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INDUCTION OF CHROMIUM (CR) STRESS TOLERANCE IN MAIZE BY FOLIAR APPLIED VANILLIC ACID: GROWTH, GAS EXCHANGE CHARACTERISTICS AND ANTIOXIDANTS

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Abstract

The effect of foliar-applied vanillic acid (VA) on biomass, photosynthesis, oxidative stress, antioxidant enzyme activities and uptake of chromium (Cr) in maize plants under varying levels of Cr stress was assessed. The experiment comprised the maize cultivar Malka-2016, three levels of Cr stress (0, 100 and 500 µM), and VA (0, 0.2 and 0.4 mg/L) applied as a foliar spray. The results showed that Cr stress markedly decreased the physiological and morphological characteristics including gas exchange attributes, non-enzymatic compounds, and the activities of enzymatic antioxidants. However, foliar-applied VA significantly increased plant dry weight and improved the gas exchange attributes such as rate of transpiration, photosynthesis and stomatal conductance of maize plants under stress and non-stress conditions. A positive influence of VA was also found on the activities of superoxide dismutase, peroxidase and catalase enzymes and the levels of glycinebetaine and proline. Overall, foliar application of VA significantly enhanced Cr stress tolerance in maize plants by improving gas exchange attributes and antioxidant defense system.

Key words: Antioxidants, Chromium, Maize, Vanillic acid, Gas exchange attributes.

Introduction

Chromium (Cr) is a toxic heavy metal that considerably contaminates most of the agricultural soils (Hussain *et al*., 2018). It is released in the environment due to many human activities such as electroplating and leather tanning effluents. It is believed to pose a potential threat to plant germination, photosynthesis, and mineral nutrition, and cause oxidative stress (Gomes *et al*., 2017). Chromium enters in the food chain through plant roots when exposed to Cr-stressed soil. It is affected by soil pH, electrical conductivity and metal competition and plant related aspects such as plant species, growth conditions and stages as well as root system (Ertani *et al*., 2017). Cr stress causes various hazardous influences on morphological parameters of plants such as shoot length, root surface area, biomass, and root hairs (Shahid *et al*., 2017). Higher concentration of Cr also causes a decrease in gas exchange attributes like transpiration rate, photosynthetic rate, water use efficiency and stomatal conductance as well as chlorophyll contents (Ma *et al*., 2016). High concentration of Cr may also result in ultra-structural changes in plants (Gill *et al*., 2015a). It also causes oxidative stress in plants due to high production of reactive oxygen species (ROS) (Ma *et al*., 2016). Plants have their own defense system which comprises both enzymatic and non-enzymatic antioxidants. But Cr toxicity alters antioxidant enzyme activities due to which overall plant growth is severely affected (Chen *et al*., 2017).

Maize is the third most cultivated grain crop after rice and wheat (Mal-covska *et al*., 2014). It is considered as the most significant staple crop in many countries around the world (Anon., 2017). Its production in arid and semi-arid regions is badly affected by various stresses such as salinity, water scarcity, heat stress, pest invasion and metal toxicity (Abd-El Majeed *et al*., 2017). Although maize is considered as a possible aspirant for phytoextraction, at high levels of metals including chromium, its growth and yield are adversely affected (Rizvi & Khan, 2018).

To cope with Cr toxicity in plants and its increased uptake, multiple strategies have been employed (Jabeen *et al*., 2016). The production of secondary metabolites such as phenolic compounds play a vital role in plant protective system against abiotic environmental problems (Keskitalo, 2003). It is believed that phenolic compounds even at very low concentrations enable plants to produce phenolic glycoside linkages which might play an efficient role in plant defense (Kleiner *et al*., 1999; Kumar *et al*., 2020). Vanillic acid (VA) is a benzoic acid derived phenolic molecule. VA is an oxidized molecule of vanillin. It is used for flavoring purposes, as a food additive and a preservative in food industry. It is obtained from cereals such as whole grains, fruits, herbs, green tea, beers, wines, and juices (Almeida *et al*., 2016). Moreover, VA has several pharmacological properties such as anti-inflammatory, antioxidant, neuroprotective, hepatoprotective and cardioprotective (Sharma *et al*., 2020). Bhuyan *et al*., (2020) stated that supply of vanillic acid improved the activities of antioxidants in Cd-stressed rice plants. It has already been reported that VA significantly improved plant growth and minimized membrane damage in tomato plants stressed with saline stress (Parvin *et al*., 2020). Phenolic compounds may stimulate protein synthesis and antioxidants even at very low concentrations (Hegab, 2005). Vanillic acid is one of the most important phenolics being used as natural antioxidants (Zhang *et al*., 2008), and VA has been identified in different plants such as *Chenopodiastrum murale* L. (Batish *et al*., 2007) and sweet clover (Macias *et al*., 1999). It was hypothesized that foliar application of vanillic acid on maize plants could improve their morphological, physiological, and biochemical responses to Cr toxicity. Owing to the importance of phenolic compounds in plant defense under stress conditions, the current research was designed to assess the ameliorating role of vanillic acid on maize plants under chromium stress.

Table 1. Physicochemical properties.

Soil type	Sandy clay loam
Sand	46
Clay	32
Silt	22
pH	7.65
EC	2.92 dS m^{-1}
SO_4^2	6.59 mmol kg^{-1}
Na^+	3.8 mmol kg^{-1}
K^+	0.07 mmol kg^{-1}
Available Cr^{2+}	0.18 mg kg^{-1}

Materials and Methods

Soil analysis and growth conditions: The sandy clay loam soil was used for the present experiment. The physicochemical characteristics are presented in Table 1. The characteristics of the soil used for experimentation were determined following Abbas *et al.*, (2017). The Bouyoucos (1962) hydrometer method was used to measure soil particle size. However, soil pH, EC, sodium adsorption ratio (SAR) and soluble ions were analyzed by using methods of Page *et al.*, (1982). Bioavailable trace elements in the soil samples were calculated by extracting the samples following the method of Soltanpour (1985). The current experiment was planned to examine the role of exogenous treatment of vanillic acid in decreasing the negative effects of chromium stress on maize (cultivar Malka-2016) plants. This maize cultivar was developed primarily for high yield, protein, and fiber content (Nawaz *et al.*, 2019), but its tolerance to various stresses including Cr stress is not known yet. A completely randomized study (four replicates of each treatment) was set-up in the Botanical Garden of the Government College University, Faisalabad, Pakistan, during February to June, 2019. Each pot was filled with 8 kg sandy clay loam soil. The seeds were carefully sown at a rate of eight seeds per pot. After seed germination, thinning of plants was done to retain five seedlings per pot. After two-week growth, inorganic fertilizers were supplied at the rate of 2.14 mg kg^{-1} of potassium sulfate, 2.19 mg kg^{-1} of urea and 0.5 mg kg^{-1} of di-ammonium phosphate in each pot as a source of potassium, nitrogen and phosphorous, respectively. After four weeks of growth, varying levels of chromium (0, 100 and 500 µM) stress were applied in the soil medium. Then, after three weeks of chromium stress, three different levels of vanillic acid (0, 0.2 and 0.4 mg L^{-1}) were sprayed to plant leaves. Vanillic acid was sprayed four times during the whole experiment. First spray was done after seven weeks of germination, whereas second, third and fourth sprays were done after 9, 11, and 13 weeks of germination, respectively. All the times, the same volume, i.e., 25 mL per plant of each treatment of vanillic acid were applied to the foliage of plants grown in each pot by carefully avoiding the addition of VA to the soil. Then, data were collected for different attributes after three months and three weeks from the start of the treatments. The plants harvested and root and shoot dry weights were accurately measured.

