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Phyto-Toxicity of Chromium in Maize: Oxidative Damage, Osmolyte Accumulation, Anti-Oxidative Defense and Chromium Uptake

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ABSTRACT

Agricultural production systems are immensely exposed to different environmental stresses in which heavy metal stress receives serious concerns. This study was conducted to explore the deleterious effects of different chromium (Cr) stress levels, *i.e.*, 0, 30, 60, 90, 120, and 150 µmol L^{-1}, on two maize genotypes, Wandan 13 and Runnong 35. Both genotypes were evaluated by measuring their growth and yield characteristics, Cr accumulation in different plant tissues, alterations in osmolyte accumulation, generation of reactive oxygen species (ROS), and anti-oxidative enzyme activity to scavenge ROS. The results showed that Cr stress decreased the leaf area, cob formation, 100-grain weight, shoot fresh biomass, and yield formation, while Cr accumulation in different maize tissues was found in the order of roots > leaves > stem > seeds in both genotypes. The increased Cr toxicity resulted in higher free proline, soluble sugars and total phenolic contents, and lower soluble protein contents. However, enhanced lipid peroxidation was noticed in the forms of malondialdehyde, hydrogen peroxide (H_2O_2) and thiobarbituric acid reactive substance accumulation, and electrolyte leakage. The hyperactivity of superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, especially glutathione peroxidase and glutathione reductase indicated that these anti-oxidative enzymes had a central role in protecting maize from Cr toxicity, especially for Wandan 13. Moreover, higher uptake and less translocation of Cr contents into the grains of Wandan 13 implied its importance as a potential candidate against soil Cr pollution.

Key Words: agronomic characteristics, anti-oxidative enzyme activity, Cr accumulation, Cr translocation, heavy metal stress, reactive oxygen species

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INTRODUCTION

Heavy metals normally enter into the food chain through plant uptakes and are accumulated and passed to the end consumers, which eventually leads to numerous health issues (Wang and Chen, 2009). The rapid development in industries and their disposal, release of xenobiotic pollutants, and use of wastewater for irrigation purposes pose serious threats to agro-ecosystem and cause food quality constraints (Wang *et al.*, 2013). Chromium (Cr) is one of the most common heavy metals accumulating in soil, ground water, and sediments due to its wider industrial usage, hence receiving serious concerns about global environment, food production systems, animals, plants, and human health (Farid *et al.*, 2013).

In general, Cr(VI) is believed to be more toxic than Cr(III) due to its higher oxidizing potential and solubility, which increase its availability to the crop plants and thus severely reduce plant growth and overall productivity. Whereas Cr(III) is less harmful and immobile owing to its bounding with organic matter in soil, which makes it less available to the plants (Ranieri *et al.*, 2013).

The uptake of Cr(VI) from rhizosphere by plants generally occurs along with the uptake of water and nutrients, which consequently leads to changes in numerous morpho-physiological and biochemical processes (Dey *et al.*, 2009; Ranieri and Gikas, 2014). Soil pH, salinity, availability of soluble salts, and the concentration of Cr in soil solution are the main factors affecting the uptake of Cr from the soil (Babula *et al.*,

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2008). After reaching in roots, only a small fraction of absorbed Cr is translocated to the aboveground plant parts (Rodriguez *et al.*, 2012).

Cr-induced oxidative stress may lead to severe phyto-toxic effects in plant. For example, high concentrations of reactive oxygen species (ROS) at cellular level lead to oxidative damage (Anjum *et al.*, 2011a, b, c), which explains the majority of the optic Cr toxicity symptoms detected at whole plant level under Cr stress (Gill *et al.*, 2015). Hyper generation of ROS can lead to significant damages to cell structures in plants such as oxidation of proteins and lipids, nucleic acid damage, enzyme inhibition, and ultimate cell death (Pérez-Pérez *et al.*, 2012; Adrees *et al.*, 2015). Thus, it is indispensable that plants possess protective mechanisms to scavenge ROS through anti-oxidative defense system. Some previous studies (Khaliq *et al.*, 2015) have explored the roles of various anti-oxidants under stress conditions. For example, increased activities of superoxide dismutase (SOD) is contemplated as the primary step to scavenge ROS at cellular levels, while defensive roles of ascorbate peroxidase (APX) and catalase (CAT) against over production of H_2O_2 have also been reported. However, under enhanced stress levels, scavenging abilities of anti-oxidative enzymes may not be enough to alleviate the phyto-toxic effects of heavy metals on plants that reduce plant growth and yield formation (Farooq *et al.*, 2013; Ashraf *et al.*, 2015).

