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RESEARCH ARTICLE

Alleviative role of exogenously applied mannitol in maize cultivars differing in chromium stress tolerance

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Abstract

A pot experiment was performed to examine the role of foliar applied mannitol (M) in chromium (Cr) stress alleviation in different maize cultivars. Two maize cultivars, one tolerant (6103) and one sensitive (9108) to chromium stress, were grown in soil treated with three concentrations of Cr (0, 5, and 10 mg kg^{-1}) and three levels of mannitol (0, 50, and 100 mg L^{-1}). Chromium stress decreased the overall growth of plants by reducing the plant height, root/shoot dry weight, chlorophyll contents, and enzymatic activities, while exacerbated the severity of reactive oxygen species in both maize cultivars. Chromium-induced reduction in growth attributes of maize plants was relatively higher in sensitive cultivar than that of tolerant one. Uptake of Cr by the plants and its translocation from roots to shoots increased with increasing concentration in the soil. However, foliar application of mannitol significantly alleviated the Cr stress and improved growth, biomass, and photosynthetic pigments of maize plants. Mannitol also considerably reduced Cr contents in leaves and roots of both cultivars. Hence, it is concluded that mannitol can be helpful for crops grown on heavy metal, especially Cr, contaminated soils for remediation purpose.

Keywords Mannitol · Maize · Chromium · Alleviation · Foliar application

Introduction

During past few decades, heavy metals have played a key role in deteriorating the quality of environment because of rapid urbanization, population growth, and industrialization (Farid et al. 2015; Rizwan et al. 2017a). In developing countries, the industrial effluent is often used to irrigate the agricultural soils due to shortage of freshwater. This industrial effluent with heavy metal contamination poses highly poisonous impacts on fauna and flora (Júnior et al. 2015; Sood et al. 2012). The accumulation of heavy metals in edible portions of plants, especially cereals, poses a major threat to human life (Anjum et al. 2016; Wang et al. 2017). These heavy metals have deleterious impacts on growth, photosynthesis, and antioxidant enzyme activities in plants (Anjum et al. 2017; Singh et al. 2017). Chromium is a nonessential and potentially deleterious heavy metal having no metabolic function in plants (Kamran et al. 2016). Chromium has 7th rank in the list of most abundant element and 21st in most abundant heavy metal in Earth's crust with specific density of 7.19 g/cm^3 (Economou-Eliopoulos et al. 2013). According to the International Agency for Research on Cancer, Cr is considered as no. 1 carcinogen (IARC 1987). Ashraf et al. (2016) reported that Cr has different oxidation states ranging from −2 to +6, but the most stable and common states present in the environment are trivalent [Cr(III)] and hexavalent chromate [Cr(VI)]. Both states show different behavior with respect to their bioavailability and sorption in soil, translocation and absorption in aboveground biomass, and toxicity inside the plant (Choppala et al. 2016). Several studies have illustrated the toxic effects of Cr on morphophysiological and biochemical processes of plants (Jabeen et al. 2016; Kamran et al. 2016). Recent

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studies reported that Cr toxicity severely affected the agronomic traits, photosynthetic pigments, gas exchange characteristics, ultrastructure of cell chloroplast and membrane, as well as proteomic and miRNA expression (Ali et al. 2015; Bukhari et al. 2016a, b). Several plant- and soil-related factors define the transfer of Cr from soil to plant, such as plant species and genotype, gas exchange attributes, root surface area, soil pH, texture, and electrical conductivity (Islam et al. 2016).

Maize (*Zea mays* L.), an important cereal crop, is one of the main staple foods and is widely cultivated under varying soil and climatic conditions all over the world. It is an important constituent of animal as well as human nutrition (Rosas-Castor et al. 2014). Maize is an important industrial crop which is used for ethanol manufacturing (Shahzad et al. 2016). China and USA are two major candidates for maize consumption (Gale et al. 2016). Maize is capable of bioaccumulation of metals from growth medium, with greater phytoextraction potential and higher transfer rates (Wuana and Okieimen 2010). According to FAOSTAT (2013), the existing production of maize is about 0.250 billion tons per annum. The demand for maize, only in East and Southeast Asia, is anticipated to reach around 0.291 billion tons in 2020 (Rosegrant et al. 2001).

Plants can fight against various biotic and abiotic stresses with the help of different organic solutes including polyols, oligosaccharides, and proline. Mannitol, a common natural polyol, is a key osmolyte synthesized by many plants which plays a pivotal role in carbon and energy storage and regulation and osmoregulation of coenzymes (Mitoi et al. 2009; Pharr et al. 1995). Because of the ability to scavenge the reactive oxygen species (ROS) and free radicals, mannitol is considered as an important antioxidant (Tandon et al. 2003; Khare et al. 2010). Mannitol is reported to have significant role in reducing salinity and osmotic stress in various plants (Bhauso et al. 2014) and maintaining the cell turgor pressure (Siringam et al. 2011). Mannitol has been reported to play an important role in stabilizing the membrane and protein structure, scavenging the ROS, maintaining the photosynthetic apparatus, and osmoprotection in various species under abiotic stress (Chan et al. 2011). Mannitol plays a vital role in salt stress tolerance (Sickler et al. 2007) and carbon utilization efficiency (Keunen et al. 2013).