Chlorophyll contents: Chlorophyll pigments were estimated following the method of Arnon (1949). The absorbance of the extract was recorded at 663, 645 and 480 nm.

Determination of free proline: Following Bates *et al.*, (1973), 0.5 g leaf sample was ground in 10 mL of 3% sulfosalicylic acid. The reaction mixture was shaken vigorously, and its absorbance recorded at 520 nm.

Determination of glycinebetaine: Dry leaves (each sample 0.5 g) were crushed in 10 mL of 5% toluene and the mixture was kept overnight at 4°C (Grieve & Grattan, 1983). To 1.0 mL of the supernatant, 2 *N* sulfuric acid solution was added. Then, to 0.5 mL of the mixture, 0.2 mL of potassium tri-iodide (KI_3) was added. After it, 2.8 mL of ice cooled distilled water and 6 mL of 1, 2 dichloroethane were added to it. Two layers appeared and lower layer was used to read at 365 nm.

Estimation of electrolyte leakage (EL), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA): Following Dionisio-Sese & Tobita (1989), the EL, Velikova *et al.*, (2000), hydrogen peroxide and the procedure of Cakmak and Horst (1991) were used to determine the lipid peroxidation (MDA) in the maize leaf and root samples.

Antioxidant enzyme activities: Fresh leaf (each 0.5 g) were ground each in 5 mL of potassium phosphate buffer of pH 7.8, centrifuged, and the supernatant was used for estimating the activities of antioxidant enzymes. The method of Giannopolitis & Ries (1977) was adopted to determine the activity of superoxide dismutase (SOD) and noted OD at 560 nm. The activities of catalase (CAT) and peroxidase (POD) were determined as described by Chance & Maehly (1955).

Gas exchange parameters: The method of Shakoor *et al.*, (2014) was employed to measure various gas exchange attributes such as photosynthetic rate, transpiration rate and stomatal conductance in maize plants using an Infra-Red Gas Analyzer (LCA-4, England). The data were recorded at maximum light intensity during day-time.

Determination of chromium (Cr): For the estimation of chromium concentration in plant shoots, by digesting the samples as reported by Rehman *et al.*, (2015). Then, the Cr level was estimated using an atomic absorption spectrophotometer (Shimadzu, *Model* AA-6300).

Statistical analysis

To work out if different treatments had a significant effect on various growth and physiological attributes, the data was subjected to ANOVA using SPSS. The Duncan's multiple range test was worked out at $p \leq 0.05$ to check significance of different treatments.

Table 2. Mean squares derived from analysis of variance (ANOVA) of data for growth and some key physio-biochemical attributes of maize plants subjected to foliar-applied varying levels of vanillic acid under different levels of chromium stress.

Source of variation	df	Root dry weight	Shoot dry weight	Chl. *a*	Chl. *b*	Transpiration rate
Chromium stress (Cr)	2	2.61***	12.53***	4.45***	1.67***	6.97***
Vinillic acid (VA)	2	1.657***	9.059***	1.240***	0.694***	2.067***
Cr x VA	4	0.0121ns	0.216ns	0.003ns	0.020ns	0.004ns
Error	18	0.028	0.125	0.016	0.018	0.030
		Photosynthetic rate	**Stomatal conductance**	**Root MDA**	**Leaf MDA**	**Root H_2O_2**
Chromium stress (Cr)	2	147.3***	3480.1***	998.1***	1348.6***	10685.0***
Vinillic acid (VA)	2	53.17***	1646.7***	987.7***	533.1***	7831.7***
Cr x VA	4	1.014ns	115.3ns	8.781ns	27.66***	91.77ns
Error	18	1.521	66.36	5.29	3.503	93.56
		Leaf H_2O_2	**Root EL**	**Leaf EL**	**Root proline**	**Leaf proline**
Chromium stress (Cr)	2	17125.7***	1617.2***	606.1***	771.1***	436.4***
Vinillic acid (VA)	2	70.98***	855.6***	663.6***	141.4***	152.1***
Cr x VA	4	312.6***	36.28**	5.441ns	1.616ns	1.737ns
Error	18	38.26	5.839	3.163	1.720	1.075
		Root glycinebetaine	**Leaf glycinebetaine**	**Root catalase**	**Leaf catalase**	**Root peroxidase**
Chromium stress (Cr)	2	7091.7***	7739.4***	2233.3***	750.7***	716.7***
Vinillic acid (VA)	2	2750.5***	1512.5***	817.6***	860.3***	195.5***
Cr x VA	4	36.37ns	48.33ns	26.68*	3.017ns	3.15*
Error	18	50.91	55.9	7.302	7.988	0.803
		Leaf peroxidase	**Root superoxide dismutase**	**Leaf superoxide dismutase**	**Cr in shoot**	
Chromium stress (Cr)	2	367.2***	1233.9***	3877.14***	26761.3***	
Vinillic acid (VA)	2	104.4***	2879.1***	1800.2***	3977.1***	
Cr x VA	4	1.052ns	34.67ns	44.0**	1032.4***	
Error	18	1.136	13.69	9.38	7.421	

