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Mannitol alleviates chromium toxicity in wheat plants in relation to growth, yield, stimulation of anti-oxidative enzymes, oxidative stress and Cr uptake in sand and soil media

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ABSTRACT

Chromium (Cr) is one of the most phytotoxic metals in the agricultural soils and its concentration is continuously increasing mainly through anthropogenic activities. Little is known on the role of mannitol (M) on plant growth and physiology under metal stress. The aim of this study was to investigate the mechanism of growth amelioration and antioxidant enzyme activities in Cr-stressed wheat (*Triticum aestivum* L. cv. Lasani 2008) by exogenously applied mannitol. For this, wheat seedlings were sown in pots containing soil or sand and subjected to increasing Cr concentration (0, 0.25 and 0.5 mM) in the form of of $K_2Cr_2O_7$ with and without foliar application of 100 mM mannitol. Plants were harvested after four months and data regarding growth characteristics, biomass, photosynthetic pigments, and antioxidant enzymes were recorded. Mannitol application increased plant biomass, photosynthetic pigments and antioxidant enzymes while decreased Cr uptake and accumulation in plants as compared to Cr treatments alone. In this study, we observed that M applied exogenously to Cr-stressed wheat plants, which normally cannot synthesize M, improved their Cr tolerance by increasing growth, photosynthetic pigments and enhancing activities of antioxidant enzymes and by decreasing Cr uptake and translocation in wheat plants. From this study, it can be concluded that M could be used to grow crops on marginally contaminated soils for which separate remediation techniques are time consuming and not cost effective.

1. Introduction

Globally wheat (*Triticum aestivum* L.) is economically one of the most important food crop and it ranks third most produced cereal crop after maize and rice worldwide (FAO, 2012). In Pakistan, wheat production was 24.21 million tons from an area of 8.66 million hectares in 2013 (Siddiqui, 2014). World population is increasing at an alarming rate and to feed the increasing population, more food is required so as a result wheat demand is increasing worldwide. This ever demanding can be fulfilled either by bringing more area under wheat cultivation or by introducing high yielding wheat varieties resistant to environmental stresses especially toxic metals.

Increased heavy metal contamination of agricultural soils has severe damaging effects on crops grown in these soils (Kanwal et al., 2014; Adrees et al., 2015a; Anwaar et al., 2014; Habiba et al., 2015; Rehman et al., 2015). Heavy metal stress in plants is characterized by the decrease in photosynthesis, nutrient uptake, damaging of roots and finally plant death (Ali et al., 2011; Rizwan et al., 2012; Shakoor et al., 2014; Gill et al., 2015; Zaheer et al., 2015). Among heavy metals, chromium (Cr) toxicity in crops has become a serious problem, especially in developing countries like Pakistan. Chromium is released into the soil environment mainly from the leather tanning (Shakir et al., 2012; Daud et al., 2014; Afshan et al., 2015) along with other anthropogenic activities such as mining and electroplating etc (Oliveira, 2012; Ali et al., 2013a; Yadav and Singh, 2013; Noman et al., 2015). However, plants growth is decreased under higher level of Cr stress and induced physio-chemical and ultrastructural changes in plants depending upon plant species (Gill et al., 2015). Chromium can reach human beings through food chain which can cause many harmful diseases in humans (Iyer and Mastorakis, 2010).

Higher concentrations of Cr in plants can lead to alterations in

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the morphology and physiology of plants due to overproduction of reactive oxygen species (ROS) from hydroxyl (OH^-) and superoxide radicals (O^{2-}) and hydrogen peroxide (H_2O_2) which cause oxidative damage in plants (Ali et al., 2012; Gill et al., 2015). Plants have developed specific adaptive mechanisms to overcome metal-induced oxidative stress through antioxidants such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). These antioxidant enzymes may be induced in order to enhance plant resistant against oxidative stress caused by potentially toxic metals (Ali et al., 2013b; Gill et al., 2015). Catalase regulates intracellular H_2O_2 levels in plants affected by SOD catalyzed reactions and APX catalyzes H_2O_2-dependent substrate oxidation and is also a useful biomarker for metal toxicity in plants (Blokhina et al., 2003; Podazza et al., 2012). Previously, we have identified that these antioxidant enzymes may play an important role in the defensive mechanism of crop plants against Cr and other heavy metals (Bharwana et al., 2014; Ehsan et al., 2014; Ali et al., 2015a). Under severe stress conditions, the antioxidant capacity of plants may not be sufficient to prevent toxic effects of metal stress which decreased plant growth and yield (Gill et al., 2015).

