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Alleviation of cadmium toxicity by silicon is related to elevated photosynthesis, antioxidant enzymes; suppressed cadmium uptake and oxidative stress in cotton

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ABSTRACT

Biotic systems face immense environmental hazards such as accumulation of heavy metals, particularly in agricultural ecosystems that might cause deterioration of yield and quality of crops. In this study, we evaluated the role of silicon (Si) in alleviating the heavy metal (Cd) stress tolerance in cotton by analyzing the induced Physio-chemical changes. Cotton plants were grown in hydroponic culture with three different Cd levels (0, 1 and 5 μM) along with two Si treatment levels (0 and 1 mM). The data showed that Cd alone reduced the plant growth as well as the efficiency of antioxidant activity as compared to control plants. Plant growth, gas exchange characteristics (net photosynthetic rate, stomatal conductance, transpiration rate, water use efficiency) chlorophyll contents, and carotenoids as well as the performance of antioxidant enzymes were improved by the exogenous application of Si. The adverse effects of Cd on plant growth were alleviated by the exogenous application of Si. It was observed that Si effectively mitigated the adverse effects of Cd on cotton plants and markedly enhanced the growth, biomass and photosynthetic parameters while decreased the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and electrolytic leakage (EL). The antioxidant enzyme activities in cotton leaves and roots increased significantly, when Si was added to control as well as Cd stressed plants. In conclusion, Si improved the growth and photosynthesis attributes of cotton plants by mitigating the adverse effects of Cd stress through reduced EL, MDA and H_2O_2 contents and improved activities of antioxidant enzymes.

1. Introduction

Heavy metals with a density higher than 5.0 g cm^{-3} such as cadmium (Cd), lead (Pb), chromium (Cr), silver (Ag) and mercury (Hg) etc., are important environmental pollutants, mainly where there are high anthropogenic activities. Soil pollution by heavy metals is an important environmental concern for the agricultural sector VacullK et al., (2009) over the past several decades, especially in the developing countries (Tandy et al., 2006; Saifullah et al., 2009). Agricultural lands that have been polluted due to the heavy metal accumulation were proved to have highly adverse effects on soil biological activity, fertility, plant metabolism, biodiversity, and the health of humans and animals (Wagner 1993; Nawrot et al., 2006; Jarup and Akesson 2009; Ali et al., 2011a, b). Even in low concentrations can cause serious threats to all living organisms via their accumulation in soil and water. Heavy metals are well known growth inhibitors and can cause detrimental effects on plant growth, develop phototoxic responses and decrease the productivity and quality of agricultural crops (Ali et al., 2012, 2013a).

Cadmium (Cd) is one of the most toxic environmental pollutant, which is quite toxic even in minute concentration. It enters the environment principally from industrial processes, mining operations, municipal wastes, and phosphate fertilizers (Wagner, 1993; Clemens, 2006). Upon exposure to Cd stress, inhibition occurs in most of the physiological and biochemical processes of plants, such as chlorophyll synthesis, photosynthesis and nutrient uptake resulting in retardation of growth and low yield (Ali et al. 2013b; Sanita and Gabbrielli, 1999). As a non-redox metal, Cd is unable to take part in Fenton-type reactions, but can produce reactive oxygen species (ROS) and cause oxidative stress in plants (Schützendübel and Polle, 2002; Zhang et al., 2009). ROS molecules are highly toxic compounds that can oxidize most of the lipids, proteins and nucleic acids causing death of the cell due to lipid peroxidation, membrane damage, and inactivation of enzymes.

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Silicon (Si) is the second most abundant element both on the surface of the earth and in the soil (Gong et al., 2006). The unavailability of Si to plants is due to its combination with other compounds to form silicates or oxides (Richmond and Sussman, 2003). Silicon is not considered as an essential element but it has been found to minimize the various biotic and abiotic stresses (Richmond and Sussman, 2003; Ma, 2004; Ma.and.Yamaji, 2006) and has beneficial effects on growth and development of many plants, particularly of gramineous plants and some cyperaceous plants (Richmond and Sussman, 2003; Epstein 1994, 1999; Liang et al., 2007; Ma et al., 2001). For example, Si enhances plant resistance to fungi, pests as well as to the drought stresses. Si also helps in mediating the adverse effects of heavy metal stresses such as chromium (Cr) (Ali et al., 2013c) aluminum (Al) (Baylis et al., 1994; Liang et al., 2001; Hammond et al., 1995), boron (B) (Gunes et al., 2007a; Inal et al., 2009) and zinc (Zn) (Kaya et al., 2009). These beneficial effects of Si to plants are attributed to both soluble Si and Si deposited in the various plant tissues. Large amount of Si that deposited in tissues may act as a physical barrier that increases the rigidity and strength of the plant tissues (Ma, Yamaji, 2006). Soluble Si plays an active role in stimulating some defense reaction mechanisms and enhancing host resistance to plant against the diseases. (Fauteux et al., 2005).

In general, silicon has been recommended for improving the resistance of plants to abiotic stresses, including heavy metal toxicity. However, to our knowledge, the possible use of Si for improving Cd stress tolerance in cotton plants has not been tested so far. In this view, a hydroponic experiment was conducted to find out the influence of Si treatments on plant growth, photosynthesis, chlorophyll contents, oxidative stress, antioxidant enzymes, and electrolyte leakage and cd uptake under Cd stress. The objective was to investigate that whether or not the exogenous application of Si activate the protective responses in cotton plants exposed to cadmium stress.

2. Materials and Methods

2.1. Experimental site

This study was carried out in wire house of Ayub Agricultural Research Institute (AARI) and, in labs of Government College University Faisalabad and Nuclear Institute for Agriculture and Biology (NIAB) Faisalabad, Pakistan.