*, **, ***= Significant at 0.05, 0.01 and 0.001, levels; ns, Non-significant

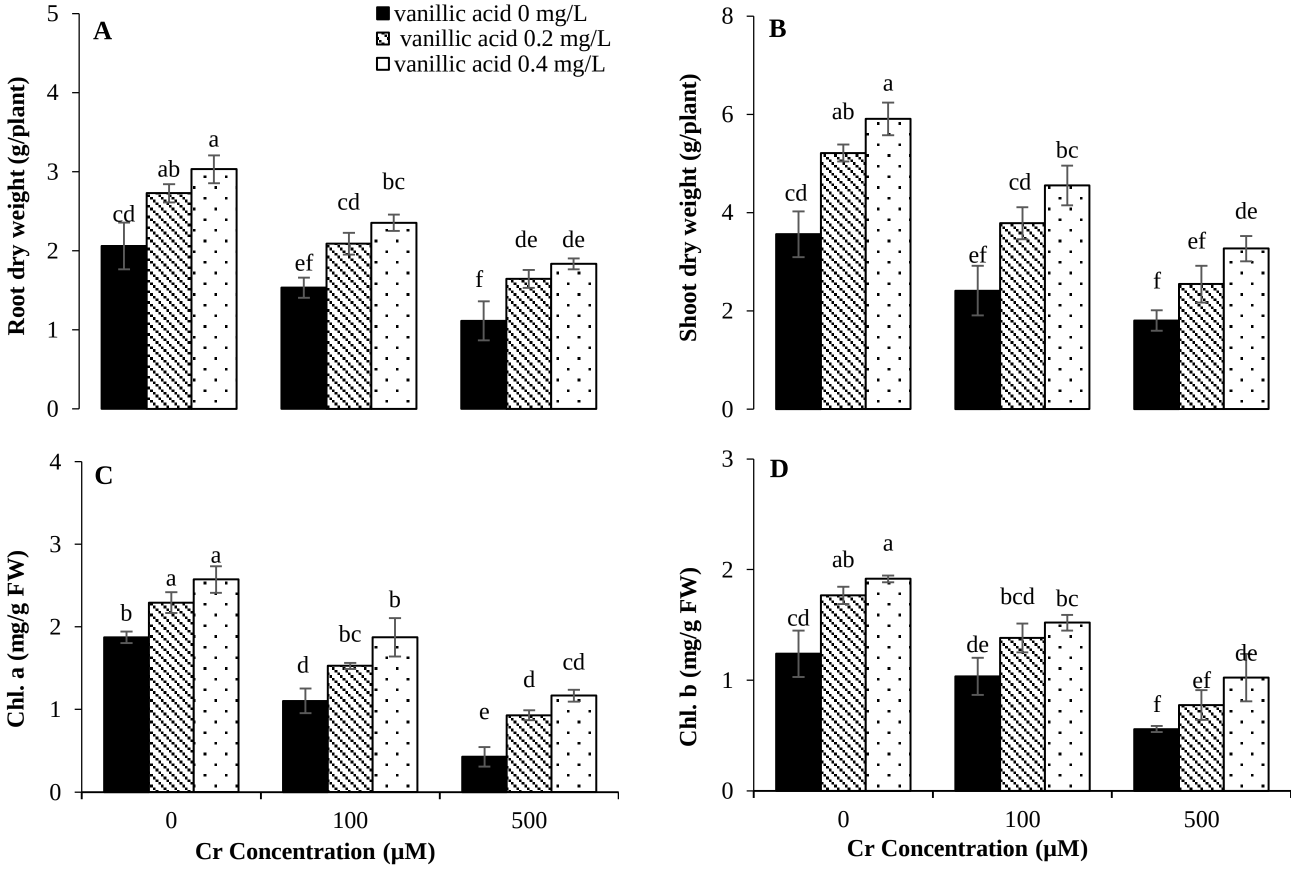

Fig. 1. Effect of foliar-sprayed varying levels of vanillic acid on root dry weight (A), shoot dry weight (B), chlorophyll *a* (C) and chlorophyll *b* (D) in maize (*Zea mays* L.) plants under different levels of chromium stress (Mean ± S.D.).

Results

Plant growth: The results showed that increasing concentration of chromium stress progressively reduced plant growth of the maize plants. The highest decrease in the above-mentioned parameters was noticed under 500 µM of chromium. Exogenous application of vanillic acid significantly improved shoot and root dry weights of all maize plants. The ameliorative effect of 0.4 mg L^{-1} VA on these growth attributes was considerably higher than that of 0.2 mg L^{-1} (Fig. 1; Table 2).

Assessment of chlorophyll pigments: Imposition of chromium (0, 100 and 500 µM) stress significantly reduced chlorophyll contents (chl. a and b) of maize cultivar Malka-2016 (Fig. 1; Table 2). However, the results showed that foliar-applied vanillic acid significantly improved the chlorophyll contents in both metal stressed and non-stressed plants. Exogenous application of vanillic acid markedly increased chl. a and chl. b contents in the maize plants grown under metal stress or non-stress regimes.

Assessment of gas exchange parameters: Of different gas exchange attributes, net photosynthetic rate, transpiration rate and stomatal conductance decreased considerably due to imposition of chromium stress (0, 100 and 500 µM). The results revealed that highest decrease in the above-mentioned attributes was noticed at 500 µM of chromium. However, vanillic acid spray significantly improved the above-mentioned photosynthetic attributes in the maize plants under both stress and control conditions. A marked ameliorative effect of vanillic acid on all gas exchange characteristics was found at 0.4 mg L^{-1} of foliar application treatment (Fig. 2; Table 2).

Effect of vanillic acid on EL, MDA and H_2O_2: Accumulation of MDA, as an indicator of lipid peroxidation, in leaf and root tissues increased markedly due to imposition of different levels of chromium stress (0, 100 and 500 µM). However, foliar-applied different concentrations of vanillic acid significantly reduced both leaf and root MDA contents in the maize plants (Fig. 3). The ameliorative effect of vanillic acid was observed to be considerably high when applied as 0.4 mg L^{-1}. Foliar-applied VA also significantly suppressed MDA in the maize plants exposed to Cr-stress. H_2O_2 and EL enhanced markedly in the maize plants due to imposition of chromium stress. However, vanillic acid as foliar spray significantly decreased both biochemical attributes in the maize plants under chromium stress.

Effect of vanillic acid on proline contents and glycinebetaine: The results showed that glycinebetaine and proline contents increased promisingly in the maize plants due to the imposition of chromium stress. However, a further increase was observed in their concentrations by foliar-sprayed vanillic acid to the maize plants grown under both control and stress conditions. A considerably high increase in both organic chemicals was noticed at the highest level of chromium (500 µM) and vanillic acid (0.4 mg L^{-1}) with respect to those in the control plants (Fig. 4; Table 2).

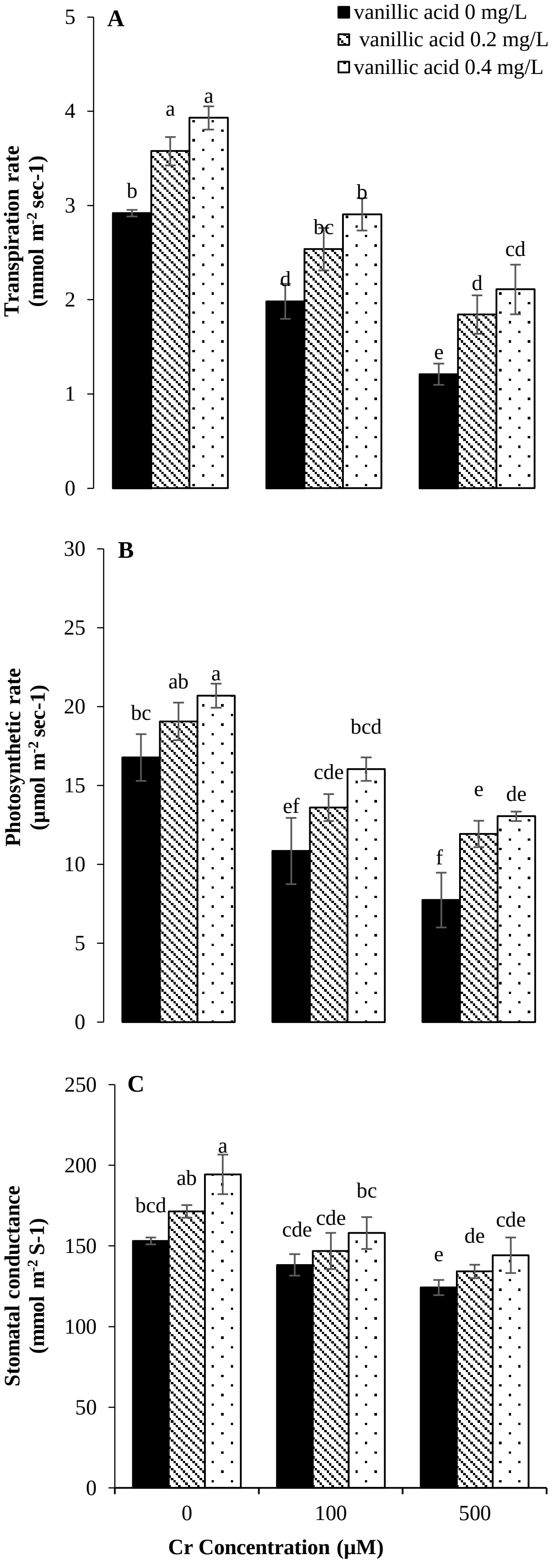

Fig. 2. Effect of foliar-sprayed varying levels of vanillic acid on transpiration rate (A), photosynthetic rate (B) and stomatal conductance (C) in maize (*Zea mays* L.) plants under different levels of chromium stress (Mean ± S.D.).

