



Full Length Article

Morpho-Physiological Responses of Maize Cultivars Exposed to Chromium Stress

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Abstract

Chromium (Cr) is non-essential element and one of the hazardous heavy metals present in environment. Among cereals, maize is used as a source of food both for plants and humans and is also being cultivated on metal-contaminated soils mainly to feed the increasing population. Therefore, the screening of low Cr accumulating maize varieties could be an environment friendly option mainly for Cr-contaminated soils. For this purpose, seeds of eight maize cultivars; 6103, Gorilla, Garanon, p1574, 8711, 6525, 6142 and 9108 were grown in soil and the plants were irrigated with different Cr concentrations after two weeks of germination for the next three weeks to reach the selected final Cr concentrations in soil (0, 100 and 500 μ M). Plants were harvested after eight weeks of sowing and different physiological and morphological traits of plants were measured including photosynthesis, biomass, growth, activity of antioxidant enzymes and oxidative stress markers. Results showed that increasing Cr concentration in the soil caused a reduction in all selected physiological and morphological parameters of maize plants. Chromium stress enhanced the electrolyte leakage, malondialdehyde, hydrogen peroxide while the production of antioxidant enzymes; catalase, peroxidase, superoxide dismutase, ascorbate peroxidase, varied with the maize cultivars in shoots and roots of maize. Furthermore, Cr addition in the growth medium increased the Cr concentration in leaves and roots in a dose dependent manner. The response of maize cultivars varied with the studied cultivars and Cr concentrations applied. Among studied cultivars, 6103 and 9108 accumulated the lowest and the highest Cr concentrations in different parts, respectively. This showed that cultivar 6103 might be used for the Cr-contaminated soils without loss in biomass and to avoid Cr entry to the food chain. However, in depth field studies are needed in future research before the practical application. © 2019 Friends Science Publishers

Keywords: Chromium stress; Antioxidant enzymes; Reactive oxygen species; Plant growth; Antioxidant enzymes

Introduction

Anthropogenic activities, urbanization, industrialization and excessive use of fertilizers and pesticides are the major sources of heavy metal entry in the soils (Adrees *et al.*, 2015; Rehman *et al.*, 2015; Rizwan *et al.*, 2017a). Besides the anthropogenic activities, some natural sources such as volcanic eruption, forest fires, sea salt aerosols production and run off are also main causes of heavy metals contamination to environment (Nagajyoti *et al.*, 2010; Farid *et al.*, 2017). The accumulation of toxic heavy metals in the soil has increased the threshold limits and is ultimately harmful to living organisms including plants and humans (Gaur *et al.*, 2014; Hassan *et al.*, 2016; Asad *et al.*, 2018).

Among various toxic heavy metals, chromium (Cr) is non-essential to plants, carcinogenic and naturally persistent metal on earth (Atta *et al.*, 2013). Chromium is industrially important metal and is being used in manufacturing of different products including those from leather industries and tanneries in Pakistan and worldwide (Shahid *et al.*, 2017). The Cr exists in different oxidation states but Cr-III and Cr-VI are more toxic forms of Cr. The Cr-VI is highly stable, water soluble, and can penetrate in to the cell and is reported to be more toxic than Cr III (Ali *et al.*, 2015a; Shahid *et al.*, 2017). It badly impacts the plant growth, for example by decreasing the plant root and shoot growth, leaf area and leaf number, plant height and total biomass (Ozdener *et al.*, 2011).

Previous published reports have indicated that Cr produces the reactive oxygen species (ROS) in plants at cell level which ultimately reduce the growth and biomass of plants (Atta *et al.*, 2013; Ali *et al.*, 2015b). Bioaccumulation of Cr in plants may also lead to various biochemical, morphological and physiological alternations which in turn significantly reduce the growth, photosynthetic activity and biomass of plants (Adrees *et al.*, 2015; Ali *et al.*, 2015a). The Cr may also adversely impact the plant physiology through reduced respiration, growth, photosynthetic interference and inhibition of enzyme actions especially at high concentration. The acceptable threshold Cr concentrations of 64 mg kg^{-1} have been suggested while maximum allowable Cr concentrations of 100, 64, and 100 mg kg^{-1} have been recommended in Austria, Canada and Serbia, respectively (Shahid *et al.*, 2017).

