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Cadmium stress in cotton seedlings: Physiological, photosynthesis and oxidative damages alleviated by glycinebetaine

M.A. Farooq [a], S. Ali [a,*], A. Hameed [b], S.A. Bharwana [a], M. Rizwan [a], W. Ishaque [b], M. Farid [c], K. Mahmood [b], Z. Iqbal [b]

[a] Department of Environmental Sciences and Engineering, Government College University, Allama Iqbal Road, 38000, Faisalabad, Pakistan
[b] Nuclear Institute for Agriculture and Biology (NIAB), P.O. Box 128, Jhang Road, Faisalabad, Pakistan
[c] Department of Environmental Sciences, University of Gujrat, Hafiz Hayat Campus, Gujrat, Pakistan

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ABSTRACT

Cadmium (Cd) level is continuously increasing in agricultural soils mainly through anthropogenic activities. Cadmium is one of the most phytotoxic metals in soils. The present study investigates the possible role of exogenously applied glycinebetaine (GB) in alleviating Cd toxicity in cotton (*Gossypium hirsutum* L.) plants in a hydroponic system. Three concentrations of Cd (0, 1.0, and 5.0 μM) were tested with and without foliar application of GB (1.0 mM). Cadmium toxicity caused a significant decrease in plant height, root length, number of leaves per plant, fresh and dry weights of leaf, stem and root and intensively increased Cd concentration in different plant parts. Cadmium toxicity also decreased photosynthetic pigments and gas exchange characteristics in leaves. Superoxide dismutase (SOD), guaiacol peroxidase (POD), catalases (CAT) and ascorbate (APX) activities increased under lower Cd stress (1.0 μM) while decreased under higher Cd stress (5.0 μM). Cadmium toxicity increased the concentration of reactive oxygen species (ROS) as indicated by the increased production of malondialdehyde (MDA), hydgrogen peroxide (H_2O_2) and electrolyte leakage in both leaves and roots. Application of GB decreased Cd concentration in different plant parts, alleviated Cd-induced inhibition in plant growth and biomass and led to a significant increase in photosynthetic pigments, protein contents and antioxidant enzymes. Glycinebetaine application alleviated the oxidative damage as evidenced by the decreased production of electrolyte leakage, H_2O_2 and MDA contents. These results revealed that GB might alleviate Cd toxicity in cotton plants through lowering Cd concentrations and regulating Cd induced oxidative stress in different plant parts possibly by increasing the performance of the antioxidant enzymatic system.

1. Introduction

Environmental pollutants, released by human activities including noxious gasses, pesticides and heavy metals, have threatened existence of biota worldwide (Wagner, 1993; Sharma and Pandey, 2014; Adrees et al., 2015a; Rizwan et al., 2015a). Among these pollutants, heavy metal contamination of agricultural soils is a serious environmental threat that affects many physiological and metabolic processes in plants and finally decreased plant growth, photosynthesis and biochemical activities (Ali et al., 2013a, 2013b; Keller et al., 2015; Adrees et al., 2015b; Rizwan et al., 2015b). Among heavy metals, cadmium (Cd) is one of the most phytotoxic elements and has no known biological function in plants and animals (Rizwan et al., 2012; Khaliq et al., 2015; Rehman et al., 2015). Cadmium stress also decreased the uptake and distribution of essential elements in plants (Ahmad et al., 2011; Hediji et al., 2015). Although Cd is not a redox active metal, it causes oxidative stress in plants by the formation of reactive oxygen species (ROS) including superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) etc. (Zhang et al., 2009). Under Cd stress, overproduction of ROS may cause physiological disorders in plants which results in growth and biomass reduction (Ahmad et al., 2011; Saidi et al., 2013; Arshad et al., 2015). In order to avoid the deleterious effect of oxidative stress, plants have evolved well developed ROS scavenging enzymatic apparatus such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (POD), and catalase (CAT) (Yin et al., 2008). It has been shown that the activities of antioxidant enzymes increased up to a certain level of Cd stress and then decreased under higher Cd stress (Saidi et al., 2013; Hediji et al., 2015). This showed that under severe Cd stress conditions, the antioxidant enzymatic capacity of plants might not be sufficient to prevent toxic effects of metal (Hossain et al., 2010; Gill et al., 2015).