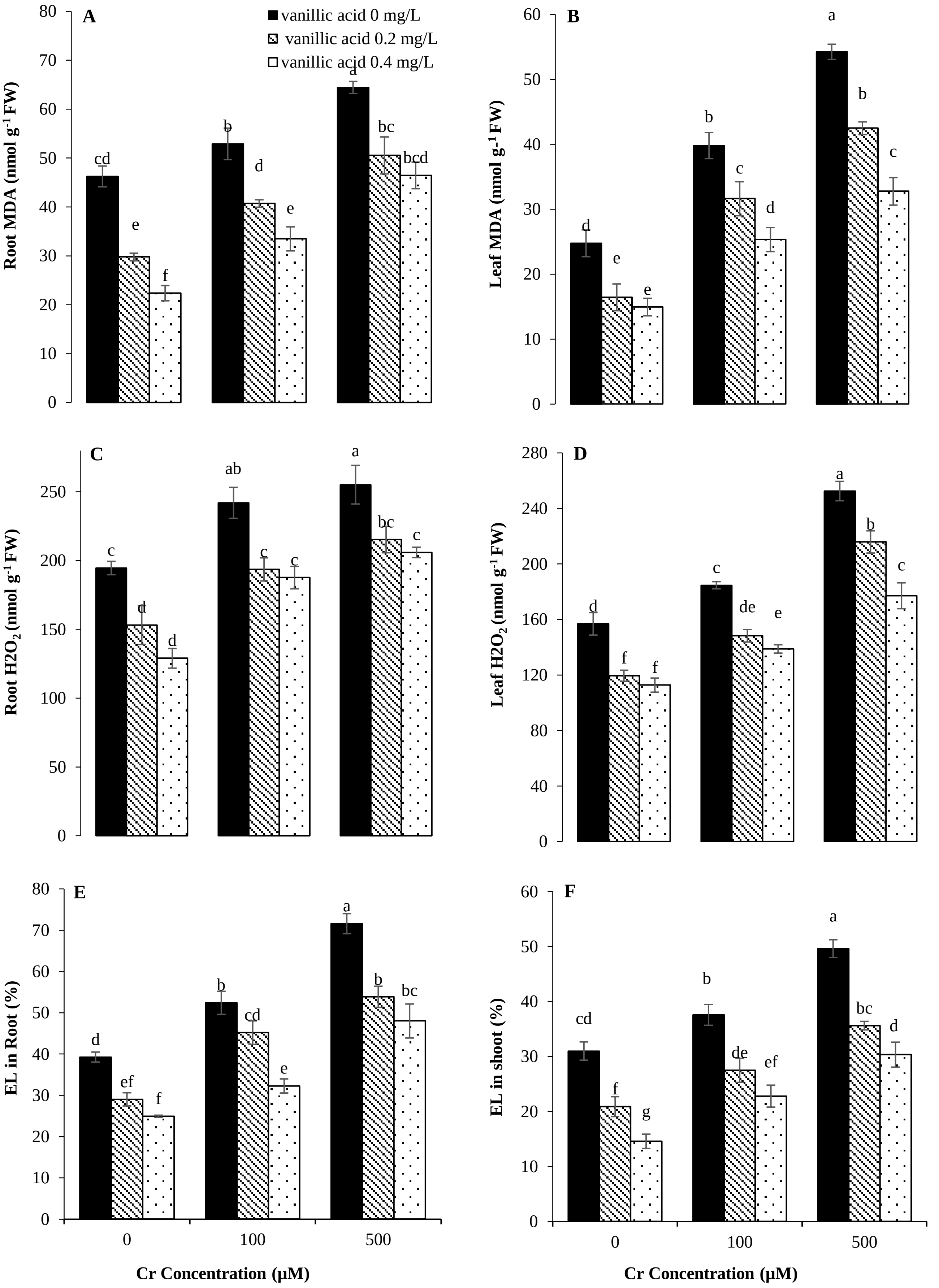

Fig. 3. Effect of foliar-sprayed varying levels of vanillic acid on root malondialdehyde (A), shoot malondialdehyde (B), root hydrogen peroxide (C), shoot hydrogen peroxide (D), root electrolyte leakage (E) and shoot electrolyte leakage (F) in maize (*Zea mays* L.) plants under different levels of chromium stress (Mean ± S.D.).

