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Indole acetic acid differently changes growth and nitrogen metabolism in *Pisum sativum* L. seedlings under chromium (VI) phytotoxicity: Implication of oxidative stress

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ABSTRACT

The present study investigated impact of exogenous application of indole acetic acid (IAA; 10 and 100 μM) in pea seedlings under hexavalent chromium (Cr VI; 50, 100 and 250 μM). Cr and 100 μM IAA alone as well as in combination decreased seed germination rate compared to control. However, under Cr phytotoxicity, addition of 10 μM IAA recovered seed germination rate to the level of control. Exposure of pea seedlings to Cr and 100 μM IAA during their early stage caused decrease in fresh mass, length, protein and nitrogen contents of roots and shoots compared to control. Treatment of pea seedlings with Cr resulted in a rapid accumulation of this metal in roots and shoots. Moreover, addition of 100 μM IAA together with Cr, further increased accumulation of this metal in roots and shoots compared to Cr treatments alone. Treatment of pea seedlings with Cr and 100 μM IAA, resulted in a marked decrease in nitrate reductase, nitrite reductase, glutamine synthetase and glutamate synthase (GOGAT) activities (except 50 μM Cr alone for GOGAT), and an increase in ammonium content and glutamate dehydrogenase activity. Parameters related with oxidative stress, i.e. superoxide radicals and reactive carbonyl groups (protein oxidation) were increased by Cr and 100 μM IAA compared to control. By contrast, addition of 10 μM IAA together with Cr, alleviated negative effect of Cr on growth, protein, nitrogen and nitrogen metabolism, and led to decrease in oxidative injuries caused by Cr. The data indicate that 10 μM IAA protects pea seedlings during the early growth period against Cr phytotoxicity by regulating Cr accumulation and oxidative damage. However, addition of 100 μM IAA together with Cr showed opposite responses.

1. Introduction

Heavy metal contamination in soil and water has become a global problem that is leading to loss in crop yield and hazardous effects on human health when these metals enter the food chain (Vernay et al., 2007). Chromium (Cr) is one of the most toxic heavy metals and seventh abundant element on the Earth (Cervantes et al., 2001). Cr compounds are widely used as industrial chemicals and contaminate soil and water thus Cr availability increases (Pandey et al., 2005). In India, several sites are contaminated with Cr that is causing considerable loss to crop yield (Chandra et al., 1997). Cr occurs in several oxidation states ranging from Cr^{2-} to Cr^{6+}. Among various forms of Cr, trivalent Cr (Cr III) and hexavalent Cr (Cr VI) are most stable and common in Cr-polluted substrates (Vernay et al., 2007). Cr (VI) is highly toxic to living organisms because it has ability to pass the membrane, penetrate the cytoplasm and react with the intracellular materials (Gikas and Romanos, 2006). Cr (VI) inhibits seed germination, growth and photosynthesis, and alters water balance and nutrient assimilation (Pandey et al., 2005; Vernay et al., 2007; Schiavon et al., 2008). The toxic effect of Cr (VI) is due to its negatively charged hexavalent Cr ion complexes, which can easily cross cellular membranes by means of sulfate ionic channels. Then these complexes immediately undergo reduction reactions and produce various reactive oxygen species (ROS), thus cause oxidative stress (Vernay et al., 2007; Pandey et al., 2009). ROS are extremely harmful to lipids, proteins and nucleic acids, and cause their oxidation (Pandey et al., 2005, 2009). To mitigate heavy metal induced generation and accumulation of ROS, plants response through changes in the levels of antioxidants (Pandey et al., 2005; Vernay et al., 2007). However, when generation of ROS exceeds ROS scavenging capacity of antioxidants, causes oxidative stress.

Nitrogen is a vital macronutrient which determines growth, development and productivity of plants. For higher plants, the most available form of this element is nitrate (NO_3^-) (Gajewska and Sklodowska, 2009). NO_3^- must be reduced to ammonium

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(NH_4^+) before its incorporation into organic compounds. Reduction of NO_3^- involves successive action of nitrate reductase (NR; EC 1.6.6.1) and nitrite reductase (NiR; EC 1.7.7.1) and produces NH_4^+ ions (Ogawa et al., 2000). NH_4^+ ions are rapidly incorporated into organic compounds by the action of glutamine synthetase (GS; EC 6.3.1.2), glutamate synthase also known as glutamine 2-oxoglutarate aminotransferase (GOGAT; EC 1.4.1.14 for NADH-GOGAT and EC 1.4.7.1 for Fd-GOGAT) and glutamate dehydrogenase (GDH; EC 1.4.1.2) (Masclaux-Daubresse et al., 2006). In the present decade, several studies have been carried out which show negative impacts of cadmium, lead, copper, nickel and salt stress on nitrogen metabolism in various crops (Kevresan et al., 2001; Chaffei et al., 2004; Yu et al., 2005; Debouba et al., 2006; Gajewska and Sklodowska, 2009). However, little is known about impact of Cr (VI) on nitrogen metabolism in pea.

Heavy metal contamination in soil and water is causing considerable decrease in crop yield (Chaffei et al., 2004). Therefore, strategies are needed to alleviate the adverse effects of heavy metal. 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). It has been reported that exogenous application of indole acetic acid (IAA) may alleviate the adverse effects of stress factors (Chakrabarti and Mukherji, 2003; Gangwar et al., 2011). However, other studies have shown that if IAA is applied in excess causes inhibition in growth and tissue damage (de Melo et al., 2004; Wang et al., 2007). Therefore, it would be interesting to know more about the impact of exogenous application of IAA in plants.