Although Cr is toxic to plants even at low concentrations, nonetheless, plant response to soil Cr is species-dependent and genotype-specific. Furthermore, different plant species show differential uptake and translocation properties. Some plants prefer roots as a major storage organ and transport a little toward shoots, whereas others accrue more in shoots than roots showing the sensitivity and tolerant abilities (Yang *et al.*, 2010; Fahad *et al.*, 2015). Research on various crops like *Hordeum vulgare* L. (Wu and Zhang, 2002), *Triticum aestivum* L. (Zhang *et al.*, 2002), *Vigna radiata* (Jabeen *et al.*, 2015), *Brassica napus* L. (Afshan *et al.*, 2015), and *Oryza sativa* L. (Cheng *et al.*, 2006) suggests that there is need to exploit Cr-tolerant genotypes accumulating minimum metal contents in the edible portion. Still a little is known about the exact mechanisms behind Cr tolerance; however, uptake and storage potential of Cr within plant tissues might be used as a major tool to unravel the complex mechanisms involved in Cr tolerance and as selection criteria for Cr-sensitive/tolerant genotypes under Cr-tainted environment.

Maize (*Zea mays* L.) is an important cereal cultivated over the world under diverse types of climate and soil. After USA, China ranked second worldwide regarding its production and consumption (Gale *et al.*, 2014). Maize has a great potential regarding phyto-extraction, bio-accumulating abilities, and variable soil-plant metal transfer rates (Wuana and Okieimen, 2010). Previously, Singh *et al.* (2015) confirmed the Cr-induced reductions in plant growth and yield, emulation of oxidative stress, membrane damage, lipid peroxidation, and anti-oxidative defense, but contradictions still exist among researchers. For example, Dey *et al.* (2009) and Ali *et al.* (2015b) found significant reductions in the activities of anti-oxidants at enhanced levels of Cr stress, while the converse was true in Khan *et al.* (2008) and Gill *et al.* (2015). This study was, therefore, conducted to study the morpho-physiological aspects of Cr tolerance in maize and the distribution pattern of Cr uptake in maize plants. That is to say, Cr accumulation in different plant tissues (roots, stem, leaves, and grains) and its consequences on morpho-physiological attributes, productivity, ROS production, and anti-oxidative defense system of maize were assessed.

MATERIALS AND METHODS

Experimentation, plant sampling and Cr determination

A pot experiment was conducted in a wire house during the summer season of 2013 at College of Agronomy and Biotechnology, Southwest University of China (29°49′32″ N, 106°26′02″ E, 220 m above sea level). Healthy, homogenous, and pure seeds of two maize genotypes, Wandan 13 and Runnong 35, were purchased from the Chongqing Guoben Seed Company and Sichuan Dragon Seed Company of China, respectively. Seeds of both genotypes were surface-sterilized with 0.1% (volume:volume) sodium hypochlorite and then rinsed with distilled water. Firstly, two seeds per hill were sown in polyvinyl chloride nursery trays, and then 15-d old seedlings were transplanted into plastic pots (34 cm diameter × 24 cm depth) filled with air-dried sandy loam soil with total N 2.14 g kg^{-1}, total P 3.66 g kg^{-1}, total K 9.39 g kg^{-1}, organic matter 15.52 g kg^{-1}, total Cr 48.05 mg kg^{-1}, and pH 6.42. The seedlings were allowed to grow in the pots under normal conditions for 20 d after transplanting and then received 30, 60, 90, 120, and 150 μmol L^{-1} of Cr solution applied as $CrCl_3$, while the uncontaminated pots (0 μmol L^{-1} Cr) served as the control. All pots were kept at 30 ± 1 °C temperature, 80% relative humidity, and 14 h photoperiod. The pots were arranged in a ra-