Maize (*Zea mays* L.) is the cereal crop that can accumulate various metals, which can cause toxicity symptoms in this plant (Atta *et al.*, 2013). This crop is considered as one of the main staple food and a cereal crop with a range of advantages and is widely cultivated under varying diversity of soil and climate (Rosas-Castor *et al.*, 2014; Farooq *et al.*, 2015). In addition, maize has also been reported for metal accumulation from soils and their transfer to the aerial parts of plants (Rizwan *et al.*, 2017b). However, the higher concentration of such metal in maize may reduce the plant growth and results in the food chain toxicity but this response varies with the cultivars and hybrid (Akhtar *et al.*, 2017). High level of Cr has already been reported across a range of soil and water ecosystems from where it can accumulate in crops ultimately disrupting the plant growth but also affecting the human health through food chain toxicity (Shahid *et al.*, 2017). Thus, alternate approaches are timely required to reduce the negative impacts of Cr accumulation on environment but also its entrance in food chain.

The selection of low metal accumulating maize cultivars might be the quick, cost-effective and environment friendly strategy for the soils contaminated with Cr to avoid Cr entry into the food chain and other environmental compartments. However, little is known regarding the physiological and morphological behavior of maize hybrid under Cr stress. In this regard, current research work was carried out to investigate the potential of different maize cultivars towards the bio-accumulation of Cr and the response of various maize functional traits under various Cr levels applied. The main objective of the present study was to examine the effects of Cr on growth, physico-chemical parameters and antioxidants activities of maize hybrids under different Cr stress levels and ultimately to screen the low Cr accumulating maize cultivars suitable for cultivation on Cr contaminated soils.

Materials and Methods

Pot Experiment

The experiment was performed in wire house at the botanical garden of Government College University Faisalabad to screen the eight cultivars of the maize (6103, Gorila, Garanon, p1574, 8711, 6525, 6142 and 9108). The experiment was arranged under complete randomized design with three replicates under natural conditions. The soil was collected from the agricultural field (0–15 cm) and then was air dried, sieved through 2.0 mm mesh size to make it homogenised and well prepared before filling the pots. Each pot contained 6 kg of soil and irrigated with tap water. The soil samples were characterized for various physico-chemical properties by standard methods (Table 1). Eight seeds of maize cultivars were sown in each pot. The recommended dose of NPK was applied as $120\text{-}50\text{-}25 \text{ kg ha}^{-1}$. Nitrogen, P and K was applied as urea, diammonium phosphate and potassium sulphate respectively. After fifteen days of germination, thinning was done to maintain five plants per pot and uprooted plants were crushed deviously in soil of the same pot. After two weeks of germination, pots were irrigated with different concentrations of Cr for the next three weeks to reach the final concentrations of Cr in each pot (0, 100 and $500 \mu\text{M}$), whereas the controlled plants were watered with tap water when needed. Potassium dichromate was used for the preparation of Cr solutions. Pots positions were randomly changed on regular basis mainly to avoid the environmental effects on the plants.

Plants Harvesting

After harvesting (after eight weeks of sowing) the plants, the roots were carefully removed from the pots. All samples of plants were carefully detached into, leaves, roots and stems and number of leaves per plant was recorded. Root length, plant height was determined by using meter rod while leaf area was calculated with leaf area meter. Plant roots, leaves, and shoots were rinsed with deionized water and oven dried at 70°C . Roots were rinsed with 0.1 M HCl to remove the Cr adhered to the surface of the roots. Samples were ground and were digested properly by using a mixture of HNO_3 and HClO_4 at 3:1 ratio for Cr determination.

Measurement of Photosynthetic Parameters

Photosynthetic contents were measured by using spectrophotometer after extracting the samples in acetone. Fully stretched leaves were taken just before harvesting the plants for the removal of pigments, aqueous acetone (85% v/v) was used to remove the chlorophyll contents from the fresh leaves. Chlorophyll contents were measured following the procedure as described by Lichtenthaler (1987). Briefly, the centrifugation of the extracted samples was completed at 4000 rpm for 10 min, afterwards the supernatant was

Table 1: Soil physiochemical properties

Texture	Clay loam
Sand (%)	52.0
Silt (%)	24.0
Clay (%)	24.0
pH (1/2.5 soil to water ratio)	7.65
EC (dS m ⁻¹)	2.86
SAR (mmol ⁻¹) ^{1/2}	5.60
Organic matter (%)	0.34
Available P (mg kg ⁻¹)	2.16
HCO ₃ ⁻ (mmol L ⁻¹)	3.68
Cl ⁻ (mmol L ⁻¹)	2.19
SO ₄ ²⁻ (mmol L ⁻¹)	6.48
Ca ²⁺ + Mg ²⁺ (mmol L ⁻¹)	3.69
Na ²⁺ (mmol L ⁻¹)	3.48
K ⁺ (mmol L ⁻¹)	0.03
Available Cu ²⁺ (mg kg ⁻¹)	0.23
Available Zn ²⁺ (mg kg ⁻¹)	0.72
Available Cr ⁺⁶ (mg kg ⁻¹)	0.17

obtained, and 85% aqueous acetone solution was utilized for dilution purposes for making a suitable volume and the readings were taken at three different absorbance of 663, 644 and 452.5 nm wavelengths by spectrophotometer.