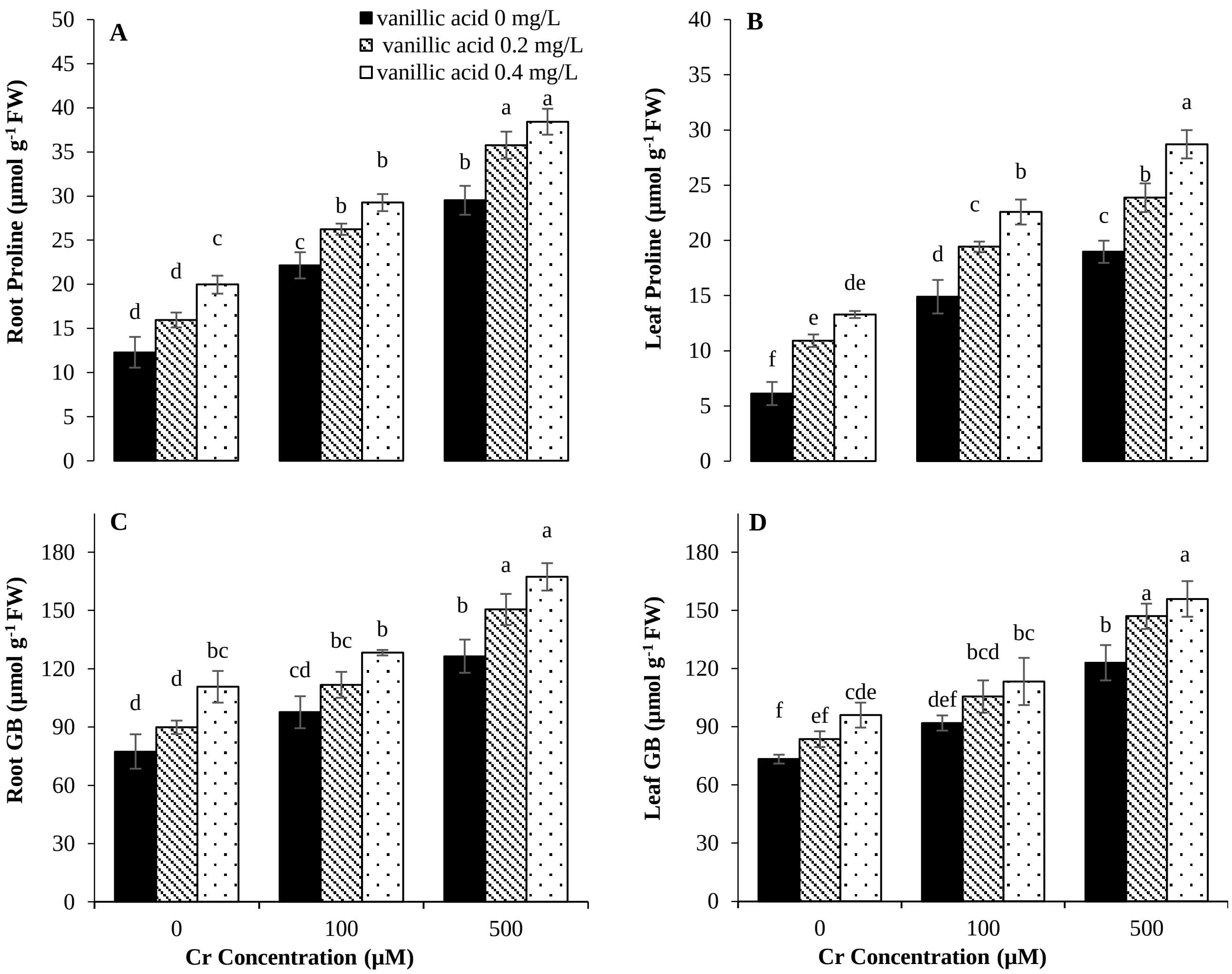

Fig. 4. Effect of foliar-sprayed varying levels of vanillic acid on root proline (A), shoot proline (B), root glycinebetaine (C) and shoot glycinebetaine (D) in maize (*Zea mays* L.) plants under different levels of chromium stress (Mean ± S.D.).

Effect of vanillic acid on antioxidant enzyme activities: Imposition of chromium stress significantly decreased the activities of SOD, POD and CAT in the maize plants (Fig. 5). However, exogenously applied vanillic acid as a foliar spray significantly accelerated the activities of all three enzymes in both metal-stressed and non-stressed maize plants. A maximal ameliorative effect of vanillic acid was observed on the activities of these enzymes at 0.4 mg L^{-1} of foliar treatment.

Assessment of chromium (Cr) concentration: Chromium (Cr) concentration in maize plant leaves increased significantly due to imposition of chromium (0, 100 and 500 µM) stress. The high Cr in the leaves was noticed at 500 µM of Cr stress. However, exogenous application of vanillic acid suppressed the tissue Cr concentration. The ameliorative effect of vanillic acid on tissue Cr concentration was considerably high at 0.4 mg L^{-1} of VA (Fig. 6; Table 2).

Discussion

In the recent study, shoot and root dry weights of maize plants decreased under chromium (Cr) stress (Fig. 1). Cr stress causes a marked decline in plant growth as observed in rice (Shahid *et al*., 2017), wheat (Ali *et al*., 2015a), and mung bean (Jabeen *et al*., 2016) plants. Suppression in plant dry weight could be due to low nutrient uptake as observed in rice (Shahid *et al*., 2017) under Cr toxicity. High levels of Cr may cause ultra-structural changes in leaves due to which plant growth could be adversely affected (Gill *et al*., 2015b). However, in the current study, foliar application of vanillic acid as a growth regulator to Cr-stressed maize plants considerably lessened the toxic effects of chromium. The role of vanillic acid as a natural antioxidant in plants under various stress conditions has been reported in different studies, e.g., Cd stress (Bhuyan *et al*., 2020), and salt stress (Parvin *et al*., 2020).

The current study also revealed that chlorophyll contents such as chlorophyll *a* and *b* as well as gas exchange attributes such as transpiration rate, photosynthetic activities and stomatal conductance decreased significantly in maize plants under Cr toxicity. Corresponding findings reported under Cr stress in various crops such as *V. radiata* (Jabeen *et al*., 2016), *H. annuus* (Farid *et al*., 2017), and *T. aestivum* (Ali *et al*., 2015b). These are in agreement with the results of another study where chlorophyll contents were shown to be declined in wheat plants with increasing concentration of lead in the growth medium (Lamhamdi *et al.*, 2013). Chlorophyll plays an important role in plant photosynthesis. Decrease in chlorophyll pigments might result in decline in photosynthetic rate in plants under chromium toxicity (Ali *et al.*, 2015b). However, the present study showed that VA considerably suppressed the toxic effects of Cr stressed maize plants. It was found that phenolic compounds such as caffeic acid, ferulic acid and vanillic acid used as allelochemicals, play a direct role in growth, survival, and self-defense of plants (Chon *et al.*, 2005), as observed in *Chenopodium murale* and tomato (Ghareib *et al.*, 2010). It has also been reported that foliar application of low concentration of vanillic acid increased chlorophyll in rice (Xuan & Khang, 2018).