In critical conditions, plants have adopted different protective processes to respond to the heavy metal stress, including Cd stress. One such adaptive mechanism under stressful conditions is the accumulation of compatible solutes including glycinebetaine (GB) and

* Corresponding author. Tel.: +92 41 9201566; fax: +92 41 9200671.
E-mail address: shafaqataligill@yahoo.com (S. Ali).

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proline (Chen and Murata, 2011). Glycinebetaine is one of the most abundant quaternary ammonium compounds produced in higher plants under stressful environments (Yang et al., 2003). Glycinebetaine is involved in the protection of plants against many stresses such as drought (Iqbal et al., 2009; Raza et al., 2014), salinity (Hossain et al., 2010; Hasanuzzaman et al., 2014) and heavy metal stress (Chen and Murata, 2011; Cao et al., 2013; Ali et al., 2015; Jabeen et al., 2015). It has been shown that GB also provides protection against oxidative stress in many plant species under stressful conditions (Hossain et al., 2010; Hasanuzzaman et al., 2014). However, natural production of GB is not enough to protect plants under severe stress conditions. Under such conditions, exogenous application of GB might be a useful strategy to overcome abiotic stresses in plants (Ali et al., 2015). It has been reported that exogenous GB enhanced salt tolerance in rice seedlings by enhancing the activities of antioxidant enzymes (Hasanuzzaman et al., 2014) and enhanced drought tolerance in wheat by improving gas exchange characteristics (Raza et al., 2014). However, its response varies with plant species and genotypes (Chen and Murata, 2011). Exogenous GB also reduced heavy metal toxicity in many plant species (Bharwana et al., 2014; Ali et al., 2015). However, little information is available behind the mechanisms of GB-mediated alleviation of metal toxicity in plants.

Thus, based upon the above discussion, this study was designed to test whether exogenous GB application is capable to improve Cd tolerance in cotton plants, an important cash crop in Pakistan and worldwide, either through a reduced Cd uptake or by affecting growth, photosynthesis and antioxidant enzymes activities under Cd stress. For this purpose, a hydroponic experiment was conducted with three concentrations of Cd (0, 1.0 and 5.0 μM) without and with 1.0 mM of GB. After harvesting, various morphological and physiological parameters were determined namely biomass, shoot and root lengths, number of leaves per plant, photosynthetic pigments and gas exchange characteristics, protein contents and Cd concentrations in different plant parts. Oxidative stress, malondialdehyde (MDA), H_2O_2 and electrolyte leakage (EL), and activities of key antioxidant enzymes, SOD, POD, APX and CAT were measured in different plant parts to evaluate the role of GB in reducing oxidative stress by affecting antioxidant enzymes activities.

2. Materials and methods

2.1. Growth conditions

Healthy seeds of cotton genotype MNH 886 were taken from Ayub Agricultural Research Institute (AARI) and immersed in concentrated sulfuric acid solution approximately 15 min just to remove the short fiber on the surface of the seed. Seeds were then rinsed with distilled water thoroughly and sown in 2" layers of sterilized quartz sand trays in a growth chamber with a photoperiod of 16 h light/8 h dark with light intensity of 400 ± 25 $\mu molm^{-2}\ s^{-1}$. The light/dark temperature was set at 30 °C/25 °C with relative humidity at 85%. After 2 weeks, uniform seedlings were wrapped with foam at a root shoot junction, and transplanted in thermopore sheets having evenly spaced holes floating on 40 L capacity iron tubs, lined with polyethylene sheet containing modified Hoagland's solution. Continuous aeration was given through an air pump in the nutrient solution by making bubbles. The solution was changed on weekly basis. Complete randomized design (CRD) was applied. Two weeks after transplanting, Cd levels (control (0 μM), 1.0 μM, and 5.0 μM) distributed as $CdCl_2$ and two levels of GB (control and 1 mM) with five replicates were applied. Solution pH was maintained at 6.0 ± 0.1 by adding 1 M H_2SO_4 or NaOH solution.