Measurement of Electrolyte Leakage, Malondialdehyde and Hydrogen Peroxide

The EC meter was used to calculate the electrolyte leakage (EL). Before harvesting, the upmost stretched leaves were cut at 5 mm length and placed vertically into test tubes containing 8 mL of deionized water. Then the incubation was done at temperature 32°C for 2 h in water bath and EC₁ were calculated. For electrolyte discharge, plant samples were autoclaved at 121°C for 20 min; then cooled at room temperature followed by the calculation of EC₂. The EL was then calculated using the following formula (Dionisio-Sese and Tobita, 1998).

$$\text{Electrolyte leakage} = (\text{EC}_1/\text{EC}_2) \times 100$$

To measure the malondialdehyde (MDA), the samples of roots and leaves (0.25 mg) were mixed with 0.1% trichloro acetic acid of 5 mL volume. Afterwards, the MDA measurements in roots and leaves were carried out by using the method reported by Heath and Packer (1968). Briefly, the reaction of thiobarbituric acid (TBA) which was further modified by Dhindsa *et al.* (1981), Zhang and Kirham (1994).

Hydrogen peroxide (H₂O₂) was measured after homogenizing the leaf and root tissues in phosphate buffer (3 mL) with concentration of 50 mM and the pH was maintained at 6.5. Afterwards, the homogenized samples were centrifuged for 25 min. The extracted solution of volume 3 mL was used with 1 mL of titanium sulphate (0.1%) along with the H₂SO₄ (20% (v/v)) and it was centrifuged for 15 min. Then the absorbance was recorded at wavelength 410 nm to calculate the supernatant intensity.

The coefficient of extinction with 0.28 μmol⁻¹ cm⁻¹ was used for the calculation of hydrogen peroxide contents (Jana and Choudhuri, 1981).

Measurement of Antioxidant Enzymes

The activities of Ascorbate Peroxidase, Superoxide Dismutase, Catalase, and Peroxidase (APX, SOD, CAT and POD, respectively) in roots and leaves were measured by using Spectrophotometer. Briefly, just before harvesting, the second most abundantly stretched leaves as well as roots were sampled in order to analyse the antioxidant enzymes. The grinded samples of roots and leaves were homogenised with phosphate buffer of molarity 0.05 M and pH 7.8, in cold *via* the use of liquid nitrogen. Then this mixture was centrifuged for time duration of 10 min at 4°C, afterwards the required absorbance was set to calculate the intensity of supernatants. The activity of the CAT was examined as described by Aebi (1984). Briefly, 3 mL of the assay mixture was taken which comprised of 300 mM of hydrogen peroxide (100 μL), the extract (100 μL) and 2.8 mL of phosphate (50 mM) and EDTA (2 mM) with pH of 7. The catalase activity was measured at spectrophotometer at wavelength of 240 nm, as the result of hydrogen peroxide disappearance ($\epsilon = 38.3 \text{ mM}^{-1} \text{ cm}^{-1}$).

The APX activity was analysed as described by Nakano and Asada (1981). In brief, the mixture used contained 300 mM of hydrogen peroxide (100 μL), the enzyme extract (100 μL) and 2.8 mL phosphate buffer (25 mM) and EDTA (2 mM) with pH 7. Then the absorbance was set at 290 nm for the calculation of APX activity ($\epsilon = 2.79 \text{ mM}^{-1} \text{ cm}^{-1}$).

Measurement of Cr Contents in Plants

Following the digestion of 1.0 g of plant sample in 10 mL of HClO₄-HNO₃ (1:3, v:v), samples were incubated overnight and the concentration of Cr in shoots and roots was measured by atomic absorption spectrophotometer after digestion as described by Rehman *et al.* (2015).

Statistical Analysis

Analysis of variance (ANOVA) was applied to analyse the data using Statistix 8.1 statistical package, with version 16.0. Tukey's post hoc test was applied on different treatments means to check the significant differences.