In the present study, pea was selected as model organism to investigate the impact of exogenous application of IAA on its growth and various biochemical parameters under Cr (VI) phytotoxicity. Pea is widely cultivated for vegetable and pulse because of its high protein content. To elucidate possible mechanism by which exogenous application of IAA influences Cr (VI) tolerance of pea seedlings, various parameters like seed germination rate, accumulation of Cr, growth, protein and nitrogen contents, nitrogen metabolism and level of oxidative stress were investigated.

2. Materials and methods

2.1. Plant materials and culture conditions

Pea (*Pisum sativum* L. cv. Azad P-1) seeds were procured from National Seed Corporation, New Delhi. Before use, uniform sized seeds were surface sterilized with 10% (v/v) sodium hypochlorite solution for 10 min, washed and soaked in distilled water for 4 h. After sterilization and soaking, healthy looking uniform sized seeds were put in Petri plates (150 mm, Riviera™) lined with Whatman No. 1 filter papers moistened either with half-strength Hoagland's solution only or with Cr and IAA alone and together prepared in half-strength Hoagland's solution (Arditti and Dunn, 1969). After this, seeds were allowed to germinate at 25 ± 2 °C for 3 days in dark. Thereafter, seedlings were grown under a photon flux density (PFD) of 150 μmol photons m^{-2} s^{-1} and relative humidity of 50–60% with a day/night cycle of 12/12 h at 25 ± 2 °C for 8 days in a growth chamber. When the seedlings were 11 days old, root and shoot samples from control and treated seedlings harvested and different parameters were analyzed.

2.2. Cr and IAA treatments

In the present study, 12 combinations of IAA and Cr were made i.e. only half-strength Hoagland's solution (control), 50 μM Cr, 100 μM Cr, 250 μM Cr, 10 μM IAA, 100 μM IAA, 50 μM Cr + 10 μM IAA, 100 μM Cr + 10 μM IAA, 250 μM Cr + 10 μM IAA, 50 μM Cr + 100 μM IAA, 100 μM Cr + 100 μM IAA and 250 μM Cr + 100 μM IAA. Thus, 12 Petri plates were used, one for each combination. Each Petri plate contained 25 healthy and uniform sized seeds. These selected treatments were repeated 4 times. All the Petri plates were kept wet by supplying Hoagland's solution daily or as per requirement to avoid limitation of nutrients.

2.3. Seed germination rate, growth and Cr content

Germination rate of seeds was recorded when control sample (only half-strength Hoagland's solution) reached to maximum level of germination. Germination rate (%) was determined by calculating number of seeds germinated with respect to control. For the determination of growth of pea seedling, ten seedlings from control and treated samples were harvested randomly and then their fresh mass and length of roots and shoots were measured. For the determination of Cr accumulation, control and treated seedlings were divided into roots and shoots. Roots and shoots were washed thoroughly with double distilled water to remove adsorbed culture medium. Oven dried sample of each treatment (50 mg) was digested in tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 ratio) at 80 °C until a transparent solution obtained. After cooling, the digested samples were filtered using Whatman No. 42 filter papers and the filtrate was maintained up to 25 ml with double distilled water. Concentrations of Cr in filtrate of digested samples were estimated using an atomic absorption spectrophotometer (ECIL, 4141). The instrument was calibrated using standard stock solution of Cr.

2.4. Measurements of protein, nitrogen and NH_4^+ contents

For total protein measurement, root and shoot samples (100 mg) from control and treated seedlings were crushed in 10 ml of 50 mM potassium phosphate buffer (pH 6.8) and centrifuged ($8000 \times g$) for 15 min. The supernatant was used for measurement of protein following the method of Lowry et al. (1951) using bovine serum albumin as standard. Total nitrogen content of each sample was estimated using Kjeldahl method (Lang, 1958). NH_4^+ content was determined spectrophotometrically using Nessler's reagent method (Molins-Legua et al., 2006). Root and shoot samples (500 mg) from control and treated seedlings were homogenized in 0.3 mM H_2SO_4 and centrifuged ($20{,}000 \times g$) for 20 min. The supernatant was used for NH_4^+ estimation. The reaction mixture (2.7 ml) contained 0.1 ml extract, 0.1 ml 10% (w/v) potassium sodium tartrate, 2.4 ml double distilled water and 0.1 ml Nessler's reagent. After 5 min of incubation, absorbance of reaction mixtures was recorded at 425 nm. NH_4^+ content was calculated using standard curve prepared with NH_4Cl.

2.5. Assay of enzymes of nitrogen metabolism

For the measurements of NR and NiR activities, method of Debouba et al. (2006) was used. Fresh root and shoot samples (500 mg) were homogenized in 0.1 M potassium phosphate buffer (pH 7.5) containing 5 mM cysteine, 2 mM EDTA and 0.5% (w/v) polyvinylpyrrolidone using mortar and pestle under cool condition. After centrifugation ($20{,}000 \times g$ at 4 °C), the supernatant was used for the determination of NR and NiR activities.

The reaction mixture of NR activity contained 0.1 M potassium phosphate buffer (pH 7.5), 5 mM EDTA, 7 mM KNO_3, 0.14 mM NADH and enzyme extract. The reaction was started by the addition of NADH. After 30 min of incubation at 27 °C, the reaction was stopped by the addition of 0.5 M zinc acetate and then the incubate was centrifuged (at $3000 \times g$) for 10 min. The nitrite (NO_2^-) formed was measured colorimetrically after diazotation with 1% (w/v) sulfanilamide (SA) and 0.01% (w/v) naphthylethylene diamine dihydrochloride (NEDD). After 20 min of incubation