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Original Research Paper

Interactive effects of salicylic acid on enzymes of nitrogen metabolism in clusterbean (*Cyamopsis tetragonoloba* L.) under chromium(VI) toxicity

Punesh Sangwan [a,*], Vinod Kumar [b], Deepika Gulati [c], U.N. Joshi [a]

[a] Department of Biochemistry, C.C.S. Haryana Agricultural University, Hisar 125001, India
[b] Department of Biochemistry, G.B. Pant University of Agriculture and Technology, Pantnagar 163145, India
[c] Department of Botany, C.C.S. Haryana Agricultural University, Hisar 125001, India

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ABSTRACT

Chromium (Cr) toxicity is a major constraint to crop production. A pot experiment was conducted to examine the ameliorating effects of salicylic acid on Cr toxicity enzymes of nitrogen metabolism in clusterbean plant parts. For this study, salicylic acid (0.25 and 0.50 mM) was applied as foliar spray on control and Cr-stressed plants at 20, 35 and 55 days after sowing and its influence on Cr toxicity at vegetative, flowering and grain filling stages was examined. Cr treatment caused decrease in specific enzyme activity of nitrogenase, nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase in various plant organs at different growth stages with an increase in Cr(VI) levels from 0 to 2.0 mg Cr(VI) Kg^{-1} soil. However, exogenously added salicylic acid (0.25 and 0.50 mM) significantly alleviated Cr toxicity effects at all growth stages by increasing in enzymes specific activity. The treatments with 0.25 and 0.5 mM salicylic acid increased the specific activity of these enzymes in leaves, stem and root, when compared to those of Cr treatments alone.

1. Introduction

Heavy metal contamination in soil and water over prolonged time becomes hazardous to plants as their release from industrial units, metallurgical operations, mining activities and disposal of sewage leads to considerable reduction in crop yields (Chaffei et al., 2004). Among heavy metals, chromium (Cr) plays a major role in polluting environment as a consequence of above activities (Mehdi et al., 2003). It can exist in the environment in several oxidation states. Cr(VI) exists predominantly in the +III and +VI oxidation states with Cr(+III) as the most stable oxidation (Zayed and Terry, 2003). The Cr(VI) is extensively used in the industries such as electroplating, leather tanning, textile printing, textile preservation and metal finishing (Dixit et al., 2002).

Cr element in very low amounts is useful for organisms but at higher concentrations, it is toxic and considered as a pollutant. Symptoms of Cr phytotoxicity include inhibition of seed germination or of early seedling development, reduction of root growth, leaf chlorosis and depressed biomass (Sharma et al., 1995). Cr affected physiological processes like seed germination, growth and nitrogen metabolism has also been reported in several studies (Seoccianti et al., 2006; Chidambaram et al., 2009; Akinci and Akinci, 2010; Sangwan et al., 2015). Nitrogen is considered to be a vital macronutrient for plants which determines growth, development and productivity of plants. Assimilation of nitrogen as NH_4^+ plays an important role in plant growth and development which determines yield and quality of grains (Balestrasse et al., 2003). Cr (VI) treatments adversely affected the growth of forage sorghum as a result of its interference with photosynthetic pigments and key enzymes of NH_4^+ assimilation (Kumar and Joshi, 2008). Cr(VI) treatments were also reported to affect the enzymes of nitrogen metabolism in clusterbean (Sangwan et al., 2014).

Therefore, strategies are needed to alleviate the adverse effects of Cr toxicity, and also to decrease the Cr level in crops which may be helpful to minimize health risks and improvement of plant growth and development (Sangwan et al. 2013). Plant hormones have been reported as active members of signal transduction cascade involved in the plant stress responses responses (Mori and Schroeder, 2004). Exogenous application of plant hormones has emerged as a potential strategy to alleviate adverse effects of various abiotic stresses including heavy metal (Chakrabarti and Mukherji, 2003; Tuna et al., 2008; Gangwar et al., 2011; Sangwan et al., 2015). Salicylic acid (SA), an endogenous plant growth regulator, is involved in regulation of a wide range of metabolic and physiological responses in plants and thereby it affects growth and developmental processes. The importance and physiological

* Corresponding author.
E-mail address: puneshsangwan@gmail.com (P. Sangwan).

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mechanism of SA in amelioration of abiotic stresses have been intensively studied (Zhou et al., 2009; Mohsenzadeh et al., 2011; Moradkhani et al., 2012). However, the possible role of salicylic acid application in alleviating Cr toxicity has not been explored yet. Therefore, it would be interesting to know more about the impact of exogenous application of SA in plants. Therefore, the present study was designed to explore and investigate the ameliorative responses of SA supplementation under Cr(VI) toxicity in clusterbean, a multipurpose fodder crop, which has recently assumed great industrial importance due to presence of gum, which is used extensively in paper, mining, explosive, food, pharmaceuticals, cosmetics, textiles and oil industries.

2. Material and methods

2.1 Chemicals, reagents and soil

The chemicals and reagents used during the present investigation were of analytical grade. A nutrient deficient loamy sand soil from Regional Research Station, Gangwa block of Hisar district was used in the present study. The characteristics of soil were: pH (1:2) 8.50; organic carbon, 0.22; N, 4.0 mg kg^{-1} soil; P, 13.0 mg kg^{-1} soil; K, 163 mg kg^{-1} soil; Zn^{2+}, 0.61 mg kg^{-1} soil; Fe^{2+}, 0.9 mg kg^{-1} soil; Cu^{2+}, 0.18 mg kg^{-1} soil; Mn^{2+}, 3.6 mg kg^{-1} soil; EC, 1.5; $CaCO_3$ 3.5; Cr^{2+}, 0.01 mg kg^{-1} soil; texture–sandy loam.