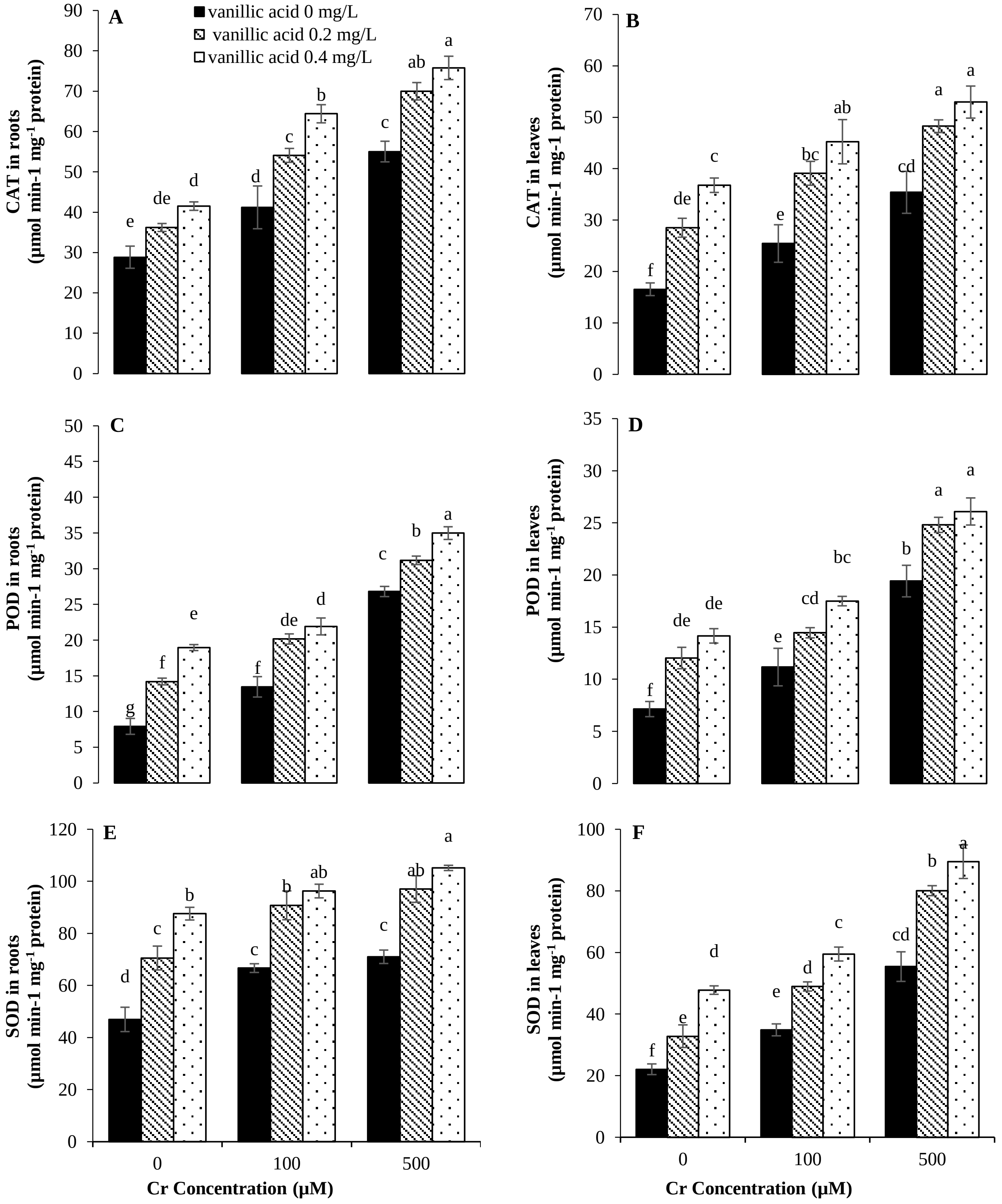

Fig. 5. Foliar application of varying levels of vanillic acid boosts the activities of catalase (CAT) in root (A) and shoot (B), peroxidase (POD) in root (C) and shoot (D) and superoxide dismutase (SOD) in root (E) and shoot (F) in maize (*Zea mays* L.) plant under different levels of chromium stress (Mean ± S.D.).

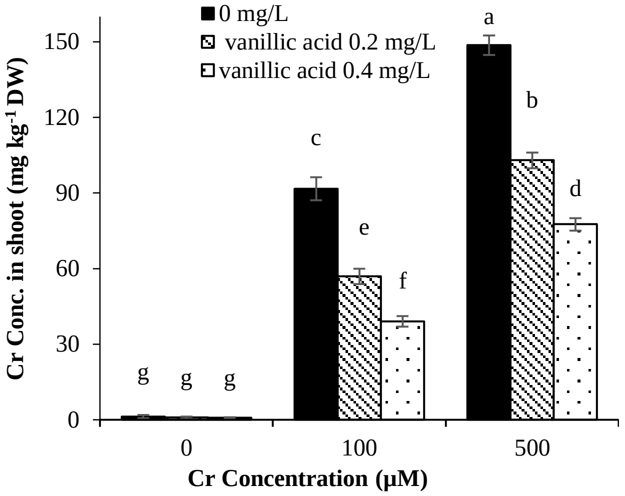

Fig. 6. Effect of foliar-sprayed varying levels of vanillic acid on shoot chromium concentration in maize (*Zea mays* L.) plants under different levels of chromium stress (Mean ± S.D.).

The hydrogen peroxide, electrolyte leakage and malondialdehyde increased in Cr-stressed maize plants. The H_2O_2 (hydrogen peroxide), EL (electrolyte leakage) and MDA (malondialdehyde) concentrations gradually increased in both roots and shoot of the maize plants with increase in external Cr levels. This increase might have been due to overproduction of reactive oxygen species (Shahid *et al*., 2017) under chromium stress. In stressed plant cells, lipid peroxidation is believed to occur frequently, which may cause severe injury to cells. MDA, an aldehydic compound, is formed by stress-induced lipid peroxidation (Liu *et al*., 2015; Davey *et al*., 2005). The lower concentration of MDA contents generally shows less damage to the cells under stress conditions (Lu *et al*., 2008). The results of our research exhibit that foliar-applied vanillic acid (a phenolic compound) improved tolerance level of the maize plants under chromium stress by reducing the MDA levels. It is imperative to note that vanillic acid application also lowered down the MDA levels in non-stressed maize plants. Thus, supplementation of vanillic acid could be beneficial for improving oxidative stress and hence growth of plants under stress and non-stress conditions. Among secondary metabolites, phenolic compounds belong to a very important class possessing anti-oxidative properties such as inhibition of lipid peroxidation in plants (Xuan & Khang, 2018).

An antioxidative enzyme system in plants generally consists of SOD, POD, CAT, APX etc. to overcome abiotic stresses by decreasing oxidative stress in plants (Damanik *et al*., 2010; Anandan *et al*., 2012). Basically, plants with high activation of antioxidants are believed to be able to considerably resist to stressful environments (Liu *et al*., 2010). However, our study exhibits that foliar application of vanillic acid increased the antioxidant enzyme activities under Cr-stressed and non-stressed conditions. The osmoprotectants like glycinebetaine play an effective role in plant defense system under stress conditions. In the present study, GB contents also increased with increasing concentration of Cr metal. Similar to our findings, GB content was found to be increased in barley (Nakamura *et al*., 2001; Muharramnejad *et al*., 2015) under drought stress. However, exogenous application of vanillic acid increased the concentration of glycinebetaine contents in the maize plants under chromium toxicity. The present study also shows that proline contents considerably increased in maize plants subjected to Cr stress. Analogous to our results, proline contents were reported to accumulate in oil-palm tree under water scarcity (Cao *et al*., 2011). However, foliar spray of vanillic acid further increased the concentration of proline contents in the metal stressed as well as non-stressed maize plants, in the present study.

The current study clearly reflects that translocation of Cr from the rooting medium to leaves increased with high soil Cr. Similar results have been reported previously in different plants, e.g., tobacco (Bukhari *et al*., 2015), and mustard (Gill *et al*., 2015c) plants. Foliar application of vanillic acid considerably reduced the transport of Cr from root to shoot of the maize plants in the current study. As a result, the deteriorating effects of chromium was reduced to some extent with the application of vanillic acid.

Vanillic acid treatment not only improved the growth and key physio-biochemical parameters of Cr-stressed maize plants, it also improved these attributes in the control (non-stressed) plants. Thus, it can be inferred that vanillic acid application can be beneficial to improve growth of plants exposed to metal stress conditions as well as those growing under normal non-stressed conditions. Such a chemical with dual benefit could be beneficial for farmers.

Conclusion

The present study concluded that Cr contamination in soil medium could impair the morphological, physiological, and biochemical parameters of maize plants. Our findings revealed that increasing concentration of Cr stress in soil progressively increased the accumulation of Cr that caused suppression in overall growth (shoot and root dry weight) of maize plants. However, exogenous spray of vanillic acid as a growth regulator markedly improved all the physiological, morphological, and biochemical processes in both the metal stressed and non-stressed maize plants. Furthermore, vanillic acid also improved anti-oxidative enzyme properties thereby decreasing the chromium-induced oxidative stress.

References

Abbas, T., M. Rizwan, S. Ali, M. Zia-ur-Rehman, M.F. Qayyum, F. Abbas, F. Hannan, J. Rinklebe and Y.S. Ok. 2017. Effect of biochar on cadmium bioavailability and uptake in wheat (*Triticum aestivum* L.) grown in a soil with aged contamination. *J. Ecotoxicol. Environ. Saf.*, 140: 37-47.

