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Amelioration of chromium phytotoxicity in spinach by withdrawal of chromium or iron application through different modes

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Abstract

To see whether the ill effects of excess Cr can be ameliorated by iron application, spinach (*Spinacea oleracea* L.) cv. Banarasi was grown at normal (control) and excess Cr (dichromate, 0.25 mM). After 14 days of metal supply, the pots of spinach with excess Cr were divided into five lots and different recovery treatments were given i.e. (i) chromium (0.25 mM) + 500 μM (as Fe-EDTA) through roots, (ii) chromium + 1000 μM Fe through spray, (iii) chromium + 250 μM Fe through roots + 250 μM Fe through spray, (iv) chromium − Cr_2 (for 8 days) (v) control + Cr_2 (0.25 mM), with a separate lot of control pots without Cr.

With all these various treatments the recovery from ill effects of chromium was observed but most conspicuous was when iron was supplied through roots (250 μM) and through spray (250 μM) both together. This was reflected by increase in biomass, concentrations of chlorophylls, total and ferrous iron, Hill reaction activity, relative water content and recovery in activity of catalase, peroxidase, ribonuclease and starch phosphorylase along with lowered chromium concentration. When chromium was withdrawn for 8 days from excess chromium treated plants, changes in all parameters was marked in absence of Cr. The removal of Cr drastically lowered the Cr concentration increased chlorophyll and iron concentration, biomass, relative water content and recovery in enzyme activities to large extent.

Keywords: Chromium; Iron; Phytotoxicity; Amelioration; *Spinacea oleracea*

1. Introduction

There are several reports of the apparent stimulation of plant growth by low concentrations (0.05–0.1 ppm) of chromium in solution culture or when added to soil [1–3] but these remain unexplained. Pratt [4] reported stimulatory effects of Cr in plant growth, although they were generally small, inconsistent, and mostly inconclusive. According to Huffman [5] there is insufficient evidence to justify Cr as an essential element or even as a generally beneficial element.

Numerous workers [6,7] have demonstrated to plants associated with high levels of Cr. Recent reviews [4,8] have indicated that 1–5 ppm Cr present in the available form in the soil solution; either as Cr(III) of Cr(VI) is the critical level for a number of plant species. Chromium once absorbed, is poorly translocated in plants, Huffman and Alloway [9] found that Cr absorbed by plants grown in culture solutions remained primarily in the roots and is poorly translocated to the leaves. Myttenaere and Mousny [10] found that >99% of the Cr(III) and Cr(VI) absorbed remained bound in the roots of rice grown in culture solution. Carry et al. reported that the barrier believed to be the cell wall to translocation of Cr from roots tops was not circumvented by supplying Cr in the form of organic acid complexes Cr(III) or Cr(VI) or by increasing the Cr(III) concentration in the nutrient solution. These workers further observed that plants or plant tissues that tend to accumulate Fe also accumulate Cr. Pratt [4] observed that the range of high Cr concentrations in plant tissues before toxicity symptoms could be observed was from about 5 ppm for barley, corn, oats and citrus to 175 ppm for tobacco.

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Concentrations in plant tissues definitely associated with toxicity symptoms are usually in the several hundreds ppm range. For the rice plants, 35–175 ppm of Cr in stem and leaf can cause a 10% reduction in yield [11]. Hunter and Vergnano [12] pointed out that while the toxic effects of most heavy metals (e.g. Ni, Co, Zn, etc.) are associated with high concentration of the element in the leaf tissue, this is not the case with Cr. It was observed that the uptake of several nutrient elements (K, Mg, O, Mn, Ca, Fe) by plants was affected by high Cr levels [13]. Chromium is known to inhibit various enzymatic reactions in vitro [14] although direct cause of Cr toxicity to a specific enzyme system has not been conclusively shown. In this paper efforts are being made to observe the effect of excess Cr on spinach metabolism and whether the toxicity of excess Cr can be ameliorated by sufficient iron application by different modes.

2. Material and methods

Spinach (*Spinacea oleracea* L.) cv. Banarsi, plants were grown in refined sand in glasshouse at an ambient temperature (15–32°) in polyethylene containers (10″), having a central drainage hole, which was covered with an inverted watch glass lined with glass wool. Plants were grown in complete nutrient solution for 77 days growth, composition of the complete nutrient solution was: 4 mM KNO_3; 4 mM $Ca(NO_3)_2$; 2 mM $MgSO_4$; 100 μM ferric ethylene diamine tetra acetic acid, Fe-EDTA; 10 μM $MnSO_4$; 30 μM H_3BO_3; 1 μM $CuSO_4$; 1 μM $ZnSO_4$; 0.2 μM Na_2MoO_4; 0.1 μM $CoSO_4$; 0.1 μM $NiSO_4$ and 0.1 mM NaCl.