The present work was designed to examine the ameliorative impact of mannitol on maize cultivars, grown under Cr stress, in terms of growth, photosynthetic pigments, and antioxidant enzymatic activities.

Materials and methods

Soil sampling and analysis

The soil samples, collected from agricultural field, were properly mixed and sieved by 2-mm sieve. Standard methods were employed to analyze the soil pH and EC (Soltanpour 1985), soil organic matter (Jackson 1962), and soil texture (Abbas et al. 2017). The physicochemical properties of soil are given in Table 1.

Pot experiment

A pot experiment was performed in the Botanical Garden of GCUF on two maize cultivars, one tolerant (6103) and the other sensitive (9108) to chromium stress (data not shown). The trial was run using completely randomized design (CRD) having six replicates. Stress was induced by using three levels of chromium (Cr VI) (0, 5, and 10 mg kg^{-1}soil) as $K_2Cr_2O_7$. Three concentrations of M (0, 50, 100 mg L^{-1}) were selected for foliar application. Every pot was filled with 6 kg soil. The pots were irrigated until they became saturated. When the soil was fully moisturized, the seeds of maize were sown uniformly by hand. After 15 days of germination, thinning was done to maintain two plants per pot. Mannitol was sprayed four times during the experiment. First spray was done after 1 week of germination; 2nd, 3rd, and 4th spray was applied after 2, 4, and 6 weeks of sowing, respectively; and Tween 80 was used as sticking agent. The pots were covered to avoid soil contamination during spray and rotated randomly throughout the growth period. After 2 weeks of germination, the N, K, and P fertilizers were applied at the rate of 120, 25, and 50 kg ha^{-1}, respectively. Urea, potassium sulfate, and diammonium phosphate fertilizers were used as sources of N, K, and P, respectively.

Table 1 Soil physiochemical properties

Texture	Sandy clay loam
Sand (%)	52.0
Silt (%)	24.0
Clay (%)	24.0
ECe (dS m^{-1})	2.86
pH (1/2.5 soil to water ratio)	7.65
Organic matter (%)	0.34
SAR ($mmol^{-1}$)1/2	5.60
HCO_3 (mmol L^{-1})	3.68
Available P (mg kg^{-1})	2.16
SO_4^{2-} (mmol L^{-1})	6.48
Cl^- (mmol L^{-1})	2.19
K^+ (mmol L^{-1})	0.03
Na^{2+} (mmol L^{-1})	3.48
Ca^{2+} + Mg^{2+} (mmol L^{-1})	3.69
Available Zn^{2+} (mg kg^{-1})	0.72
Available Cu^{2+} (mg kg^{-1})	0.23
Available Cr^{+6} (mg kg^{-1})	0.17

Growth traits

Plants were harvested after 10 weeks of treatment. The growth characteristics, such as leaf area, number of leaves per plant, root length, and plant height, were measured on fresh plant samples. The roots and shoots were separated, followed by oven drying at 70 °C to measure their dry biomass.

Chlorophyll and carotenoid contents

After 8 weeks of treatment, fresh leaf samples were harvested to measure chlorophyll (a, b, total chlorophylls) and carotenoid contents according to the methodology given by Lichtenthaler (1987).

Electrolyte leakage, H_2O_2, MDA, and antioxidant enzymes

After 8 weeks of treatment, the electrolyte leakage (EL), H_2O_2, MDA, and antioxidant enzymatic activities were measured on plant leaves. The samples were autoclaved at 32 °C for 2 h, and EC_1 was noted. The same samples were again autoclaved at 121 °C for 20 min to measure EC_2 thereafter. Then, EL was calculated as described by Dionisio-Sese and Tobita (1998) using the following equation:

$$\mathrm{EL} = \mathrm{EC}_1/\mathrm{EC}_2 \times 100$$

The H_2O_2 contents were measured by homogenizing the samples with 50 mM phosphate buffer having pH 6.5. The samples were centrifuged for 25 min, followed by addition of 20% H_2SO_4 (*v*/*v*). The specimens were again centrifuged for 15 min. The H_2O_2 contents were measured by running the samples at 410 nm absorbance (Jana and Choudhuri 1981). The MDA contents were measured by using the technique described by Zhang and Kirham (1994).

The activities of SOD, POD, CAT, and APX were estimated spectrophotometrically. The leaf samples were mixed with 0.05 M phosphate buffer (pH 7.8) and centrifuged for 10 min. POD and SOD contents were obtained as described by Zhang (1992), while APX and CAT contents were measured by following Nakano and Asada (1981) and Aebi (1984), respectively.

Measurement of Cr contents

The samples were rinsed with dilute HCl, dried at 70 °C, and grinded to fine powder. The plant samples (1 g each) were burnt to ashes at 450 °C for 12 h and digested with10 mL of HNO_3-$HClO_4$ (3:1 *v*/*v*) thereafter. The specimens were kept overnight and further digested by adding 5 mL of the same solution (Rehman et al. 2015). The Cr contents were estimated by atomic absorption spectrophotometer.

Statistical analysis

Data was presented with means of the three replicates. Analysis of variance (ANOVA) was done using the statistical package (SPSS, version 16.0), followed by the Tukey's post hoc test among means of different treatments to estimate significant difference.

