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Differential physiological, ultramorphological and metabolic responses of cotton cultivars under cadmium stress

M.K. Daud [a,b], Shafaqat Ali [a,c], M.T. Variath [a,d], S.J. Zhu [a,*]

[a] Department of Agronomy, College of Agriculture and Biotechnology, Zijingang Campus, Zhejiang University, Hangzhou, PR China
[b] Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan
[c] Department of Environmental Sciences, GC University, Faisalabad, Pakistan
[d] Department of Botany, University of Delhi, Delhi 110007, India

HIGHLIGHTS

- Biomass, diameter, volume and area significantly inhibited in all cultivars at 1000 μM Cd.
- Subcellular changes were found in nucleus, vacuoles, mitochondria and chloroplast.
- Ultrastructural alterations were greater in BR001 followed by GK30 and Coker 312.
- Greater incline in SOD activity occurred in leaves of BR001 and GK30 at 1000 μM Cd.
- POD activity in roots of BR001 and Coker 312 was greater at all Cd levels.

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ABSTRACT

Cadmium (Cd) stress may cause serious physiological, ultramorphological and biochemical anomalies in plants. Cd-induced physiological, subcellular and metabolic alterations in two transgenic cotton cultivars (BR001, GK30) and their parent line (Coker 312) were evaluated using 10, 100 and 1000 μM Cd. Germination, fresh biomass of roots, stems and leaves were significantly inhibited at 1000 μM Cd. Root volume tolerance index significantly increased (124.16%) in Coker 312 at 1000 μM Cd. In non-Cd stressed conditions, electron micrographs showed well-configured root meristem and leaf mesophyll cells. At 1000 μM Cd, greater ultramorphological alterations were observed in BR001 followed by GK30 and Coker 312. These changes were observed in nucleus, vacuoles, mitochondria and chloroplast. Dense precipitates, probably Cd, were seen in vacuoles, which were also attached to the cell walls. A considerable increase in number of nuclei, vacuoles, starch granules and plastoglobuli was observed in the electron micrographs of both roots and leaves at 1000 μM Cd. MDA contents were higher in roots of BR001 at 1000 μM Cd. Mean values of SOD activity in leaves of both BR001 and GK30 at 1000 μM Cd significantly increased as compared to the controls. POD activity in roots of BR001 and Coker 312 was greater at all Cd (10, 100, 1000 μM) levels over the control. Regarding APX, highest percent increase (71.64%) in roots of GK30 at 1000 μM Cd was found. Non-significant differences in CAT activity were observed at all levels of Cd stress in leaves of BR001 and GK30. Both transgenic cotton cultivars and their parental line invariably responded towards Cd stress. However, Coker 312 showed Cd-resistant behavior as compared to its progeny lines (BR001 and GK30).

1. Introduction

Heavy metal pollution has become a global environmental threat (Xu et al., 2012), which is caused by a number of heavy metals such as cadmium, lead, arsenic, or chromium. Among them, cadmium (Cd) is considered as one of highly phytotoxic (DalCorso et al., 2010) element and its toxicity has been a great agricultural problem in soil (Hasan et al., 2011). It is an important persistent inorganic pollutant having high toxicity for humans, animals and plants even at low concentrations (Benavides et al., 2005; Fojta et al., 2006; Strydom et al., 2006; Mobin and Khan, 2007; Wahid and Ghani, 2008). Its presence in our environment is due to various anthropogenic activities such as mining, industrialization, or production of phosphate fertilizers. Due to its greater solubility in water and high mobility in the soil–plant system (Groppa et al.,

Abbreviations: APX, ascorbate peroxidase; CAT, catalase; MDA, malondialdehyde; POD, guaiacol peroxidase; ROS, reactive oxygen species; SOD, superoxide dismutase; H_2O_2, hydrogen peroxide.

* Corresponding author. Tel.: +86 13067922851.
E-mail address: shjzhu@zju.edu.cn (S.J. Zhu).

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2012), it can be taken up by plant root system via apoplasmic system of cell and easily enter in plant body.

Inside the plant, Cd causes physiological disturbances such as water transport, absorption and transport of essential elements, oxidative phosphorylation in mitochondria, photosynthesis, reduced mitochondrial respiration, chlorophyll content, stunted plant growth and reproduction (Djebali et al., 2005; Vitória et al., 2006; Tukaj et al., 2007; Lage-Pinto et al., 2008; Gill et al., 2011). Thus, it significantly influences both the qualitative and quantitative traits of crops as well as their by-products (Myśliwa-Kurdziel and Strzałka, 2005).