Results

Growth and Physiological Traits

Results revealed that the Cr levels and their interaction showed significant differences ($p \leq 0.05$) towards the various agronomic (plant height, root length, number of leaves per plant, leaf area) and physiological traits

(chlorophyll a, b, total chlorophyll and carotenoids) (Fig. 1 and 2). It was observed that these parameters declined with the increasing level of Cr where maximum reduction was observed at the highest Cr stress (500 μM) compared with control. Different maize cultivars denoted variable susceptibility to Cr stress based upon the concentration of applied Cr. However, Maize-6103 was found to be the most tolerant cultivar while Maize-9108 was the least tolerant to Cr stress for all studied traits at all Cr levels. The tolerance level order of different maize cultivars for the applied Cr stress was observed as 6103 > Gorila > Garanon > P1574 > 8711 > 6525 > 6142 > 9108 for all above studied parameters.

Agronomic Traits

The applied Cr stress reported a significant effect ($p \leq 0.05$) on plant agronomic traits including; leaves dry weight, stem dry weight and root dry weight (Fig. 3). All agronomic traits gradually decreased with increasing Cr concentration. The highest decline was noticed at 500 μM Cr stress. The tolerance showed by the maize cultivars against Cr stress with respect to dry weight was given as 6103 > Gorila > Garanon > P1574 > 8711 > 6525 > 6142 > 9108 for the studied agronomic traits.

Oxidative Stress Related Traits

The oxidative stress among various maize cultivars was assessed by determining the concentration of H_2O_2 , EL and MDA in leaf and roots of maize. The applied Cr stress reported a significant effect ($p \leq 0.05$) on all these parameters used as proxy for the oxidative stress measurement both in plant leaves and root (Fig. 4). All studied oxidative stress parameters increased with the increasing level of applied Cr. For example, the most significant increase was observed to EL in leaves for the cultivar 6142, MDA in leaves for the cultivar Gorila and H_2O_2 in leaves for the cultivar P1574 at Cr level of 500 μM , while H_2O_2 in leaves for cultivar 9108 at Cr level of 100 μM , as compared with other cultivars. Overall, the observed concentrations for all these oxidative traits followed the trend as; 9108 > 6142 > 6525 > 8711 > P1574 > Garanon > Gorila > 6103 for the studied oxidative stress markers.

Antioxidant Enzymes Activities

The activities of various antioxidant enzymes were assessed by determining the concentration of POD, SOD, APX and CAT in leaves and roots of maize cultivars. The applied Cr levels showed a significant impact ($p \leq 0.05$) on all the studied antioxidant enzymes activities in plant leaves and roots (Fig. 5). Moreover, all antioxidant enzymes activities were decreased or increased with the increase in the applied Cr levels depending upon maize cultivars where the most

significant decrease was noticed for the cultivar 9108 at maximum Cr stress (500 μM) for all antioxidant enzymes activities. This also showed that the maize cultivar 9108, in contrast to other cultivars, was the least tolerant against the Cr stress under maximum applied Cr level.

Chromium Concentration in Plants

The applied Cr stress reported a significant effect ($p \leq 0.05$) on Cr concentrations in root and leaves of maize cultivars (Fig. 6). The Cr translocation concentrations in leaves and roots were increased with a dose-additive manner of applied Cr which was maximum at high Cr level (500 μM) for all the studied maize cultivars. However, it was observed that all cultivars accumulated more Cr concentration in roots as compared to leaves. The Cr concentrations in leaves and roots of all maize cultivars followed the increasing order of 6103 > Gorila > Garanon > P1574 > 8711 > 6525 > 6142 > 9108.

Discussion

In the present study, eight maize cultivars were tested in soil medium to observe the morpho-physiological responses of these cultivars to Cr stress. A significant decrease in the plant growth traits, dry weight and chlorophyll contents was observed with increasing level of Cr to different extent in all studied maize cultivars (Fig. 1, 2 and 3). However, the highest toxicity was observed at maximum level of applied Cr. The maize cultivars have the differential effects on growth and physiology under heavy metal stress (Akhtar *et al.*, 2017; Rizwan *et al.*, 2017b). The Cr-mediated growth inhibition in maize might be due to the disturbance in leaf photosystem and reduction in the activities of plant photosynthesis (Chen *et al.*, 2017). Also, the growth disruption because of the Cr toxicity can be attributed to the Cr transportation from roots to shoots which may have direct effects on the plant cellular metabolism ultimately contributing to growth inhibition (Gomes *et al.*, 2017). Some recent studies have revealed that the Cr stress reduces the crop yield and productivity (Hussain *et al.*, 2018). Here, it was observed that the plant biomass and chlorophyll contents were decreased by increasing the Cr level in growth medium (Fig. 1 and 2). Reduction in plant growth and chlorophyll contents due to Cr toxicity had previously been reported in various plant species (Ashfaq *et al.*, 2017; Hussain *et al.*, 2018). Interestingly, the maize cultivar 6103 showed the highest plant growth and chlorophyll contents as compared to the other cultivars, which might be because of its greater tolerance and adaptability towards the applied Cr stress as compared to other maize cultivars. The Cr-induced photosynthetic reduction may be associated to the lower chlorophyll contents under Cr stress (Ashfaq *et al.*, 2017; Maqbool *et al.*, 2018).