2.2. Measurements of plant growth and biomass

Plants were harvested after 6 weeks of growth under Cd stress. After measuring shoot and root lengths, plants were separated into leaves, stem and roots, washed thoroughly with distilled water, wiped the plant material and fresh weight of these plant parts was determined. After this, samples were oven dried at 70 °C for about 72 h and then weighed.

2.3. Gas exchange parameters and chlorophyll contents

After 6 weeks of growth with Cd and GB treatments, photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), water use efficiency (A/E) of a fully expanded youngest leaf of each plant were determined by using infrared gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). These measurements were taken from 10:00 am to 11:30 am with the growth conditions as describe above (Section 2.1).

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were determined by spectrophotometrically (Metzner et al., 1965). After 6 weeks of treatment, the topmost fully expanded fresh leaves were weighed and dipped overnight in 85% (v/v) acetone for the extraction of the chlorophyll pigments. Supernatant taken was centrifuged at 4000 rpm for 10 min and diluted with 85% acetone to the suitable concentration for spectrophotometric measurements. The disappearance was calculated at absorbance of 452.5, 644 and 663 nm alongside blank of 85% liquid acetone. Chl a, b, total chlorophyll and carotenoids were determined by spectrophotometer (Halo DB-20/DB-20S, Dynamica Company, London, UK). The chlorophylls and carotenoids contents were calculated by using the adjusted extinction coefficients and equations (Lichtenthaler, 1987).

2.4. Determination of H_2O_2, MDA and electrolyte leakage

The H_2O_2 content was colorimetrically determined as described by Jana and Choudhuri (1981). $H_2O_2$was extracted by homogenizing 50 mg leaf or root tissues with 3 ml of phosphate buffer (50 mM, pH 6.5). To measure H_2O_2 content, 3 ml of extracting solution was mixed with 1 ml of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and the mixture was centrifuged at 6000 × g for 15 min. The intensity of the yellow color of the supernatant was measured through spectrophotometer at 410 nm. H_2O_2 content was computed by using the absorbance coefficient of 0.28 $\mu mol^{-1}\ cm^{-1}$.

The level of lipid peroxidation in the leaf tissue was measured in terms of malondialdehyde content (MDA, a product of lipid peroxidation) determined by the thiobarbituric acid (TBA) reaction using the method of Heath and Packer (1968), with minor modifications as described by Zhang and Kirham (1994). A 0.25 g leaf sample was homogenized in 5 ml 0.1% TCA. The homogenate was centrifuged at 10 000 × g for 5 min. To 1 ml aliquot of the supernatant, 4 ml of 20% TCA containing 0.5% TBA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10 000 × g for 10 min, the absorbance of the supernatant at 532 nm was read and the value for the nonspecific absorption at 600 nm was subtracted. The MDA content was calculated by using an absorbance coefficient of 155 $mM^{-1}\ cm^{-1}$.

Electrolyte leakage was estimated by using the method of Dionisio-Sese and Tobita (1999). After treatment for 6 weeks, leaves samples were cut into small parts of 5 mm length and put in test tubes containing 8 ml deionized and distilled water. The tubes were placed in a water bath at 32 °C for two hours. Initial electrical conductivity of the medium (EC_1) was assessed (Conductivity Model 720, INCO -LAB Company, Kuwait). For second electrical conductivity (EC_2), samples were placed in autoclave at 121 °C for 20 min to expel all electrolytes. Samples were cooled at 25 °C. Total electrolyte leakage was calculated by using the following formula:

$$EL = (EC_1/EC_2) \times 100.$$

2.5. Assay of antioxidant enzymes and soluble protein contents

Antioxidant enzymes such as SOD, POD, CAT and APX in roots and leaves were determined by spectrophotometrically. After 6 weeks of