2.2 Plant growth and environmental conditions

Seeds of clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) cv HG 2-20 were procured from Forage Section, Department of Genetics and Plant Breeding, C.C.S. Haryana Agricultural University, Hisar and raised in pots filled with 5 kg of sandy loam soil in a naturally lit net house. Ten pots were used for each treatment. Two concentrations of SA i.e., 0.25 and 0.50 mM were prepared for foliar spray and 5 treatments of SA and Cr(VI) were made as follows; T_0=0 mg Cr(VI) Kg^{-1} soil; T_1=2.0 mg Cr(VI) Kg^{-1} soil; T_2=(0 mg Cr(VI) Kg^{-1} soil+0.25 mM SA); T_3=(2.0 mg Cr(VI) Kg^{-1} soil+0.25 mM SA); T_4=(0 mg Cr(VI) Kg^{-1} soil+0.50 mM SA); T_5=(2.0 mg Cr(VI) Kg^{-1} soil+0.50 mM SA). The seeds were surface sterilized with mercuric chloride and after proper washing with distilled water, inoculated with *Rhizobium* culture. Equal amount of nutrient solution was supplied at weekly interval to each pot. The plants were irrigated with equal quantities of tap water as and when required. Foliar spray of SA was given at 20, 35 and 55 days after sowing. Plant samples from each treatment were collected at vegetative (30 DAS), flowering (50 DAS) and grain filling stages (65 DAS). The temperature and relative humidity during the experiment ranged from 11.0 to 35.6 °C and 34.5% to 95.2%, respectively. The light intensity ranged from 36,100 to 84,000 lx.

2.3 Enzyme activity measurement

Specific activity of nitrogen metabolism enzymes i.e. Nitrogenase (E.C.1.7.99.2), nitrate reductase (NR; E.C.1.6.6.1), nitrite reductase (NiR; E.C. 1.7.7.1), glutamine synthetase (GS; E.C.6.3.1.2), glutamate dehydrogenase (GDH; E.C.1.4.1.4) and glutamate synthase (GOGAT; E.C.1.4.1.14) in leaves, shoot and root (Nitrogenase activity in nodules only) at different growth stages were measured by the standard methods as described in Sangwan et al. (2014). All observations were measured up to 2.0 mg kg^{-1} soil because plants treated with more than 2.0 mg Cr(VI) kg^{-1} soil concentration did not survive 20 days after sowing.

2.4 Protein estimation

The soluble protein in the sample extract was precipitated by 20% TCA, centrifuged and resultant residue was dissolved in 0.1 N sodium hydroxide (NaOH) solution for its estimation following method of Lowry et al. (1951).

2.5 Chromium estimation

One gram powdered sample was digested with 15 ml of di-acid mixture (4 HNO_3:1 $HClO_4$) in a conical flask by heating on hot plate in open space till clear white precipitates settled down at the bottom of the conical flask. The precipitates were then dissolved in 1 N HCl prepared in double glass distilled water, filtered and volume of the filtrate was made 50 ml with double glass distilled water. The content of Cr was estimated from the extract prepared by Atomic Absorption Spectrophotometer (Perkin-Elmer Model 2380).

2.6 Statistical analysis

A two-factorial ANOVA in complete randomized block design was used to confirm the validity of the data using OPSTAT software available on CCSHAU website home Page (http://hau.ernet.in/opstat.html). The values used in graphs are mean of three replicates and shown as ± standard error.

3. Results

3.1 Effect of SA foliar spray on nitrogen metabolism enzymes

Foliar spray of 0.25 mM SA (T_3) on 2.0 mg Cr(VI) kg^{-1} soil stressed clusterbean plants leads to an increase in NR specific activity in leaves, stem and roots (Fig. 1). It was noticed that specific activity of NR increased from 0.016 to 0.018 units in leaves, 0.030 to 0.033 units in stem and 0.133 to 0.138 units in root at 50 DAS, respectively. In similar way, specific activity of NR also increased from 0.016 to 0.019 units in leaves, 0.030 to 0.037 units in stem and 0.133 to 0.146 units in root with spray of 0.50 mM SA (T_5) over the 2.0 mg Cr(VI) kg^{-1} soil treated plants at 50 DAS, respectively (Fig. 1).

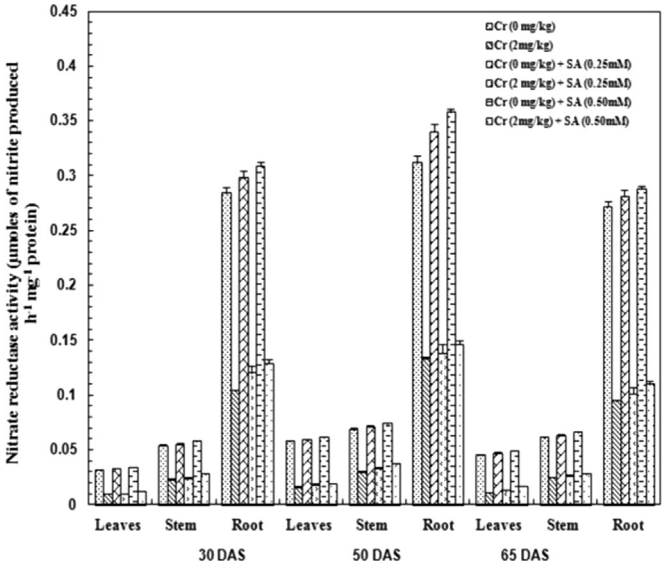

Fig. 1. Effect of SA foliar spray for amelioration of Cr(VI) toxicity on nitrate reductase activity in clusterbean plant parts at different stages of growth.