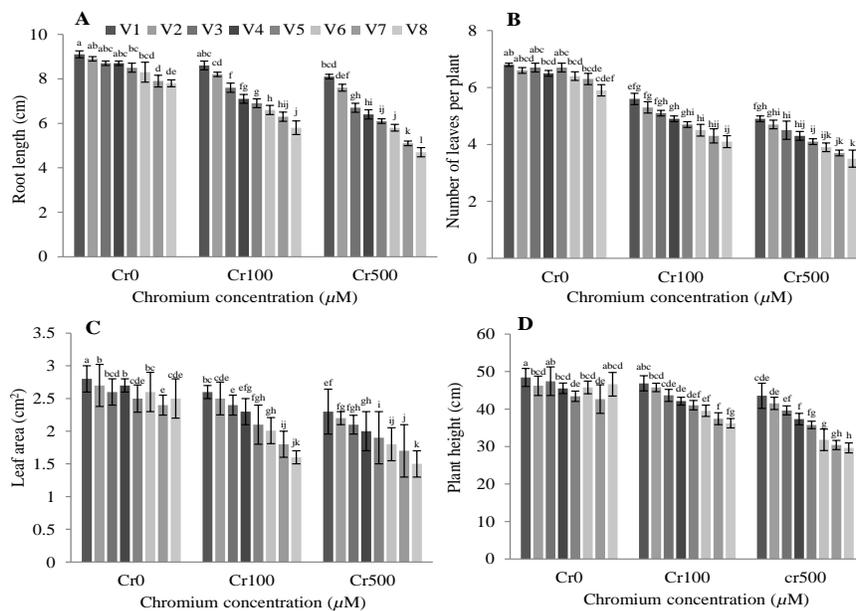


Fig. 1: Impact of chromium levels on root length (A), number of leaves per plant (B), leaf area (C), and plant height (D) of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability

Here $V_1 = 6103$, $V_2 = \text{Gorila}$, $V_3 = \text{Garanon}$, $V_4 = \text{P1574}$, $V_5 = 8711$, $V_6 = 6525$, $V_7 = 6142$, $V_8 = 9108$

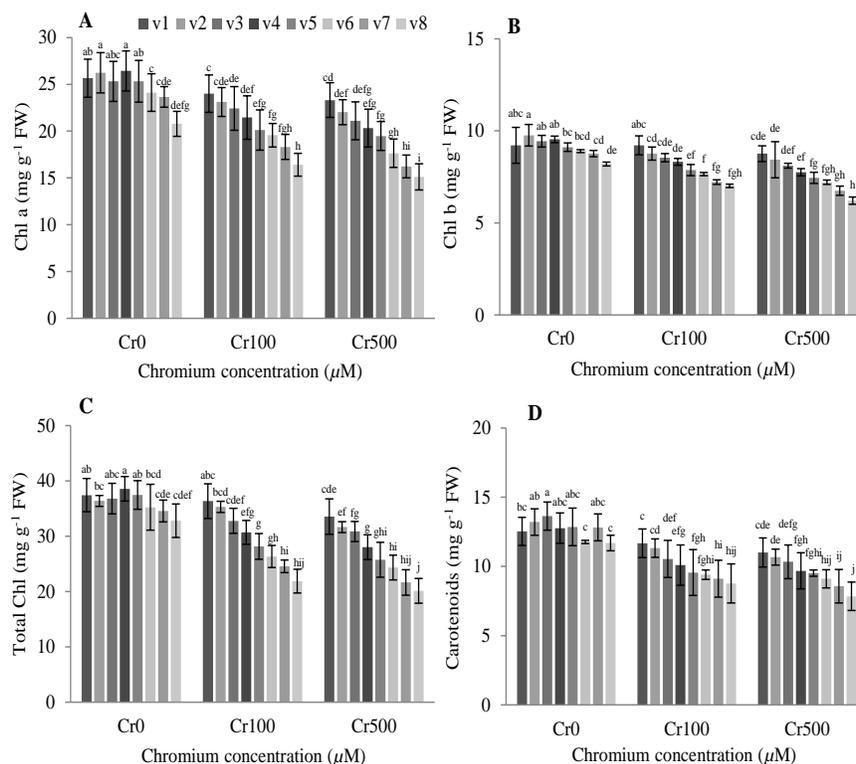


Fig. 2: Impact of chromium levels on chlorophyll *a* (A), chlorophyll *b* (B), total chlorophyll (C), and carotenoids concentrations (D) in leaves of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability

Here $V_1 = 6103$, $V_2 = \text{Gorila}$, $V_3 = \text{Garanon}$, $V_4 = \text{P1574}$, $V_5 = 8711$, $V_6 = 6525$, $V_7 = 6142$, $V_8 = 9108$

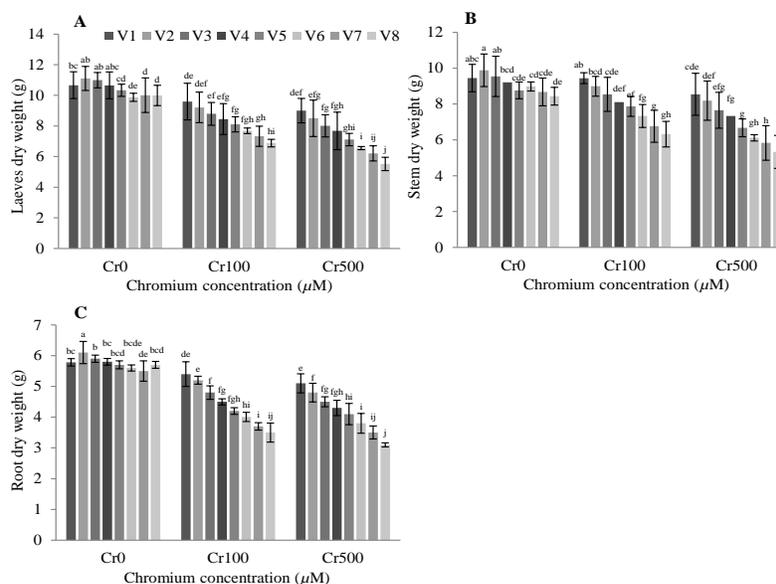


Fig. 3: Impact of chromium levels on dry weights of leaves (A), stem (B), and roots (C) of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability Here $V_1 = 6103$, $V_2 = \text{Gorila}$, $V_3 = \text{Garanon}$, $V_4 = \text{P1574}$, $V_5 = 8711$, $V_6 = 6525$, $V_7 = 6142$, $V_8 = 9108$

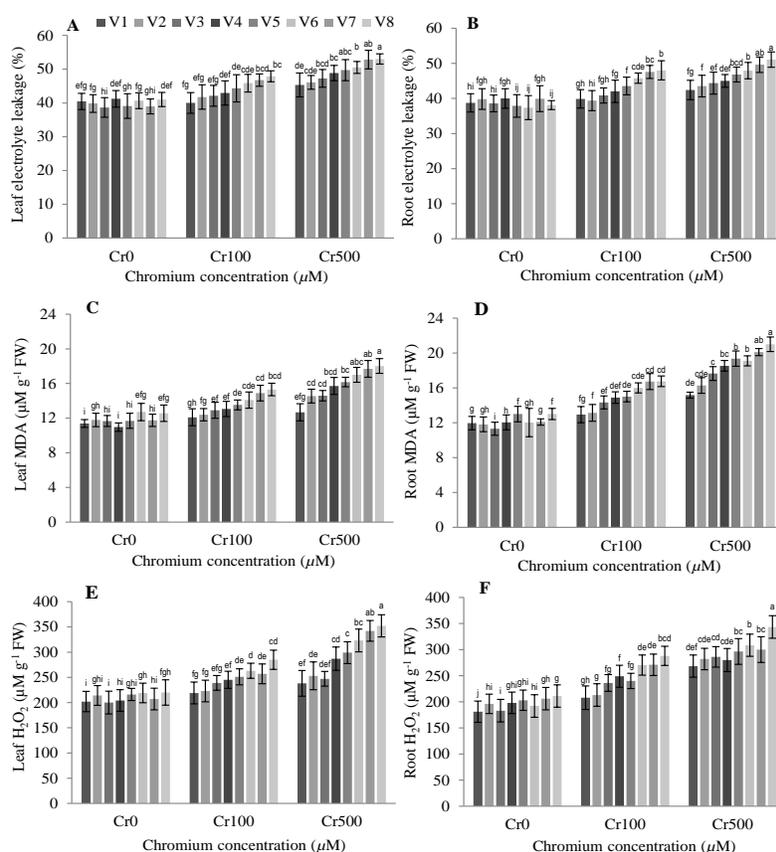


Fig. 4: Impact of chromium levels on leaf EL (A), root EL (B), leaf MDA (C), root MDA (D), leaf H_2O_2 (E), and root H_2O_2 (F) of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability Here $V_1 = 6103$, $V_2 = \text{Gorila}$, $V_3 = \text{Garanon}$, $V_4 = \text{P1574}$, $V_5 = 8711$, $V_6 = 6525$, $V_7 = 6142$, $V_8 = 9108$