A number of strategies are used to enhance metal tolerance in economically important crops such as wheat (Adrees et al. 2015b; Ali et al., 2015a; Arshad et al., 2015; Keller et al., 2015). These may include the production of metal-tolerant crops through conventional breeding and genetic engineering for the production of enhanced levels of different organic solutes. Some other shotgun approaches are also in practice to induce metal tolerance in plants including the exogenous application of some organic and inorganic compounds through different methods (Ashraf and Foolad, 2007; Ali et al., 2015a, b). In response to a variety of stresses, plants build up low molecular weight compatible solutes, osmolytes, and sugar alcohols like mannitol (Rajam et al., 1998). Mannitol is an important osmolyte and is normally synthesized in numerous plant species but not in wheat (Abebe et al., 2003; Mitoi et al., 2009). It plays an important role in storage of energy and carbon, osmoregulation and regulation of coenzymes (Pharr et al., 1995). Mannitol plays a major function as an antioxidant due to its ability to search free radicals (Tandon et al., 2003; Yu et al., 2003). It has been reported that the performance of a mannitol-accumulating transgenic plant enhanced because of the searching of reactive oxygen, rather than osmoregulatory effects, as the plant did not build up adequate M to maintain the osmotic potential (Abebe et al., 2003; Khare et al., 2010). It has also been reported that M plays a fundamental role in reducing osmotic and salinity induced stresses in many plants species (Tang et al., 2005; Bhauso et al., 2014).

The function of M in metal stress tolerance has not been evaluated in plants of agronomic importance such as wheat. More precisely, the function of exogenously applied M has not been evaluated in wheat plants, which cannot produce M, under Cr stress despite the fact that wheat is more sensitive to Cr toxicity as compared to other crops (Dey et al., 2009; Diwan et al., 2012). Thus, the present work was done to study whether exogenous application of M to wheat plants could improve growth and photosynthetic pigments and upto what extent M could alter the activities of some key antioxidant enzymes involved in counteracting the ROS production in wheat plants under Cr stress.

2. Materials and methods

2.1. Plant materials and growth conditions

The wheat plants were sown in two growth mediums including sand and soil. The soil was a clay loam (27% sand, 20% silt, 53% clay) collected at 0–15 cm depth from botanical garden of government college university Faisalabad, Pakistan. The soil was air dried under room temperature and passed through 2 mm mesh and then thoroughly mixed and characterized (Table 1). Sand was also collected from botanical garden of government college university Faisalabad, Pakistan and washed thoroughly with distilled water, air-dried and passed through 2-mm mesh. Each pot was filled with 5 kg of sand or soil separately.

Table 1
Properties of soil used for the pot experiment.

Physicochemical properties	
Texture	Clay loam
Sand (%)	27
Silt (%)	20
Clay (%)	53
pH (1/2.5 soil to water ratio)	6.7
EC_e (dS m^{-1})	2.9
SAR $(mmol^{-1})^{1/2}$	6.5
Organic matter (%)	0.31
Available P (mg kg^{-1})	2.17
HCO_3 (mmol L^{-1})	3.55
Cl^- (mmol L^{-1})	2.34
$SO_4{}^{2-}$ (mmol L^{-1})	6.67
$Ca^{2+}+Mg^{2+}$ (mmol L^{-1})	3.5
Na^{2+} (mmol L^{-1})	3.7
K^+ (mmol L^{-1})	0.06
Available Cu^{2+} (mg kg^{-1})	0.35
Available Zn^{2+} (mg kg^{-1})	0.85

The wheat (Triticum aestivum L. cv. Lasani, 2008) seeds were surface sterilized with 3% H_2O_2 for 10 minutes and then thoroughly washed with double distilled water. According to Punjab Seed Corporation of Pakistan (PSC, 2008), yield potential of the wheat variety used in this study was about 6.75 t per hectare and average yield production was 4.69 t per hectare. Recently, this variety is cultivated in many parts of the country and has strong resistance against diseases and pests. The pot experiment was conducted in a botanical garden at 18–25 °C and 70% humidity at the time of sowing and 30–35 °C and 85% humidity at the time of harvesting. Pots were initially seeded at a density of twenty seeds per pot, then thinned to fifteen individuals per pot after fifteen days of germination and the pulled up plants were crushed carefully into the same pot. All treatments were performed in three replicates. Each soil pot was fertilized with a 500- ml solution containing 2.19 g L^{-1} N (as $(NH_2)_2CO$), 0.5 g L^{-1} P (as $(NH_4)_2HPO_4$) and 2.14 g L^{-1} K (as K_2SO_4). Half of the fertilizer solution was applied after 15 days of germination and remaining half was applied after 30 days of germination. Each sand pot was fertilized with modified Hoagland solution (Sigma) containing following macro elements: 0.2 M $Ca(NO_3)_2$, 0.09 M $MgSO_4$, 0.4 M KH_2PO_4, 0.01 M $FeSO_4$, 0.3 M KNO_3, and micro elements: 0.4 mM $CuSO_4$, 1.4 mM $ZnSO_4$, 0.5 mM H_3BO_3, 10 mM H_2MoO_4 when required (Ali et al., 2015b). All plastic and glass wares were rinsed with 10% HNO_3 and then washed with distilled water till neutral pH.

2.2. Treatments

After 30 days of germination, both soil and sand pots were exposed to increasing concentrations of Cr, 0, 0.25 and 0.5 mM, in the form of $K_2Cr_2O_7$ (Sigma) solution (500 ml pot^{-1}) at 7-days interval, respectively, with three replications. The M solution was foliar applied, at 0 and 100 mM, on the leaves until runoff at both tillering and booting vegetative stages. Mannitol concentration was selected based on the literature (Seckin et al., 2009). The experiment was designed as a complete randomized design (CRD). Pots were regularly rotated and weeds were removed when present.