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Research article

Sulphur alters chromium (VI) toxicity in *Solanum melongena* seedlings: Role of sulphur assimilation and sulphur-containing antioxidants

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ABSTRACT

The present study investigates modulation in hexavalent chromium [Cr(VI) 25 μM] toxicity by sulphur (S; 0.5, 1.0 and 1.5 mM S as low (LS), medium (MS) and high sulphur (HS), respectively) in *Solanum melongena* (eggplant) seedlings. Biomass accumulation (fresh and dry weights), photosynthetic pigments, photosynthetic oxygen evolution and S content were declined by Cr(VI) toxicity. Furthermore, fluorescence characteristics (JIP-test) were also affected by Cr(VI), but Cr(VI) toxicity on photosystem II photochemistry was ameliorated by HS treatment via reducing damaging effect on PS II reaction centre and its reduction side. Enhanced respiration, Cr content and oxidative biomarkers: superoxide radical, hydrogen peroxide, lipid peroxidation and membrane damage were observed under Cr(VI) stress. Though Cr(VI) enhanced adenosine triphasphate sulfurylase (ATPS) and *o*-acetylserine(thiol)lyase (OASTL), glutathione-S-transferase (GST), glutathione reductase (GR) and ascorbate peroxidase (APX) activity, and content of total glutathione, cysteine and NP-SH, however, their levels/activity were further enhanced by S being maximum with HS treatment. The results show that Cr(VI) toxicity does increase under LS treatment while HS protected Cr(VI)-induced damaging effects in brinjal seedlings. Under HS treatment, in mitigating Cr(VI) toxicity, S assimilation and its associated metabolites such as cysteine, glutathione and NP-SH play crucial role.

1. Introduction

Chromium is a naturally occurring toxic metalloid with chromium (III) and chromium (VI) oxidation states. Among them, Cr(VI) is more mobile and carcinogenic and/or mutagenic for human beings (Hu et al., 2016). Chromium as well as other heavy metals is non-biodegradable in nature and thus, they persist in the environment forever via entering into the food chain as a result of their uptake by plants (Ghani et al., 2015). Cr(VI) is heavily produced in metal mining, smelting, battery manufacturing, paper and pulp production, chrome plating, pigments, dyes, wood preservation, pesticides, tanning of animal skins, and fertilizers (Marieschi et al., 2015; Singh et al., 2015a; Hu et al., 2016). In plants, its uptake depends on oxidative state of Cr, pH and concentration of dissolved salts in aqueous media (Babula et al., 2008). In agricultural soil, 10–50 mg Cr kg^{-1} has been reported and in serpentine soils its concentration may reach up to 125 g Cr kg^{-1} (ATSDR, 2012; Bashri

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Abbreviations: ABS/RC, the energy fluxes for absorption of photon per active RC; APX, ascorbate peroxidase; ATPS, adenosine triphasphate sulfurylase activity; CAT, catalase; DI_0/RC, energy dissipation flux per active RC; ET_0/RC, electron transport flux per active RC; F_v/F_m or Phi_P_0 or φP_0, quantum yield for primary photochemistry; GST, glutathione-*S*-transferase; GR, glutathione reductase; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; NP-SH, non-protein thiols; OASTL, *o*-acetylserine(thiol)lyase; Phi_E_0 or φE_0, quantum yield of electron transport; PI_{ABS}, performance index of PS II; POD, peroxidase; PS II, photosystem II; Psi_0 or Ψ_0, yield of electron transport per trapped excitation; RC, reaction center; ROS, reactive oxygen species; SOD, superoxide dismutase; $O_2^{\cdot-}$, superoxide radical; TR_0/RC, trapped energy flux per active RC.

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et al., 2016).

Several symptoms of Cr(VI) phytotoxicity have been reported by researchers such as restricted seed germination, leaf chlorosis and altered photosynthesis, respiration, transpiration, sulphur and nitrogen metabolism, etc. (Sharma et al., 1995; Bashri et al., 2016). Furthermore, damaging effect of Cr(VI) leads to overproduction of reactive oxygen species (ROS) in the plant system. The metal stress-induced overproduction of ROS causes imbalance antioxidants and oxidants i.e. ROS leading to lipid peroxidation and membrane damage by damaging proteins and lipids (Gill and Tuteja, 2010; Singh et al., 2015a). To counteract damaging effect of ROS, plants have developed two important antioxidant defense mechanisms that can be classified as enzymatic (SOD, POD, CAT, GST, GR, APX, etc.) and non-enzymatic (ascorbate, glutathione, cysteine, NP-SH, etc.) antioxidant systems, which scavenge and/or reduce excess ROS and improve performance of plants by counteracting toxic ROS (Gill and Tuteja, 2010; Singh et al., 2015a).