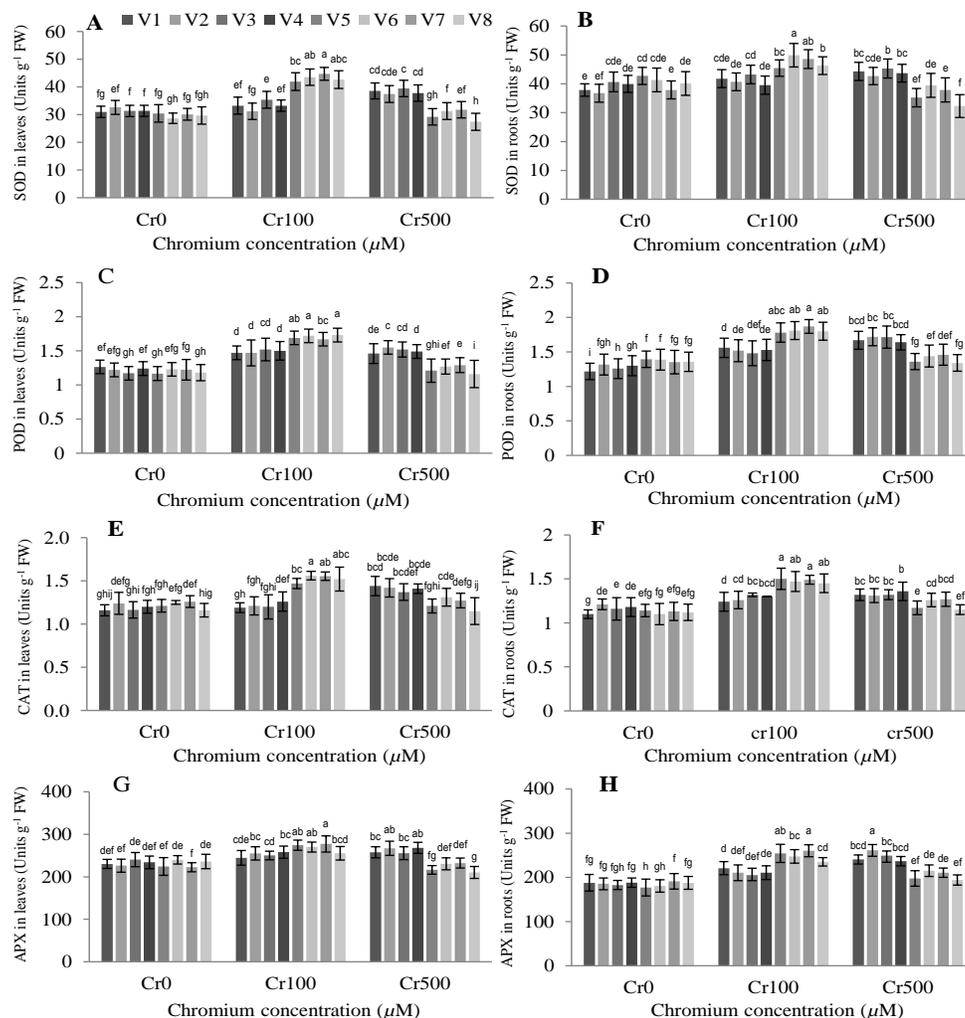


Fig. 5: Impact of chromium levels on the activities of SOD in leaf (A), SOD in root (B), POD in leaf (C), POD in root (D), CAT in leaf (E), CAT in root (F), APX in leaf (G), APX in root (H), of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability. Here V₁ = 6103, V₂ = Gorila, V₃ = Garanon, V₄ = P1574, V₅ = 8711, V₆ = 6525, V₇ = 6142, V₈ = 9108