Abd El-Mageed, T.A., M.A. Ahmed, M.A. Mahmoud and H.A. Mohamed. 2017. Combined effect of deficit irrigation and potassium fertilizer on physiological response, plant water status and yield of soybean in calcareous soil. *J. Arch. Agron. Soil Sci.*, 63: 827-840.

Ali, S., A. Chaudhary, M. Rizwan, H.T. Anwar, M. Adrees, M. Farid, M.K. Irshad, T. Hayat and S.A. Anjum. 2015b. Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). *J. Environ. Sci. Pollut. Res.*, 22, 10669-10678.

Ali, S., S.A. Bharwana, M. Rizwan, M. Farid, S. Kanwal, Q. Ali, M. Ibrahim, R.A. Gill and M.D. Khan. 2015a. Fulvic acid mediates chromium (Cr) tolerance in wheat (*Triticum aestivum* L.) through lowering of Cr uptake and improved antioxidant defense system. *J. Environ. Sci. Pollut. Res*., 22: 10601-10609.

Almeida, I.V., F.M.L. Cavalcante and V.E.P. Vicentini. 2016. Different responses of vanillic acid, a phenolic compound, in HTC cells: cytotoxicity, antiproliferative activity, and protection from DNA-induced damage. *Genet. Mol. Res*., 19, DOI: 10.4238/gmr15049388.

Anandan, A. and P. Arunachalam. 2012. Relative proportion of antioxidative enzyme activity in locally grown Indian rice cultivars (*Oryza sativa* L.) under submergence condition. *J. Plant Interact*., **7**: 183-192.

Anonymous. 2017. Crop Statistics, FAOSTAT. Rome (Italy): Food and Agriculture Organization of the United Nations (FAO). 2017.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoxidase in *Beta vulgaris* L. *J. Plant Physiol*., 2: 1-15.

Bates, L.S., R.P. Waldren and L.D. Teare. 1973. Rapid determination of free proline for water stress studies. *J. Plant Soil*, 39: 205-207.

Batish, D.R., K. Lavanya, H. Pal Singh and R.K. Kohli. 2007. Root-mediated allelopathic interference of nettle-leaved goosefoot (*Chenopodium murale*) on wheat (*Triticum aestivum*). *J. Agron. Crop Sci*., 193: 37-44.

Bhuyan, M.H.M., K. Parvin, S.M. Mohsin, J.A. Mahmud, M. Hasanuzzaman and M. Fujita. 2020. Modulation of cadmium tolerance in rice: Insight into vanillic acid-induced upregulation of antioxidant defense and glyoxalase systems. *Plants* (Basel), 9: 188.

Bouyoucos, G.J. 1962. Hydrometer method improved for making particle size analyses of soils. *J. Agron*., 54: 464-465.

Bukhar, S.A.H., R. Wang, W. Wang, I.M. Ahmed, W. Zheng and F. Cao. 2016. Genotype dependent effect of exogenous 24-epibrassinolide on chromium-induced changes in ultrastructure and physicochemical traits in tobacco seedlings. *J. Environ. Sci. Pollut. Res*., 23: 18229-18238.

Cakmak, I. and J.H. Horst. 1991. Effect of Aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max* L.). *J. Plant Physiol*., 83: 463-468.

Cao, H.X., C.X. Sun, H.B. Shao and X.T. Lei. 2011. Effects of low temperature and drought on the physiological and growth changes in oil palm seedlings. *Afr. J. Biotech*., 10: 2630-2637.

Chance, B. and A.C. Maehly. 1955. Assay of catalases and peroxidases. *J. Meth. Enzymol*., 2: 764-775.

Chen, Q., X. Zhang, Y. Liu, J. Wei, W. Shen, Z. Shen and J. Cui. 2017. Heminmediate alleviation of zinc, lead and chromium toxicity is associated with elevated photosynthesis, antioxidative capacity; suppressed metal uptake and oxidative stress in rice seedlings. *J. Plant Growth Regul*., 81: 253-264.

Chon, S., H. Jang, D. Kim, Y. Kim, H. Boo and Y. Kim. 2005. Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *J. Hortic. Sci*., 106: 309-317.

Damanik, R.I., M. Maziah, M.R. Ismail, S. Ahmad and A.M. Zain. 2010. Responses of the antioxidative enzymes in Malaysian rice (*Oryza sativa* L.) cultivars under submergence condition. *Acta Physiol. Plant*., 32: 739-747.

Davey, M.W., E. Stals, B. Panis, J. Keulemans and R.L. Swennen. 2005. High-throughput determination of malondialdehyde in plant tissues. *Anal. Biochem*., 347: 201-207.

Dionisio-Sese, M.L. and S. Tobita. 1998. Antioxidant responses of rice seedlings to salinity stress. *J. Plant Sci*., 135: 1-9.

Ertani, A., A. Mietto, M. Borin and S. Nardi. 2017. Chromium in agricultural soils and crops: a review. *Water Air Soil Poll*., 228: 1-12.

Farid, M., S. Ali, N.A. Akram, M. Rizwan, F. Abbas, S.A.H. Bukhari and R. Saeed. 2017. Phyto-management of Cr-contaminated soils by sunflower hybrids: physiological and biochemical response and metal extractability under Cr stress. *J. Environ. Sci. Pollut. Res*., http://dx.doi.org/ 10.1007/ s11356-017-9247-3.

Ghareib, H.R.A., M.S. Abdelhamed and O.H. Ibrahim. 2010. Antioxidative effects of acetone fraction and vanillic acid from *Chenopodium murale* on tomato plants. *Weed Biol. Manag*., 10: 64-72.

Giannopolitis, C.N. and S. Ries. 1977. Superoxide dismutase. 1. Occurrence in higher plants. *Plant Physiol*., 59: 309-314.

Gill, R.A., B. Ali, F. Islam, M.A. Farooq, M.B. Gill, T.M. Mwamba and W. Zhou. 2015a. Physiological and molecular analyses of black and yellow seeded *Brassica napus* regulated by 5-aminolivulinic acid under chromium stress. *Plant Physiol. Biochem*., 94: 130-143.

Gill, R.A., L. Zang and B. Ali. 2015c. Chromium-induced physiochemical and ultrastructural changes in four cultivars of *Brassica napus* L. plant. *Chemosphere*., 120: 154-164.

Gill, S.S., N.A. Anjum, R. Gill, S. Yadav, M. Hasanuzzaman, M. Fujita, P. Mishra, S.C. Sabat and N. Tuteja. 2015b. Superoxide dismutase mentor of abiotic stress tolerance in crop plants. *J. Environ. Sci. Pollut. Res*., 22: 10375-10394.

Gnanasekaran, N. and S. Kalavathy. 2017. Drought stress signal promote the synthesis of more reduced phenolic compounds (chloroform insoluble fraction) in *Tridax procumbens*. *Free Radic. Antiox.*, 7: 128-136.

Gomes, M.A.D., R.A. Hauser-Davis, M.S. Suzuki and A.P. Vitória. 2017. Plant chromium uptake and transport, physiological effects and recent advances in molecular investigations. *Ecotoxicol. Environ. Saf*., 140: 55-64.

Grieve, C.M. and S.R. Grattan. 1983. Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil*, 70: 303-307.