2.2. Growth conditions

Healthy seeds of cotton genotype MNH 886 were immersed in concentrated sulfuric acid solution for 15 min to remove the short fibers on the surface of the seeds. Seeds were then rinsed with distilled water thoroughly and sown in 2 in. layers of sterilized quartz sand trays were put in a growth chamber with a photoperiod of 16 h light/8 h dark with light intensity of 400 ± 25 μmol m^{-2} s^{-1}. The light/dark temperature was set at 30 °C/25 °C with relative humidity at 85%. After two weeks of sowing, the uniform seedlings were wrapped with foam at a root shoot junction, and transplanted in thermopole sheets having evenly spaced holes (15 in. × 17 in. in size) floating on 40 L capacity iron tubs, lined with polyethylene sheet containing modified Hoagland's solution. The basic nutrient medium had composition: $(Ca(NO_3)_2$ 2.5 mM, $MgSO_4$ 1 mM, KCl 0.5 mM, KH_2PO_4 0.5 mM, $FeCl_3$ 0.1 μM, $CuSO_4$ 0.2 μM, $ZnSO_4$1 μM, H_3BO_3 20 μM, H_2MoO_4 0.005 μM, $MnSO_4$ 2 μM). Continuous aeration was given through an air pump in the nutrient solution by making bubbles. The solution was changed every week. Thereafter two weeks of transplantation, Cd levels (control, 1 and 5 μM) were developed with $CdCl_2$ and two levels of Si as sodium silicate (Na_2SiO_3) (control and 1 mM) with three replicates were applied. Complete randomized design (CRD) was applied. By adding 1 M H_2SO_4 and NaOH, solution pH (6.0 ± 0.1) was maintained.

2.3. Measurements of plant growth and biomass

Plants were harvested after five weeks of growth under Cd stress. Data regarding shoot, root length, shoot, and root fresh and dry weight were recorded.

2.4. Leaf area

Leaf area was estimated with a leaf area meter (LI-2000, LI-COR, USA).

2.5. Gas exchange parameters

Five weeks after application of the treatment, photosynthetic rate (*A*), stomatal conductance (gs), transpiration rate (*E*), water use efficiency (*A*/*E*) was determined by using Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England).

2.6. Determination of chlorophyll contents

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were determined spectrophotometrically (Metzner et al., 1965). After five weeks of Cd treatment, the topmost fully expanded fresh leaves were weighed and dipped overnight in 85% (v/v) aqueous acetone for the extraction of the chlorophyll pigments. Supernatant taken was centrifuged at 4000 rpm for 10 min and diluted with 85% aqueous acetone to the suitable concentration for spectrophotometric measurements. The disappearance was calculated at absorbance of 452.5, 644 and 663 nm alongside blank of untainted 85% liquid acetone. Chl a, b, total chlorophyll and carotenoids were analyzed using the following equations:

Chlorophyll a (μg/ml) $= 10.3^{*}E_{663} - 0.98^{*}E_{644}$
Chlorophyll b (μg/ml) $= 19.7^{*}E_{644} - 3{:}87^{*}E_{663}$
Total chlorophyll = chlorophyll a+chlorophyll b
Total carotenoids (μg/ml) $= 4.2^{*}E_{452.5} - \{(0.0264^{*}$chl a)+(0:426*chl b)$\}$

Finally, these pigment fractions were calculated as mg/g fresh weight.

2.7. Estimation of electrolyte leakage

Electrolyte leakage was estimated by using the method of Dionisio-Sese, Tobita (1998). After five weeks of Cd treatment, leaves (1 g) 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. The initial electrical conductivity of the medium (EC_1) was assessed. The samples were placed in an autoclave at the 121 °C for 20 min to expel all electrolytes. Samples were cooled at 25°C and second electrical conductivity (EC_2) was measured. Total electrolyte leakage was calculated by using the following formula.

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

2.8. Assay of antioxidant enzymes

Anti-oxidant enzymes such as superoxide dismutase (SOD), guaiacol peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) of roots and leaves were determined spectrophotometrically.

After five weeks of Cd treatment fresh samples (0.5 g) of leaves and roots were ground with the help of a motor and pestle and homogenized in 0.05 M phosphate buffer (pH 7.8) under chilled condition. The homogenized mixture was filtered through four layers of muslin cloth and centrifuged at 12,000 × *g* for 10 min at 4 °C.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was analyzed by the nitroblue tetrazolium (NBT) method (Beauchamp and Fridovich, 1971) at 560 nm by calculating the photoreduction of NBT. The reaction mixture (3 ml) consisted of 50 mM sodium phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 10 μM EDTA, 2 mM riboflavin and enzyme extract (100 μl). Reaction was started by placing tubes below two 15 W fluorescent lamps for 10 min.

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed according to method of (Putter, 1974) with some modification. The reaction mixture (3 ml) consisted of 100 μl enzyme extract, 100 μl guaiacol (1.5%, v/v), 100 μl H_2O_2 (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). Increase in the absorbance due to oxidation of guaiacol was measured spectrophotometrically at 470 nm ($\varepsilon = 26.6$ mM^{-1} cm^{-1}).

Catalase (CAT, EC 1.11.1.6) activity was determined by the method of (Aebi, 1984). The assay mixture (3.0 ml) was comprised of 100 μl enzyme extract, 100 μl H_2O_2 (300 mM) and 2.8 ml 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was assayed by monitoring the reduction in the absorbance at 240 nm as a consequence of H_2O_2 disappearance ($\varepsilon = 39.4$ mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to the method of (Nakano and Asada, 1981). The reaction mixture consisted of 100 μl enzyme extract, 100 μl ascorbate (7.5 mM), 100 μl H_2O_2 (300 mM) and 2.7 ml 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). The oxidation of ascorbate was observed by the change in absorbance at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}).

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