Results

Growth and physiological traits

Growth characteristics of maize plants such as height, leaf area, root length, and number of leaves plant^{-1} were considerably decreased due to Cr stress at 10 mg kg^{-1}. With increasing Cr concentration in growth medium, there was a regular reduction in all growth parameters of both varieties (Tolerant 6103, Sensitive 9108) (Fig. 1.). Foliar application of M (50, 100 mg L^{-1}) on stressed plants improved root length, leaf area, plant height, and number of leaves plant^{-1} as compared to Cr treatment alone.

Agronomic traits

Agronomic characteristics of maize plants such as dry weight of leaves, roots, and stems were depressed with increasing Cr concentration in both varieties (Tolerant 6103, Sensitive 9108). Maximum reduction in biomass was noticed at 10 mg kg^{-1} Cr treatment in both varieties (Fig. 2.) However, foliar application of mannitol enhanced the dry weight of all parts of plants with dose-additive manner in both maize varieties.

Oxidative stress

Increasing Cr concentration imposed the oxidative stress on plants by increasing the EL, H_2O_2, and MDA contents in root and leaves of both cultivars (Fig. 3). Foliar application of mannitol, at both concentrations (50, 100 mg L^{-1}), considerably ameliorated the oxidative stress in roots and leaves of plants. However, the severity of oxidative stress was more pronounced in Cr-sensitive cultivar (9108) than the tolerant one (6103) as shown by higher concentration of EL, H_2O_2, and MDA.

Antioxidant enzymatic activities

Under Cr stress, a significant reduction in the activities of antioxidant enzymes was observed both in leaves and root of maize plants. But foliar application of mannitol, at both concentrations (50, 100 mg L^{-1}), on Cr-stressed plants considerably improved the activities of antioxidant enzymes (SOD,

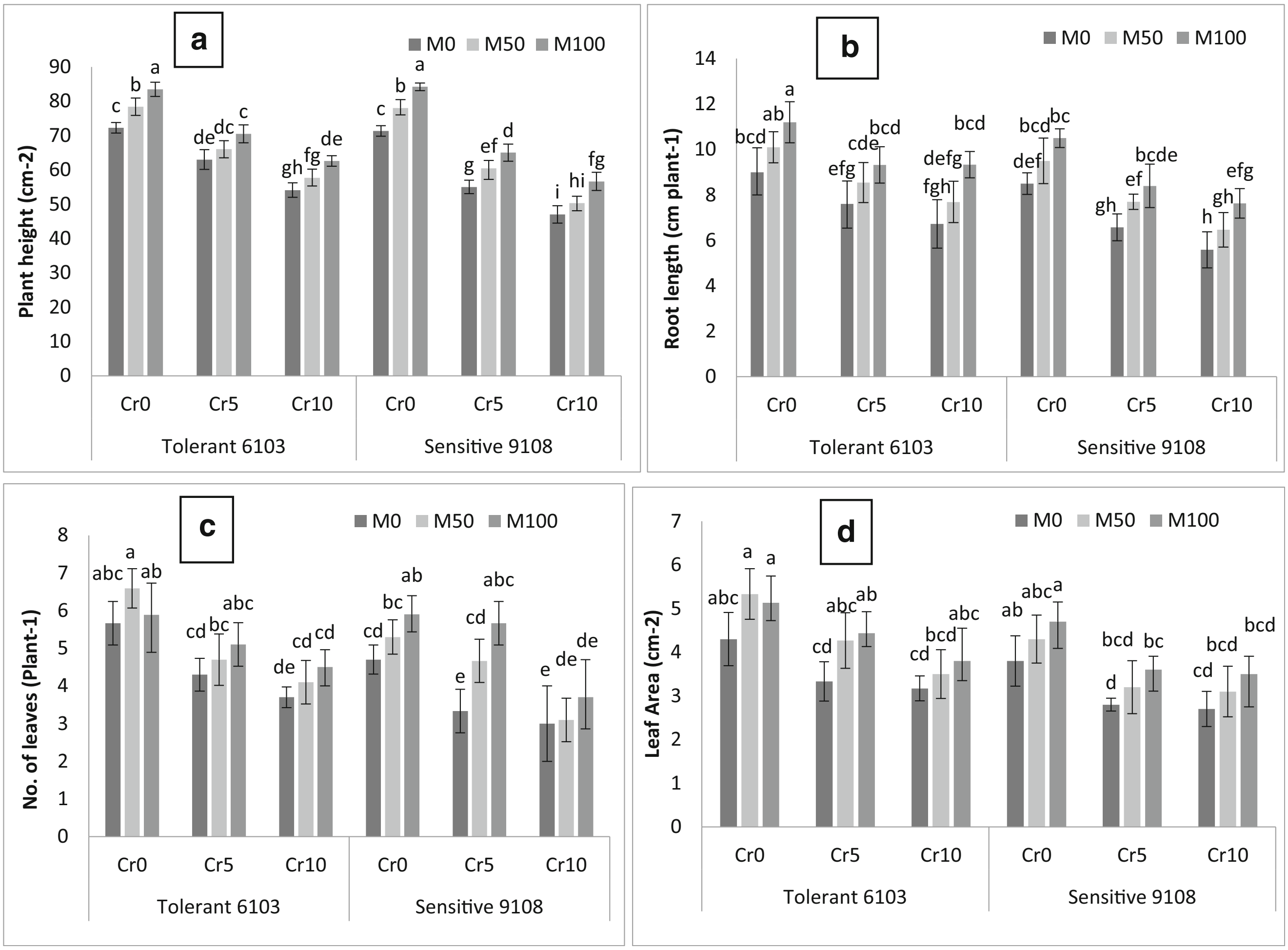


Fig. 1 Impact of chromium on plant height (**a**), root length (**b**), number of leaves (**c**), and leaf area (**d**) in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters

POD, CAT, and APX) in both cultivars, as compared to those treated with same Cr concentration without mannitol. The highest increase in antioxidant enzymatic activities was observed at higher mannitol concentration (100 mg L^{-1}) (Fig. 4).

Photosynthetic characteristics

Photosynthetic traits including chlorophyll a, b, total chlorophyll, and carotenoids were considerably reduced in leaves of plants grown under Cr stress at both levels (5 and 10 mg kg^{-1}) in comparison with the control (Fig. 5) in both verities (Tolerant 6103, Sensitive 9108). But, foliar applied mannitol on Cr-stressed plants significantly enhanced chlorophyll pigments and carotenoids, as compared to those facing Cr stress without mannitol. However, mannitol was more effective in enhancing chlorophyll contents at lower Cr concentration (5 mg kg^{-1}), as compared to the higher one (10 mg kg^{-1}) in both varieties.

Chromium contents

Chromium contents in maize plants increased with increasing Cr concentration in the growth medium in a dose-additive way in roots and leaves of both maize cultivars (Fig. 6). Highest Cr contents were obtained at maximum applied Cr level (10 mg kg^{-1}). There were higher Cr contents in roots than that in leaves. But the exogenous application of mannitol significantly reduced the Cr contents in roots and leaves of both maize varieties. The highest reduction in Cr contents was observed at maximum mannitol level (100 mg L^{-1}).

Discussion

In present study, the growth and biomass of maize were severely suppressed by Cr toxicity as compared with control (Figs. 1 and 2). This growth and biomass reduction might be due to ultrastructural changes in mesophyll cells of leaves