Cd not only affects the physiology but also the ultra-morphology of plants. A number of ultrastructural changes in roots and leaves have been documented. 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 (Liu and Kottke, 2004; Aravind and Prasad, 2005; Daud et al., 2009b) has been reported due to Cd stress. Similarly, Cd caused disruption of grana and an increase in the number and size of plastoglobuli in chloroplasts, wavy appearance of grana and stroma thylakoids and dilation of thylakoid membranes (Daud et al., 2009c). Cd stress also inhibits various metabolic events that results in cellular energy deficiency and oxidative stress (Lannig et al., 2006; Cherkasov et al., 2010) and may cause the production of free radicals and reactive oxygen species (ROS). ROS like superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) can cause peroxidation of poly unsaturated fatty acid of lipid membranes (Tian et al., 2012; Kumar et al., 2013), protein oxidation and DNA damage (Gill and Tuteja 2010). Various mechanisms have been proposed to reduce Cd-induced ROS effects in plants. They are, decreasing Cd absorption and uptake, binding and sequestration to biomolecules, synthesizing antioxidant molecules (Pál et al., 2006; Lyubenova et al., 2007; Romero-Puertas et al., 2007; Jin et al., 2008).

ROS-scavenging antioxidant defense mechanisms are composed of both enzymatic and non-enzymatic antioxidants. Important antioxidants are comprised of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) (Ahsan et al., 2009).

World over, upland cotton (*Gossypium hirsutum* L.) is an important fiber crop; however, its productivity is adversely affected by various biotic and abiotic stresses (Li et al., 2012). Cadmium stress is an important abiotic stress particularly for those cotton cultivars, which are grown in the vicinities of industrial areas. How does Cd affect the biometric traits of these cultivars and how can these cultivars can be exploited for the remediation of Cd contaminated soil need research? The present study was designed with the aims to study the physiological and ultramorphological alterations as well as to investigate the antioxidant enzymes status in two transgenic cotton cultivars (BR001 and GK30) and their parent line (Coker 312) under Cd stress.

2. Materials and methods

2.1. Plant materials and culture conditions

Two transgenic cotton cultivars (BR001 and GK30) and their parent line (Coker 312) were used in the present study. BR001, a gluphosinate resistant transgenic cotton cultivar possessing the *Bar* gene developed in our laboratory (Daud et al., 2009a). GK30, an insect resistant *Bt* cotton, was kindly provided by Chinese Academy of Agricultural Sciences. Uniform grade matured coatless seeds of all cultivars were first immersed in 70% ethanol for 3 min and then in 0.1% $HgCl_2$ for 8–10 min. The seeds were washed with ddH_2O for three times and were directly treated with three different levels (10, 100, and 1000 μM) of Cd (as $CdCl_2{\cdot}2.5H_2O$) solution for approximately 2 h. Control group was without Cd. Other treatment processes were same for both Cd stressed and non-stressed group. The treated seeds were immediately transferred to sterilized petri plates (90 mm) having double-layered filter papers. There were three replications having single petri plate per replication for all Cd treatments (10, 100, and 1000 μM) along with control. Control was provided with distilled water. All experiments for Cd-mediated physiological, biochemical and ultrastructural changes in different parts of cotton seedlings were performed in three independent replicates with 10 seeds per petri plate. For the first 3 d of experiment, 5 mL of Cd and water solutions were added to each plate, while for another 3 d, the solution volume was 8–9 mL per petri plate. Solution was changed every other day. Petri plates were properly sealed with parafilm tape and placed in dark for 48 h followed by 4-d exposure to a 16 h photoperiod of 50 μmol m^{-2} s^{-1} under white fluorescent light. Throughout the experiment 28 ± 2 °C culture temperature and 60% relative humidity were maintained.

2.2. Growth studies

Various physiological parameters like germination, fresh biomass of roots, stems and leaves, root-leaf morphology parameters and cadmium uptake were used as end points to investigate the effect of Cd on the selected cotton cultivars. Replicated data were used to determine inhibitory rates, tolerance indices, translocation factor and concentration index.

Inhibitory rates of germination and fresh biomass of roots, stems and leaves were determined according to Liu et al. (2005) as describe below.

$$IR = (1 - X/Y) \times 100$$

where IR is inhibitory rate; X is mean values in the relevant treatment; Y is mean values in the relevant control.

We also measured root and leaf morphology-based tolerance indices (Daud et al., 2009b). They were calculated based on mean replicated data of respective root and leaf morphological traits using the following formula:

$$TI = MV^t/MV^c \times 100$$

where MV^t is the mean values in treatment and MV^c represents mean values in control. Moreover, to better comprehend the translocation of Cd from roots to shoots as well as shoot/root Cd concentration ratios, translocation factor (TF) and concentration index (CI) were studied according to Mattina et al. (2003) and Kiekens and Camerlynck (1982), respectively. Following formula for translocation factor (TF) was used.

$$TF = Cd^s/Cd^r$$

where Cd^s = Cd concentration in shoots, Cd^r = Cd concentration in roots.

In order to determine the concentration index (CI), we used the following formula. Where Cd^{tp} reveals Cd concentration in treated plants, Cd^{np} means Cd concentration in normal plants.

$$CI = Cd^{tp}/Cd^{np}$$

To study the translocation factor and concentration index in the present cotton cultivars, Cd contents in both roots and leaves were first determined using their dry biomasses. Their dried samples were digested in a 5 mL mixture of HNO_3:$HCLO_4$ (2:1, v/v), which