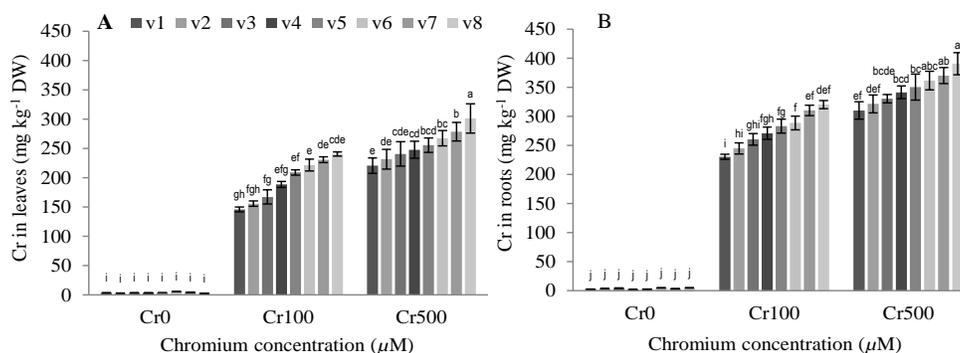


Fig. 6: Impact of chromium levels on chromium concentrations in leaves (A), and root (B) of different maize hybrids. The values are means of three replicates \pm S.E. Different letters on the bars indicate significant differences from each other at 5% level of probability. Here V₁ = 6103, V₂ = Gorila, V₃ = Garanon, V₄ = P1574, V₅ = 8711, V₆ = 6525, V₇ = 6142, V₈ = 9108

The Cr not only hampers the plants morpho-physiological processes but also challenges the plants by the generation the ROS in various parts of the plants, which is considered to be the result of heavy metal stress in plants (Dubey, 2010; Rizwan *et al.*, 2017a). In our work, it was found that the levels of EL, H₂O₂ and MDA in leaves and roots of all maize cultivars increased with increasing Cr concentration depending upon cultivars (Fig. 4). The increase in ROS due to the higher Cr concentrations in plants has earlier been reported by several studies (Farid *et al.*, 2017; Maqbool *et al.*, 2018). It was observed that the maize cultivar 9108 showed the maximum ROS values that may be ascribed to its lower tolerance to applied Cr stress among all studied maize cultivars. The maximum ROS values were observed for the highest applied Cr stress (500 μ M). The generation of ROS previously led to the non-equilibrium in plants defense system and ultimately caused the oxidative damage to various plants (Adrees *et al.*, 2015).

It has been documented that plants have a well-developed defense system against ROS production (Rizwan *et al.*, 2017b) and this defense system might consist of several antioxidants, chelate synthesis, osmolyte production, suberin lamella formation and increased cell wall lignification (Adrees *et al.*, 2015; Devi *et al.*, 2017). In this study, it was observed that the lowest values for antioxidant enzymatic activities were recorded at higher Cr level (500 μ M) as highlighted in Fig. 5. The mechanism behind this observation can be related to the fact that the higher concentration of accumulated Cr might have affected the activities of antioxidant enzymes by overproduction of ROS (Ali *et al.*, 2014, 2015b). However, plants can also tolerate the stress up to a certain extent through increased antioxidant enzymes activities as well as through the stimulation of other tolerance mechanisms (Devi *et al.*, 2017). Whereas, this tolerance differs with plant genotype, species as well as the applied metal (Parrotta *et al.*, 2015). It was observed that the Cr uptake by leaves and roots varied among the cultivars and this uptake linearly increased with increasing applied Cr concentration (Fig. 6). The varying accumulation and translocation of Cr in plant species is well reported in literature (Anjum *et al.*, 2017; Hussain *et al.*, 2018). In our case, the varying Cr accumulation in maize cultivars may also be due to genetic makeup differences among the different plants. Interestingly, it was found that a higher Cr accumulation in maize roots as compared to leaves (Fig. 6). This higher accumulation of Cr in roots than the aerial parts of maize plants might be ascribed to the restriction or immobilization of metal ions within the cortex of the roots to reduce the detrimental effects of metals towards plants (Adrees *et al.*, 2015). Moreover, the cell wall of plant roots also acts as the barrier to stop the heavy metals entrance in plant cell and is known as fundamental storage site for heavy metals (Polle and Schützendübel, 2003). In addition, the Cr toxicity on physiological mechanisms of plants also depends upon the concentration of Cr uptake, its mobility and deposition within the plant

(Ali *et al.*, 2015a; Shahid *et al.*, 2017). This study clearly demonstrated that the cultivar 6103 showed the highest while the cultivar 9108 denoted the lowest tolerance to applied Cr stress. Thus, cultivar 6103 might be effective for the cultivation of soils contaminated with Cr to avoid its entry to the food chain as well as other environmental compartments.

Conclusion

High Cr concentration resulted in increased levels of ROS but decreased the overall anti-oxidant enzymatic activities and plant growth traits. Among the studied maize cultivars, the cultivar 9108 and 6103 accumulated the highest and the lowest Cr concentration respectively, both in roots and leaves. The maize cultivars accumulating low level of Cr may be recommended for cultivation in Cr contaminated soils ultimately to reduce and/or avoid Cr entrance in the food chain.

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