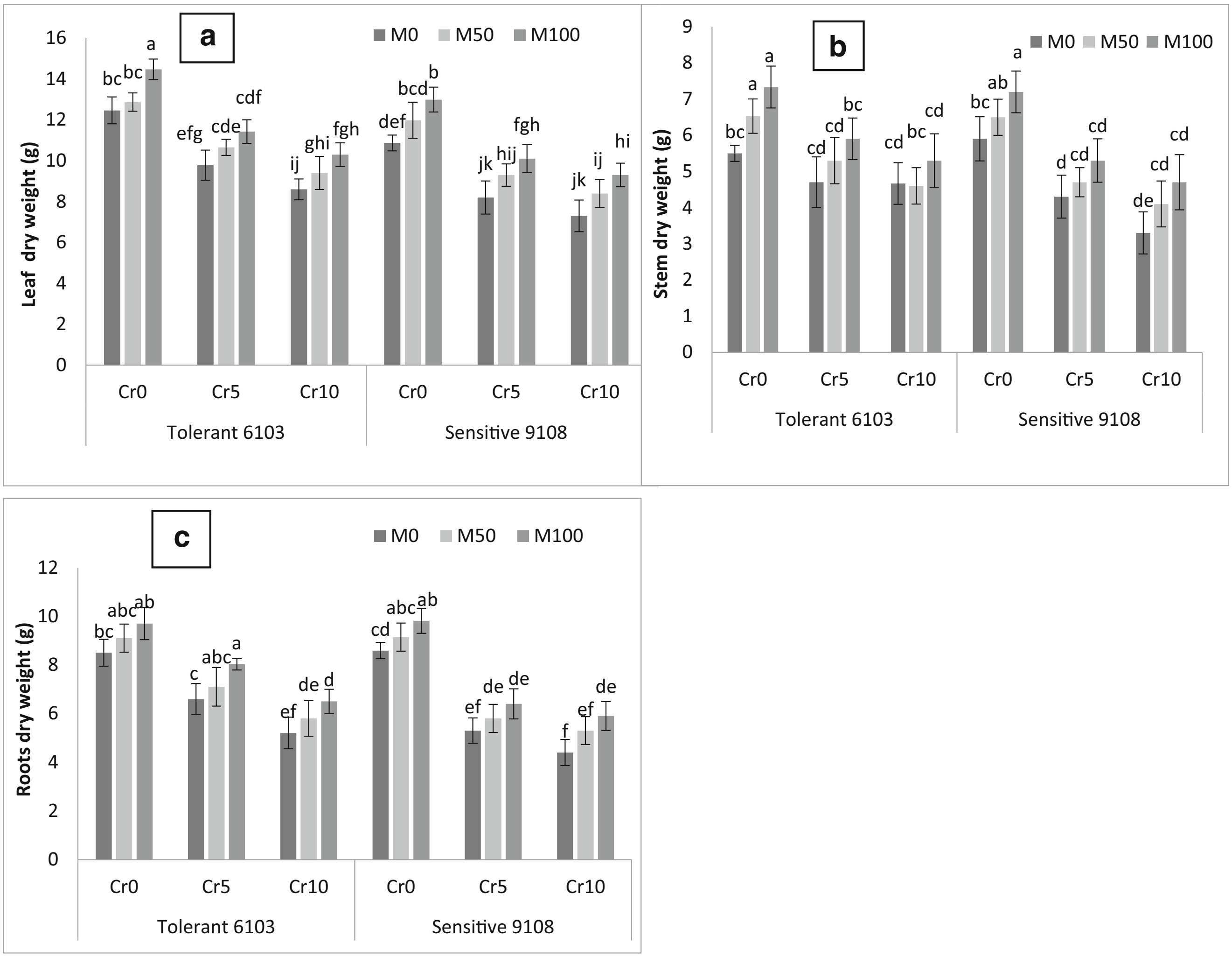


Fig. 2 Impact of chromium on leaf (**a**), stem (**b**), and root (**c**) dry weights in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters

(Gill et al. 2015; Antoniadis et al. 2017). Also, the reduction in nutrient uptake by plants due to Cr stress might have resulted in plants' biomass reduction (Tauqeer et al. 2016). Foliar application of mannitol significantly alleviated the Cr-induced deterioration in growth and biomass of maize plants. This increase in biomass and growth, because of foliar applied mannitol, has also been noticed in various plant species under drought (Ullah et al. 2014) and salinity stress (Kaya et al. 2013). The improvement in growth and biomass of plants with mannitol might be ascribed to its utilization in plant leaves, where it might have served as a source of nitrogen and carbon (Mitoi et al. 2009). Chlorophyll contents and carotenoids were reduced by Cr toxicity both in leaves and root of maize (Fig. 5). Similar results have been found in many other plants like sunflower (Singh et al. 2013), wheat, and mung bean under Cr stress (Jabeen et al. 2016). Many other heavy metals like Cd, Ni, and Cu also decreased the chlorophyll and carotenoid contents in various plants species (Farooq et al. 2016). The reduction in photosynthetic pigments might be associated with ultrastructural changes in chloroplasts (Bukhari et al. 2016a; Najeeb et al. 2011). However, the foliar applied mannitol significantly alleviated the Cr-induced stress and improved photosynthetic pigments of maize plants. It has been reported previously that the mannitol enhanced the photosynthetic pigments under Cr stress in maize (Kaya et al. 2013), which might be due to higher photosynthetic rate.

Under Cr stress, maize plants faced oxidative stress induced by overproduction of ROS (Fig. 3). Same results were observed in various plant species under stressful condition (Habiba et al. 2015; Ahmad et al. 2017). Under heavy metal stress, many plant species revealed the higher production of ROS like sunflower (Rizwan et al. 2017b), barley, and wheat (Gill et al. 2016). Like heavy metals, salinity and drought also increased the ROS production in plants (Arshad et al. 2016). In the current study, foliar application of mannitol significantly decreased the ROS

