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Response of silicon on metal accumulation, photosynthetic inhibition and oxidative stress in chromium-induced mustard (*Brassica juncea* L.)

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ABSTRACT

Silicon plays an important role in the amelioration of heavy metal stress. We have examined the effects of exogenously applied Si (500μM and 700 μM) in the metal accumulation, photosynthetic protection, and oxidative stress under chromium (Cr) stress in mustard (*Brassica juncea* L.) cv. Varuna. The experimental work was carried out in the naturally illuminated green house in the Department of Botany, Aligarh Muslim University, Aligarh. Plants grown with Cr (100 μM) alone revealed increased oxidative stress via enhancement in H_2O_2 content and lipid peroxidation. Si protects the plant under Cr stress by reducing transportation of Cr from root to aerial part and also protected photosynthetic activity through increased activity of the net photosynthetic rate, chlorophyll, and carotenoid content. Application of 700 μM Si significantly alleviated the Cr-induced photosynthetic inhibition oxidative stress more than 500 μM Si. The treatment of Si increased proline and ascorbate content which might help increase photosynthetic and growth attributes.

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1. Introduction

Contamination of soil by toxic heavy metals rising day by day has become a burning issue for the environment, at private as well as government level. The toxic effect of heavy metals, especially on the agricultural environment presents a challenge to the plant scientist concerned with the quality and yield of crop production. Some heavy metals such as Cr, Cd, Pb, and Hg, especially in large amounts, could affect growth and productivity of plants (Najafian et al., 2012). These metals reduce the efficiency of farming by retarding growth and development of crop productivity, which ultimately affects the animal and human health through the food chain (Mudgal et al., 2010). However, accumulation of metals in the food chain has been evidenced, lead to serious threats to ecological and health problems (Verstraeten et al., 2008). Among heavy metals, Cr is one of the non-essential and most toxic heavy metals release from the industries, mainly leather industry, accounting for 40% of the total industrial use (Barnhart, 1997). Cr has detrimental effects on plant growth and development even at very low concentration (Marschner, 1999). Phytotoxicity of Cr inhibits seed germination, seedling growth, alters water balance, photosynthetic processes, pigment status, nutrient balance and antioxidant enzymes (Kumar and Joshi, 2008; Schiavon et al., 2008). It has been reported that the toxicity of Cr originates from the production of excess ROS causing oxidative stress leading to cellular redox imbalance, ionic transport imbalance, damage lipids, proteins, and DNA which leads to programmed cell death (Choudhury and Panda, 2005; Panda and Choudhury, 2005). Plants develop several strategies to combat heavy metal toxicity by up-regulating the production of ROS scavenging enzymatic and non-enzymatic antioxidants and maintains high rates of photosynthesis (Foyer and Shigeoka, 2011; Noctor et al., 2012). The significant role of proline in cell osmotic adjustment and membrane stabilization, buffers cellular redox potential by scavenging free radicals and detoxification of toxic ions in plants exposed to stress has been recognized (Ashraf and Foolad, 2007).

Silicon (Si), the second most abundant elements in the earth's crust, plants require as a macronutrient like calcium, magnesium, and phosphorus (Epstein, 1999). Although Si has not been recognized as an essential element for higher plants, it has beneficial effects on growth and development (Liang et al., 2001). Si can enhance resistance and tolerance of many higher plants grown under stress environment by the stimulation of both enzymatic and non-enzymatic antioxidants (Vaculik et al., 2012). A number of mechanisms have been documented by which Si alleviates heavy metal toxicity via reduction of metal availability to plants in growth medium, regulation of metal uptake from root-to-shoot transport, modulation of the cation binding capacity of the cell wall and complexation and co-precipitation of toxic metal ions in cytoplasm followed by sequestration of metal in the vacuole (Liang et al., 2007; Cunha and do Nascimento, 2009).

However, the potential contribution of Si in the regulation of the elevated level of Cr toxicity in mustard has not been clearly established. Therefore, methods are needed to alleviate Cr toxicity, and also to

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decrease the Cr content in crops which may be helpful to minimize health risks. The present study was carried out to assess the potential involvement of Si in the alleviation of Cr stress in mustard (*Brassica juncea* L.) and study Cr accumulation, proline content, lipid peroxidation, photosynthetic activity, nitrogen metabolism, and growth characters by the application of Cr and Si treatments.

2. Materials and methods

2.1. Plant material and growth condition

Seeds of mustard (*B. juncea* L. Czern & Coss.) cultivar "Varuna" were surface sterilized with 0.1% $HgCl_2$ solution followed by repeated washing with distilled water and were sown in 23-cm diameter earthen pots filled with 5 kg of reconstituted soil (sand:clay:peat; 70:20:10; EC: 8.8 dS m^{-1}; pH: 6.8) in the net house of Botany Department, Aligarh Muslim University, Aligarh, India under natural day/night conditions with a photosynthetically active radiation (PAR), 960 μmol/m^2/s. Day and night temperatures were 24/18 ± 3 °C, and relative humidity 68 ± 5%. After seedling establishment, three healthy plants nearly of equal size were maintained. Plants were treated with control (0), 100 μM Cr, 500 μM Si, 700 μM Si, 100 μM Cr + 500 μM Si, and 100 μM Cr + 700 μM Si in the soil 20 days after sowing (DAS). Cr and Si were given in the form of $K_2Cr_2O_7$ and Na_2SiO_3 respectively. The experiments were arranged in a factorial randomized block design, and the number of replicates for each treatment was maintained three (n = 3). The sampling of plants was done at 40 DAS to record physiological and growth parameters.

