 312) grown for 6 days under various Cd treatments.

| Cultivars | Cd Treatments (μM) | Mean values and their relative increase/decrease over the controls | | | |
|---|---|---|---|---|---|
| | | Root Length (cm plant$^{-1}$) | Stem Length (cm plant$^{-1}$) | Leaf Length (cm plant$^{-1}$) | Leaf Width (cm plant$^{-1}$) |
| BR001 | 0 | 2.42 ± 0.03a (100) | 3.47 ± 0.12a (100) | 1.47 ± 0.01a (100.00) | 2.52 ± 0.01a (100.00) |
| | 10 | 1.99 ± 0.07b (−17.49) | 2.97 ± 0.12b (−14.42) | 1.43 ± 0.02ab (−2.72) | 2.42 ± 0.01b (−3.97) |
| | 100 | 1.20 ± 0.15c (−50.55) | 2.52 ± 0.02c (−27.40) | 1.33 ± 0.01c (−9.52) | 2.11 ± 0.05c (−16.14) |
| | 1000 | 0.73 ± 0.03d (−69.83) | 1.83 ± 0.02d (−47.12) | 1.40 ± 0.02b (−4.76) | 2.37 ± 0.02b (−5.95) |
| GK30 | 0 | 2.99 ± 0.05a (100) | 3.67 ± 0.28a (100) | 1.52 ± 0.02a (100.00) | 2.56 ± 0.02a (100.00) |
| | 10 | 2.85 ± 0.04b (−5.01) | 3.06 ± 0.05b (−16.61) | 1.45 ± 0.02b (−4.62) | 2.47 ± 0.01b (−3.65) |
| | 100 | 2.43 ± 0.04c (−18.91) | 2.74 ± 0.02b (−25.32) | 1.36 ± 0.01c (−10.33) | 2.25 ± 0.02c (−12.11) |
| | 1000 | 1.61 ± 0.03d (−46.27) | 2.08 ± 0.05c (−43.29) | 1.34 ± 0.02c (−11.87) | 2.17 ± 0.02d (−15.36) |
| Coker 312 | 0 | 1.69 ± 0.04a (100) | 2.76 ± 0.11a (100) | 1.38 ± 0.01a (100.00) | 2.28 ± 0.02 a (100.00) |
| | 10 | 1.58 ± 0.02b (−6.52) | 2.69 ± 0.02a (−2.42) | 1.34 ± 0.01a (−2.91) | 2.14 ± 0.01b (−5.86) |
| | 100 | 1.33 ± 0.03c (−20.95) | 2.31 ± 0.02b (−16.20) | 1.27 ± 0.01b (−7.99) | 2.23 ± 0.02a (−2.05) |
| | 1000 | 1.18 ± 0.01d (−29.84) | 1.98 ± 0.02c (−28.17) | 1.21 ± 0.02c (−11.86) | 2.17 ± 0.01b (−4.83) |

Values are the means of three replications ± SE. Variants possessing the same letter are not statistically significant at $P < 0.05$. Values in the parenthesis show relative increase (+)/decrease (−) over the respective controls.