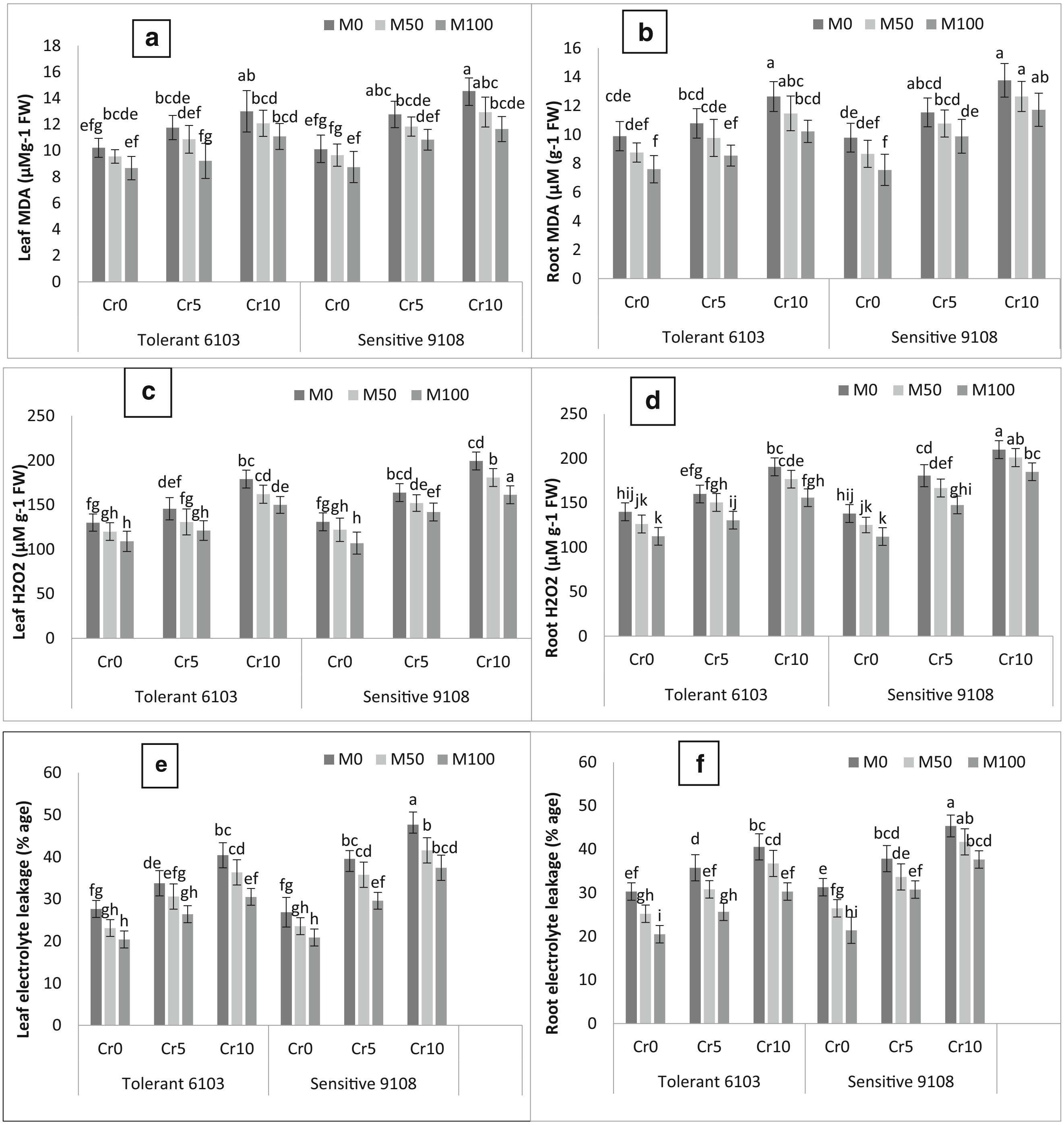
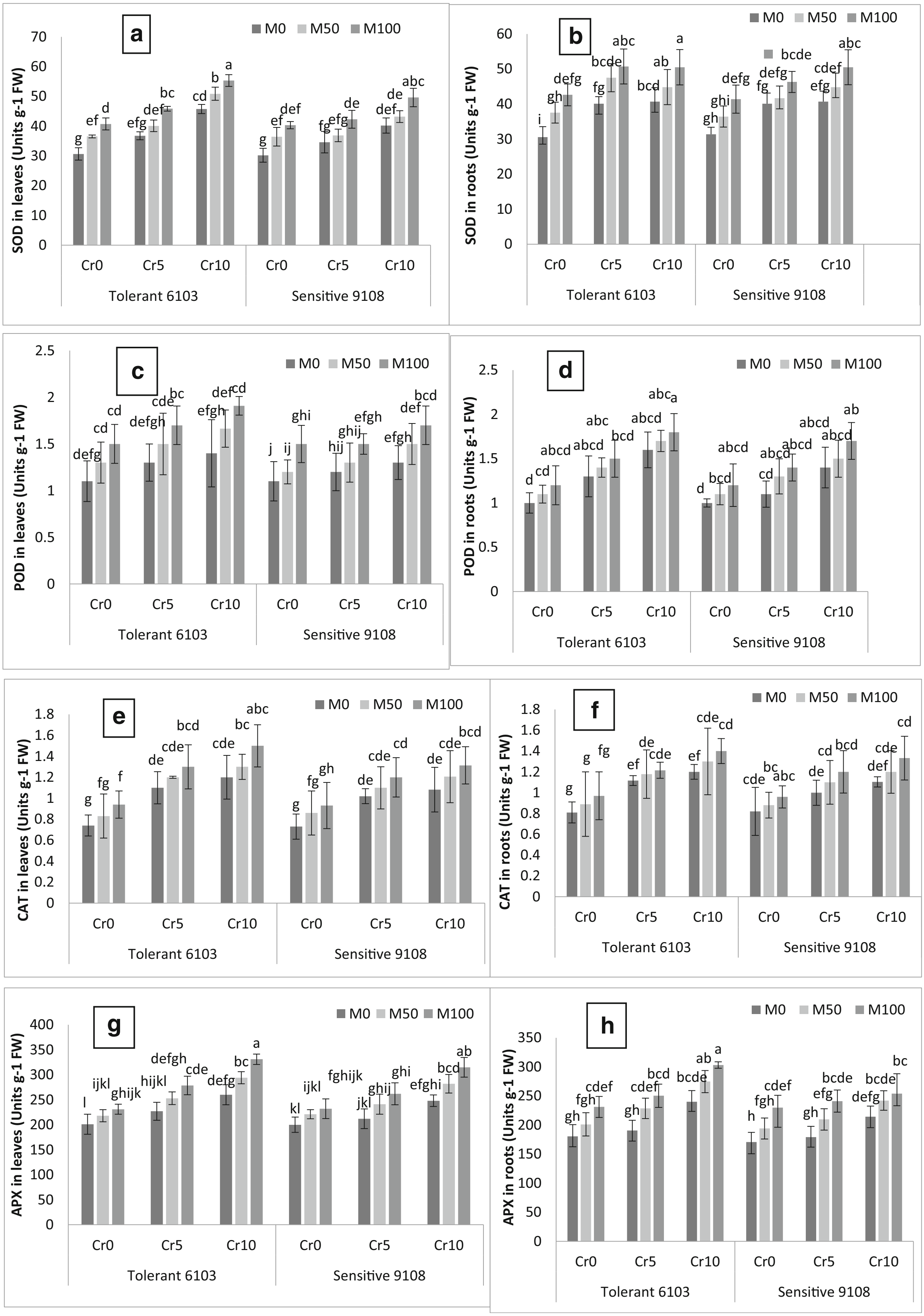