Iron was supplied as Fe-EDTA prepared by the method of Jacobson [15]. Pots with two plants in each pot were divided into two lots. Plants in first lot was grown with complete nutrient solution to serve as control, in second lot 0.25 mM Cr were supplied as potassium dichromate for 14 days. On a 15th day spinach plant at each Cr (0.25 mM) level were given the following treatments:

1. Control + Cr (0.25 mM).
2. Control + Cr (0.25 mM) + 500 μM Fe through root.
3. Control + Cr (0.25 mM) + 1000 μM Fe through spray.
4. Control + Cr (0.25 mM) + 250 μM Fe through root + 250 μM through spray.
5. Control + Cr (0.25 mM) − Cr.

There were six replicates in each treatment. Finally on 99th day plants (22nd day after metal supply and 8th day after recovery) were sampled for biomass. On this day, the young leaves of spinach were assayed for the activity of catalase, peroxidase, ribonuclease, starch phosphorylase, concentration of chlorophyll a and b, Hill reaction activity, active iron and relative water content. Chlorophyll concentration was measured by the method of Arnon [16] in the acetone extracts and measured in Milton Roy Spectronic 1201 Spectrophotometer at 663 and 645 nm and the concentration of chlorophyll was expressed as mg chlorophyll per g fresh weight.

In spinach Hill reaction activity was measured colorimetrically at 620 nm as the reaction of 2,4,6-dichlorophenol indophenol (DCPIP) with isolated chloroplast preparation [17] and results were expressed as change on OD per 10 min per 100 mg fresh weight.

At day 99, harvested plants were rinsed with 0.01N HCl, washed under running tap water and finally rinsed with deionized water to avoid any surface contamination. The plants of all treatments were separated into top and root. Further, the plant parts were chopped and dried in an electric oven at 70 °C for 48 h. The concentration of chromium and iron in top and root in spinach were measured by atomic absorption spectrophotometer after diacid digestion (nitric acid:perchloric acid 10:1) [18] and expressed as μg per g dry weight. Homogenates of fresh leaf tissue (20%) was prepared in glass distilled water for enzyme extraction and protein determination by grinding the fresh leaf lamina in chilled pestle and mortar kept in ice bath. The homogenate was filtered with two-fold muslin cloth and the supernatant was stored at 2 °C and used for enzyme assays within 4 h.

The catalase activity (E.C. 1.11.1.6) was measured by the method of Euler and Josephson [19] in 10 ml reaction mixture, standardized against 0.1N $KMnO_4$, containing 0.5 mM H_2O_2 and 1 mM phosphate buffer pH 7.0 was stabilized at 25 °C. The reaction was initiated by adding 1 ml of suitably diluted enzyme extract to the reaction mixture and was allowed to proceed for 5 min after which 5 ml of 2N H_2SO_4 was added to stop the reaction. Corresponding blanks were run simultaneously in which sulfuric acid was added prior to the addition of enzyme extract. The amount of H_2O_2 reduced by the enzyme was determined by titrating the reaction mixture against 0.1N $KMnO_4$. After making the blank corrections, results have been expressed as μmoles H_2O_2 decomposed.

The peroxidase (E.C. 1.11.1.7) was assayed by the method of Luck [20]. The assay system contained 5 ml 0.1 M phosphate buffer (pH 6), 1 ml 0.01% (v/v) H_2O_2 and 1.0 ml 0.5% *p*-phenylanadiamine. The reaction was run by the addition of suitably diluted enzyme extract at 25 °C for 5 min and was stopped with 2 ml 5N H_2SO_4. Blanks with added H_2SO_4 was also taken. After centrifugation, OD of the supernatant was measured at 485 nm. The change in OD of 0.01 per min represents one unit of peroxidase activity.

Ribonuclease (E.C. 3.1.1.22) was assayed by the method of Tuve abd Anfinson [21]. The reaction mixture contained 0.5 ml enzyme extract with 0.1 M citrate buffer (pH 6.0) and 2.5 mg RNA in 2 ml. The reaction was run at 30 °C for 30 min and was stopped by the addition of 0.5 ml 0.75% (w/v) uranyl acetate in 25% perchloric acid. Zero hour blanks were also taken. After centrifugation, OD of the supernatant was measured at 260 nm. The change in OD of 0.01 per min corresponds to a unit of ribonuclease activity.