Hegab, M.M. 2005. Assessment of the allelopathic effect of some phenolic compounds on some physiological processes of pea plant (*Pisum sativum*) (PhD thesis). Faculty of Science, Beni-Sueif University, Beni-Sueif, Egypt.

Hussain, A., S. Ali, M. Rizwan, M.Z. Abdur Rehman, A. Hameed, F. Hafeez and L. Wijaya. 2018. Role of zinc-lysine on growth and chromium uptake in rice plants under Cr stress. *J. Plant Growth Regul*., 37: 1413-1422.

Jabeen, N., Z. Abbas, M. Iqbal, M. Rizwan, A. Jabbar, M. Farid, S. Ali, M. Ibrahim and F. Abbas. 2016. Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. *Arch. Acker. Pflazenbau. Bodenkd*., 62: 648-662.

Julkunen-Titto, R. 1985. Phenolic constituents in the leaves of northern willows methods for the analysis of certain phenolics. *J. Agric. Food Chem*., 33: 213-217.

Kaya, C., M. Ashraf and O. Sonmez. 2018. Combination of nitric oxide and thiamin regulates oxidative defense machinery and key physiological parameters in salt-stressed plants of two maize cultivars differing in salinity tolerance. *Adv. Agric. Sci*., 6: 34-44.

Keskitalo, M. 2003. Crop plants a: Proceedings of the NJF's 22nd Congress "Nordic Agriculture in Global Prespective, 1-4. http://www.Nif.dk/nif/reports/nifreports.htm.

Kleiner, K.W., K.F. Raffa and R.E. Dickson. 1999. Partitioning of 14C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover. *Oecologia*, 119: 408-418.

Lamhamdi, M., O. El Galiou, A. Bakrim, J.C. Nóvoa-Muñoz, M. Arias-Estévez, A. Aarab and R. Lafont. 2013. Effect of lead stress on mineral content and growth of wheat (*Triticum aestivum*) and spinach (*Spinacia oleracea*) seedlings. *Saudi J. Biol. Sci*., 20: 29-36.

Liu, A., S. Chen, Y. Mi, Z. Zhou and G.J. Ahammed. 2010. Effects of hypoxia stress and different level of Mn^{2+} on antioxidant enzyme of tomato seedlings. *Amer. J. Plant Sci*., 1: 24-31.

Liu, M., M. Chu, Y. Ding, S. Wang, Z. Liu, S. Tang and G. Li. 2015. Exogenous spermidine alleviates oxidative damage and reduce yield loss in rice submerged at tillering stage. *Front. Plant Sci*., 6: 919.

Lu, P., W.G. Sang and K.P. Ma. 2008. Differential responses of the activities of antioxidant enzymes to thermal stresses between two invasive *Eupatorium* species in China. *J. Integr. Plant Biol*., 50: 393-401.

Ma, J., C. Lv, M. Xu, G. Chen, C. Lv and Z. Gao. 2016. Photosynthesis performance, antioxidant enzymes, and ultrastructural analyses of rice seedlings under chromium stress. *J. Environ. Sci. Pollut. Res*., 23: 1768-1778.

Macias, F.A., A.M. Simonet, J.C.G. Galindo and D. Castellano. 1999. Bioactive phenolics and polar compounds from *Melilotus messanensis*. *Phytochemistry*., 50: 35-46.

Malcovska, S.M., Z. Ducaiova and M. Backor. 2014. Impact of silicon on maize seedlings exposed to short-term UV-B irradiation. *Biology*., 69: 1349-1355.

Moharramnejad, S., O. Sofalian, M. Valizadeh, A. Asgari and M. Shiri. 2015. Proline, glycine betaine, total phenolics and pigment contents in response to osmotic stress in maize seedlings. *J. Biosci. Biotechnol*., 4: 1245-1250.

Nakamura, T., M. Nomura, H. Mori, A.T. Jagendroff, A. Ueda and T. Takabe. 2001. An isozyme of betaine aldehyde dehydrogenase in barley. *Plant Cell Physiol*., 42: 1088-1092.

Nawaz, K., M. Shahid, F. Ahmad, M. Sagheer, Mansoor-ul-Hasan, M.A. Saleem, U. Naeem-Ullah and M. Sadique. 2019. Assessment of resistant varieties of maize against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in laboratory conditions. *J. Agric. Sci*., 3: 6-8.

Page, A.L., R.H. Miller and D.R. Keeny. 1982. Methods of soil analysis (part 2). ASA and SSSA, Madison.

Parvin, K., K. Nahar, M. Hasanuzzaman, M.B. Bhuyan, S.M. Mohsin and M. Fujita. 2020. Exogenous vanillic acid enhances salt tolerance of tomato: insight into plant antioxidant defense and glyoxalase systems. *Plant Physiol. Biochem*., 150: 109-120.

Rehman, M.Z., M. Rizwan, A. Ghafoor, A. Naeem, S. Ali, M. Sabir and M.F. Qayyum. 2015. Effect of inorganic amendments for in situ stabilization of cadmium in contaminated soils and its phyto-availability to wheat and rice under rotation. *J. Environ. Sci. Pollut. Res*., 22: 16897-16906.

Rizvi, A. and M.S. Khan. 2019. Heavy metal-mediated toxicity to maize: oxidative damage, antioxidant defence response and metal distribution in plant organs. *Int. J. Environ. Sci. Tech*., 16: 4873-4886.

Robards, K., P.D. Prenzler, G. Tucker, P. Swatsitang and W. Glover. 1999. Phenolic compounds and their role in oxidative processes in fruits. *Food Chem*., 66: 401-436.

Shahid, M., S. Shamshad, M. Rafiq, S. Khalid, I. Bibi, N.K. Niazi, C. Dumat and M.I. Rashid. 2017. Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. *Chemosphere*, 178: 513-533.

Shakoor, M.B., S. Ali, A. Hameed, M. Farid, S. Hussain, T. Yasmeen, U. Najeeb, S.A. Bharwana and G.H. Abbasi. 2014. Citric acid improves lead (Pb) phytoextraction in *Brassica napus* L. by mitigating Pb-induced morphological and biochemical damages. *Ecotoxicol. Environ. Saf*., 109: 38-47.

Sharma, N., N. Tiwari, M. Vyas, N. Khurana, A. Muthuraman and P. Utreja. 2020. An overview of therapeutic effects of vanillic acid. *Plant Arch*., 20(2): 3053-3059.

Soltanpour, P.N. 1985. Use of AB-DTPA soil test to evaluate elemental availability and toxicity. *Commun. Soil Sci. Plant Anal*., 16: 323-338.

Velikova, V., I. Yardanov and A. Edreva. 2000. Oxidative stress and some antioxidants systems in acid rain treated bean plants: protective role of exogenous polyamines. *Plant Sci*., 151: 59-66.

Wahid, A. and A. Ghazanfar. 2006. Possible involvement of some secondary metabolites in salt tolerance of sugarcane. *Plant Physiol*., 163: 723-730.

Xuan, T.D. and D.T. Khang. 2018. Effects of exogenous application of protocatechuic acid and vanillic acid to chlorophylls, phenolics and antioxidant enzymes of rice (*Oryza sativa* L.) in submergence. *J. Mol*., 620: *23* https://doi.org/10.3390/molecules23030620.

Zhang, Z., L. Liao, J. Moore, T. Wu and Z. Wang. 2008. Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.). *Food Chem*., 113: 160-165.

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