Cr(VI) may affect uptake and translocation of essential macro and micronutrients in plants (Dube et al., 2003; Oliveira, 2012). Among nutrients, S is taken up by plant through two processes, one is from growth medium and other is through air (SO_2) via stomata thereafter, it is converted into sulphur containing amino acids or sulfate (Rausch and Wachter, 2005; Hu et al., 2015). It is a vital macronutrient needed by plants (Marschner,1959; Nazar et al., 2011; Mera et al., 2014; Marieschi et al., 2015). Plant growth and development are largely regulated by sulphur. Unfortunately, there is an increasing problem of S deficiency in agricultural soil (Hawkesford, 2000; Muneer et al., 2014). It involves in plant defence mechanisms by producing cysteine-rich peptides such as reduced glutathione (GSH), phytochelatins (PCs) and metallotioneins (MTs) (Marieschi et al., 2015). Assimilation of S begins with sulfate, utilizing activities of ATP sulfurylase and *O*-acetylserine (thiol) lyase (OASTL) to form cysteine (Rausch and Wachter, 2005; Noctor et al., 2012; Hu et al., 2015; Liang et al., 2016).

Cr(VI) mediated generation of ROS may be reduced by sulphur containing amino acids (cysteine, NP-SH), non-enzymatic antioxidant (glutathione) and enzymatic antioxidant (GST, GPX, etc.) using S containing metabolites in scavenging of ROS, which collectively help in evolving metal chelation and ROS scavenging potential in the plant (Zhang et al., 2011; Mera et al., 2014; Liang et al., 2016). Studies showed that sulphur enhances tolerance in plants under different biotic and abiotic stresses (Nazar et al., 2011; Mera et al., 2014; Marieschi et al., 2015; Fang et al., 2016; Liang et al., 2016), but in plants mechanisms involved in S-mediated reduction of Cr(VI) toxicity are still poorly known.

Vegetables are important constituent of human diet. They serve as a source of several vitamins and minerals. Among different vegetable crops, *Solanum melongena* L. (brinjal) is an important vegetable crop, grown worldwide (Sihachakr et al., 1994). Keeping in view diverse physiological roles played by sulphur, present study has investigated the regulation of Cr(VI) toxicity in brinjal by analyzing biomass accumulation, photosynthetic activities, PS II photochemistry (JIP-test), sulphur metabolism, enzymes involved in oxidative defence system. Further, this study also investigated about mechanisms which involved in the regulation of Cr(VI) toxicity by sulphur nutrition.

2. Material and methods

2.1. Plant material and growth conditions

Seeds of *Solanum melongena* L. var. Neelam (brinjal) were purchased from certified supplier of local market. Healthy seeds were surface sterilized in 2% (v/v) sodium hypochlorite solution for 5 min and then washed thoroughly and soaked in distilled water for 2–4 h. After surface sterilization and soaking, uniform sized sprouted seeds were sown in plastic trays containing sterilized sand. Subsequently, trays were kept in the dark for seed germination at 25 ± 2 °C. When germination reached maximum, seedlings were grown in growth chamber (CDR model GRW-300, DGe, Athens) under photosynthetically active radiation (PAR) of 350 μmol photons m^{-2} s^{-1} and 60% relative humidity with 16:8 h day-night regime at 26 ± 1 °C for 20 days, until the secondary leaves emerged.

2.2. Sulphur and Cr(VI) treatments

Twenty days old uniform sized seedlings having secondary leaves softly up rooted from plastic tray and their roots were washed in tap water. After that, seedlings were acclimatized in half strength Hoagland's nutrient medium for 24 h. Thereafter, Cr(VI) and sulphur treatments were given. The 25 μM (1.29 mg/L) of Cr(VI) as $K_2Cr_2O_7$ was selected for the present study. Treatments include low sulphur (LS, 0.5 mM equal to 16 mg/L), control (MS, 1 mM equal to 32 mg/L S present in normal half strength Hoagland medium), high sulphur (HS, 1.5 mM equal to 48 mg/L), 25 μM Cr(VI) + LS, 25 μM Cr(VI) + control (MS), 25 μM Cr(VI) + HS. In case of Cr(VI) + S treatments, seedlings were pretreated with Cr(VI) for 24 h and then treated with S. During the treatment three uniform weighed plants were transferred in each glass bottle containing 10 ml of half strength Hoagland nutrient medium solution supplemented with selected concentrations of Cr(VI) and S. For control treatment plants were placed in only 10 ml of half strength Hoagland solution with MS and without Cr(VI). Just after Cr(VI) and S treatments, seedlings were further grown for 7 days in growth chamber. After 7 days of treatments, seedlings harvested and various parameters were analyzed.

2.3. Estimation of growth

Growth of tested seedlings was measured in terms of fresh and dry weight, shoots and roots length of plant. Six seedlings were selected randomly from control and treated samples, divided into root and shoot and then their fresh weight was measured. The length of roots and shoots was measured by using a meter scale and subsequently weighed to record fresh weight of roots and shoots by using single pan digital balance (Model CA 223, Contech, India). After taking fresh weight of treated and untreated plant sample it dried in an oven maintained at 70 °C for 48 h before recording dry weight.

2.4. Estimation of chlorophylls and carotenoids

Photosynthetic pigments were estimated as per procedure of Lichtenthaler (1987).

2.5. Estimation of Cr and S contents

Estimation of elements was performed as procedure described by Allen et al. (1986).

2.6. Estimation of photosynthetic oxygen evolution and respiration rate

Photosynthetic oxygen evolution (photosynthesis) and respiration were determined in terms of oxygen evolution/consumption in presence and absence of light, respectively as described by Kurra-Hotta et al. (1987).