Fig. 3 Impact of chromium on leaf MDA (**a**), root MDA (**b**), leaf H_2O_2 (**c**), root H_2O_2 (**d**), EL in leaves (**e**), and EL in roots (**f**) in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters

production under Cr stress. This decrease in ROS production with mannitol application might be ascribed to enhanced performance of antioxidant defense system (Islam et al. 2016). It has also been reported that the application of mannitol decreased lipid peroxidation in wheat plants under salt stress (Seckin et al. 2009).

Fig. 4 Impact of chromium on SOD leaf and root (**a**, **b**), POD leaf and root (**c**, **d**), CAT leaf and root (**e**, **f**), and APX leaf and root (**g**, **h**) in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters ▶


a
M0 M50 M100
SOD in leaves (Units g-1 FW)
Cr0 Cr5 Cr10 Cr0 Cr5 Cr10
Tolerant 6103 Sensitive 9108
b
SOD in roots (Units g-1 FW)
c
POD in leaves (Units g-1 FW)
d
POD in roots (Units g-1 FW)
e
CAT in leaves (Units g-1 FW)
f
CAT in roots (Units g-1 FW)
g
APX in leaves (Units g-1 FW)
h
APX in roots (Units g-1 FW)

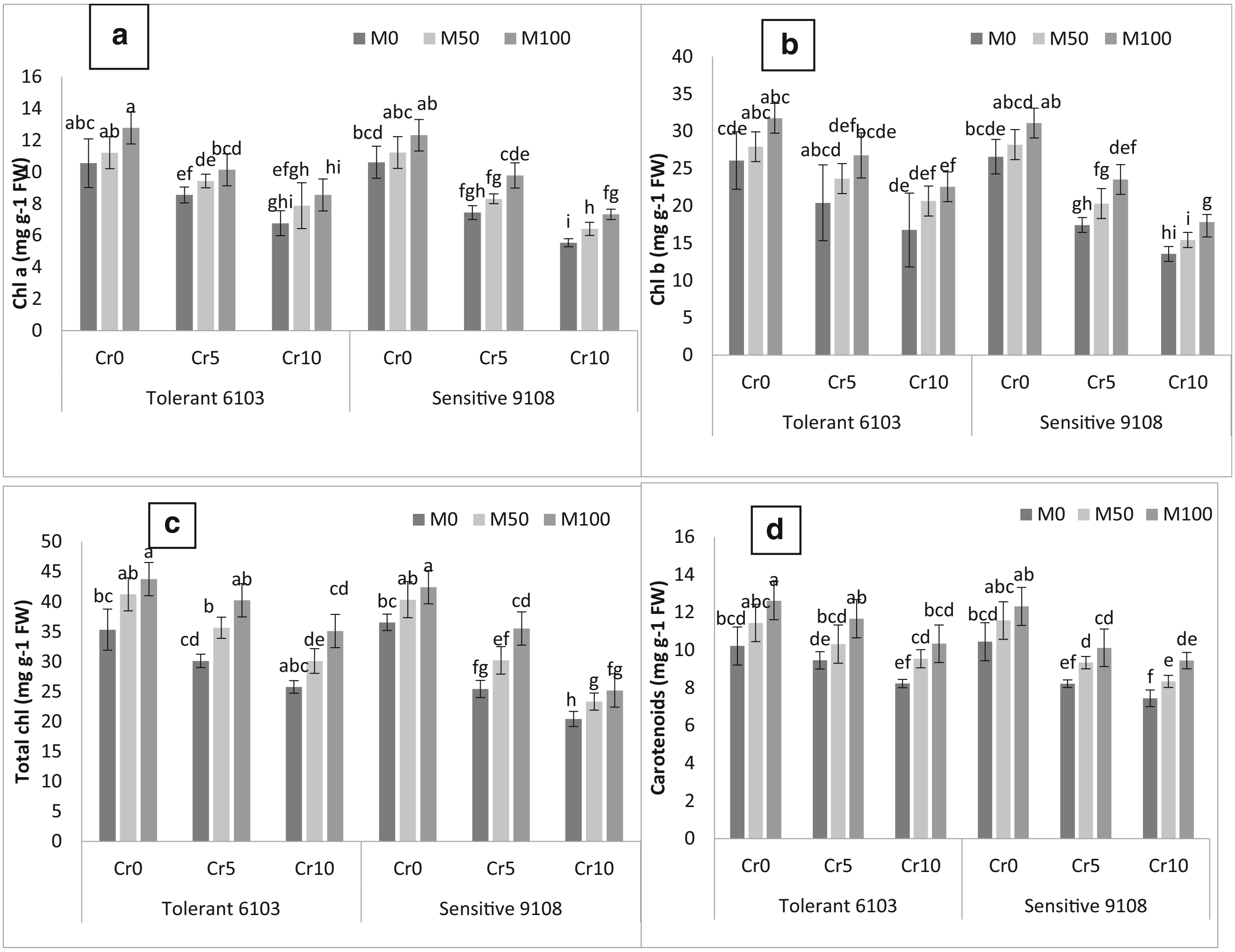


Fig. 5 Impact of chromium on Chl a (**a**), Chl b (**b**), total Chl (**c**), and carotenoids (**d**) in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters

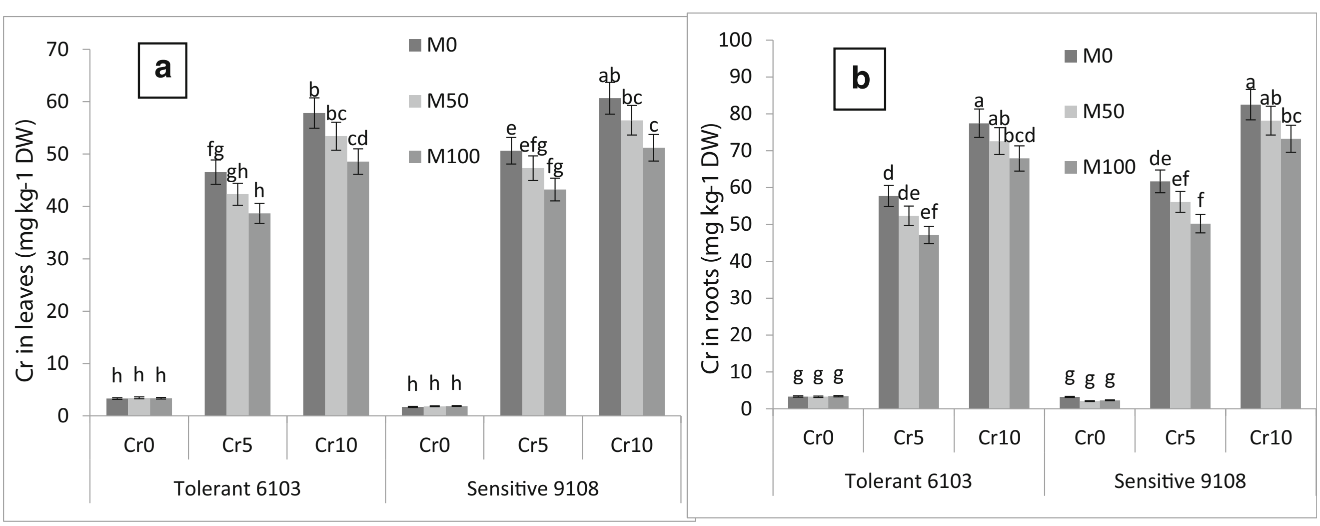


Fig. 6 Chromium uptake in leaves (**a**) and roots (**b**) in two different hybrids of maize, one is tolerant (6103) and other is sensitive (9108), cultivated in soil along with three different chromium levels (0, 5, and 10 mg kg^{-1}) and three different mannitol levels (0, 50, and 100 mg L^{-1}) with three replicates. The significant difference between the values is of $P < 0.05$ which is shown by different letters

Plants develop their defense system through antioxidant enzymatic activities against various environmental stresses (Kanto et al. 2015). The Cr toxicity decreased the antioxidant enzyme activities in both leaves and roots of maize plants (Fig. 4). Similar findings have also been noticed in various plants under Cr stress (Farid et al. 2017). The decrease in antioxidant enzymatic activities might be attributed to higher Cr uptake by maize plants (Fig. 4), which might have reduced the plants' self-defense system. However, the mannitol application significantly alleviated the Cr-induced reduction in antioxidant enzymatic activities of maize plants. It has also been reported that the mannitol enhanced the activities of antioxidant enzymes in peanut under drought and salt stress, which might be attributed to the reduction in ROS production (Bhauso et al. 2014).

In recent study, the increasing Cr concentration in soil media significantly increased the uptake and accumulation of Cr, both in leaves and root of maize plants (Fig. 6). These findings are similar to those reported in *Brassica napus* (Gill et al. 2015), barley (Ali et al. 2013), and tobacco (Bukhari et al. 2015). The higher Cr concentration was observed in roots than that in leaves. However, mannitol significantly reduced the Cr uptake and translocation from roots to leaves. This decline in Cr uptake might be associated with the protective role of mannitol in cell membrane stability, which might have resulted in reduced entry of Cr in cytoplasm (Bhauso et al. 2014).

Conclusion

Our study concluded that Cr application to soil media significantly reduced the physiological and morphological parameters of maize plants. But the foliar application of mannitol effectively alleviated the Cr-induced toxic effects in maize plants. The mannitol application enhanced the morphophysiological parameters, chlorophyll contents, carotenoids, and anti-oxidant enzymatic activities, while reduced the ROS production and Cr uptake and translocation both in leaves and root of plants, which suggested the protective role of mannitol in maize under heavy metals stress. However, the mechanism of Cr stress alleviation by mannitol and its uptake still bears a question mark which should be further studied.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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