2.2. Determination of lipid peroxidation and H_2O_2 content

The level of lipid peroxidation was assayed in terms of Malondialdehyde (MDA) content determined by the thiobarbituric acid (TBA) reaction as described by Cakmak and Horst (1991). A total of 0.5 g of fresh leaf was homogenized in 10 ml of 0.1% tri-carboxylic acid (TCA) and centrifuged at 12,000 × g for 5 min. 1 ml of the supernatant was added to 4 ml of 0.5% TBA. The mixture was heated at 95 °C for 30 min, cooled and centrifuged at 12,000 rpm for 5 min. The absorbance of the supernatant was read at 532 nm with a UV–VIS Spectrophotometer (SL 164, Elico, Hyderabad, India) and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. The content of MDA was calculated using the extinction coefficient (155 mM^{-1} cm^{-1}).

The content of H_2O_2 was determined following the method of Okuda et al. (1991). Leaf tissues (250 mg) were ground in ice cold 200 mM perchloric acid. After centrifugation at 1200 × g for 10 min, a perchloric acid of the supernatant was neutralized with 4 M KOH. The insoluble potassium perchlorate was eliminated by centrifugation at 500 × g for 3 min. The reaction was started by the addition of peroxidase and the increase in absorbance was recorded at 590 nm.

2.3. Determination of net photosynthetic rate and photosynthetic pigments

Net photosynthetic rate (P_N) was measured in fully expanded uppermost leaves of plants in each treatment using an infrared gas analyzer (CI-340, Photosynthesis System, CID Bio-Science, USA). The measurements were done between 11.00 and 12.00 h at the light saturating intensity and at 370 ± 5 μmol mol^{-1} atmospheric CO_2 concentrations. Total chlorophyll and carotenoid content was extracted by using the method of Hiscox and Israelstam (1979) by using dimethyl sulfoxide (DMSO) as an extraction medium and estimated and calculated by the method of Arnon (1949). Fresh leaves (100 mg) were cut into small pieces and collected in the test tube containing 7 ml of DMSO. The test tubes were covered with black paper and incubated at 45 °C for 40 min for the extraction. The content was transferred to a graduated tube and the final volume was made to 10 ml of DMSO. Extract measuring 3 ml was transferred to cuvette and absorbance was read at 645 and 663 nm for chlorophyll content and at 480 and 510 nm for carotenoid content on a UV–VIS Spectrophotometer (SL 164, Elico, Hyderabad, India).

2.4. Determination of nitrate reductase activity and leaf nitrogen content

Nitrate reductase (NR) activity in leaves was measured by preparing an enzyme extract using the method of Kuo et al. (1982). Leaves (1.0 g) were frozen in liquid N_2, ground to a powder with a chilled mortar and pestle, and then stored at −80 °C. The powder was thawed for 10 min at 4 °C and was homogenized in a blender in 250 mM Tris–HCl buffer, pH 8.5, containing 10 mM cysteine, 1 mM EDTA, 20 M FAD, 1 mM DTT, and 10% (v/v) glycerol. The homogenate was centrifuged at 10,000 × g for 30 min at 4 °C. NR activity was assayed as the rate of nitrite production at 28 °C adopting the procedure of Nakagawa et al. (1984). The assay mixture contained 10 mM KNO_3, 0.065 M HEPES (pH 7.0), 0.5 mM NADH in 0.04 mM phosphate buffer (pH 7.2) and enzyme in a final volume of 1.5 ml. The reaction was initiated by adding NADH. After 15 min the reaction was terminated by adding 1 ml of 1 N HCl solution containing 1% sulfanilamide followed by the addition of 1 ml of 0.02% aqueous N-1-napthyl ethylene-di-amine-dihydrochloride (NED). The absorbance was read at 540 nm after 10 min.

Leaf N content was determined in acid–peroxide digested material using the method of Lindner (1944). 2 ml of 2.5 N NaOH solution and 1 ml of 10% sodium silicate solution were added to 10 ml aliquots of the digested material and volume was made up to 50 ml using DDW. Nessler's reagent was added to this solution. The OD was recorded at 525 nm.

2.5. Determination of protein content

Protein content in leaves was measured by adopting the Coomassie brilliant Blue G250 dye method of Bradford (1976) using the bovine serum as standard. Leaf tissue (200 mg) homogenized in phosphate buffer solution and centrifuge at 10,000 rpm for 10 min. 5 ml of dye solution was added to the supernatant and read the absorbance at 595 nm at not more than 30 min.

2.6. Determination of proline and ascorbate content

Proline content was determined by the ninhydrin method as proposed by Bates et al. (1973). Fresh leaves (300 mg) were homogenized in 3 ml of 3% sulfosalicylic acid. The homogenate filtrate was reacted with 1 ml each of acid ninhydrin and glacial acetic acid for 1 h in a test tube placed in a water bath at 100 °C. The reaction was terminated by placing the test tube on ice. The mixture was extracted with toluene and the absorbance was measured at 520 nm using L-proline as a standard.

Ascorbate content was estimated by 2,6-dichlorophenol-Indophenol's dye method as determined by Keller and Schwanger (1997). 500 mg fresh leaf sample was homogenized in the ice bath with 20 ml of extracting solution. The homogenate was centrifuged at 6000 × g for 15 min. 1 ml of the supernatant and 5 ml of working 2,6-dichlorophenol-Indophenol solution were mixed with constant shaking and the O.D. of the solution was determined at 520 nm wavelength.

2.7. Determination of growth characteristics

Plants were uprooted carefully from the pots, washed to remove dust. Shoot length and root length were measured by a meter scale. After recording fresh weight, plants were dried in a hot air oven at 80 °C for 48 h till constant weight, dry weight of plants was determined. Leaf area was measured by a leaf area meter (LA 211 Systronics, New Delhi, India) and leaf area index was also measured.

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