EXOGENOUSLY APPLIED GLYCINEBETAINE ALLEVIATES CHROMIUM TOXICITY IN PEA BY REDUCING CR UPTAKE AND IMPROVING ANTIOXIDANT DEFENSE SYSTEM

FAISAL, M.[1] – IMRAN, K.[2] – MUHAMMAD, B. C.[3] – RIZWAN, M.[2] – ATHAR, M.[2] – RASHID, W. K.[4] – ARBAB, J.[5] – HAIFA, A. S. A.[6] – SABRY, H.[7] – SAJID, U.[8] – MUHAMMAD, U. H.[9] – SAMEER, H. Q.[10*]

[1]Department of Environmental Sciences & Engineering, Government College University, Faisalabad, Pakistan

[2]Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan

[3]Department of Agronomy, Faculty of Agricultural Sciences, University of the Punjab Lahore, Lahore 54590, Pakistan

[4]Insititute of Horticultural Sciences, University of Agriculture, Faisalabad 38040, Pakistan

[5]Agronomy (Forage Production) Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan

[6]Biology Department, College of Science, Jouf University, Sakaka 2014, Saudi Arabia

[7]Department of Biology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia

[8]Insititute Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan

[9]Research Center on Ecological Sciences, Jiangxi Agricultural University, Nanchang 330045, China

[10]Department of Biology, Al-Jumum University College, Umm Al-Qura University, Makkah 21955, Saudi Arabia

**Corresponding author*
e-mail: shqari@uqu.edu.sa

(Received 27th Mar 2022; accepted 26th Jul 2022)

Abstract. Soils with chromium (Cr) pollution are significantly increasing globally. Therefore, proper measures should be opted for to restrict its entry into food crops. *Glycinebetaine* (GB)-induced tolerance in plants against different abiotic stresses has been well documented, but still little evidence is available concerning its potential to increase the tolerance of pea against Cr stress. Therefore, this study determined the impact of GB application in increasing Cr tolerance in pea (*Pisum sativum*). The experiment was comprised of three concentrations of Cr (0 mM, 0.25 mM, and 0.50 mM) and two levels of foliar-applied GB (0 and 50 mM). Chromium stress led to a significant reduction in growth, biomass production, and photosynthetic pigments however, Cr stress increased reactive oxygen species (ROS) production. Exogenously applied GB reduced the deleterious impacts of Cr stress on pea and increased growth, biomass production, and photosynthetic pigments. Moreover, GB reduced ROS production and Cr accumulation in plant roots and shoots by increasing the activities of antioxidant enzymes (APX, CAT, POD and SOD) and consequently increased growth and plant biomass production. In conclusion, beneficial effects of GB under Cr stress were attributed to reduced Cr uptake, enhanced photosynthetic pigment and enzymatic activities and less ROS production.

Keywords: *chromium, glycinebetaine, growth, photosynthetic pigments, ROS*

DOI: http://dx.doi.org/10.15666/aeer/2005_42954307

Introduction

Chromium magnification in agricultural soils has become a serious concern worldwide owing to its non-degradable nature and unfavorable consequences for plant development and productivity (Singh and Gautam, 2013). Cr is considered as a non-essential element for plants and exposure of plants to Cr stress cause significant reduction in plant growth (Samantaray et al., 2015; El-Baz et al., 2021). It enters into the soil through different sources i.e., bed rocks, volcanoes, human activities, agricultural use of domestic and municipal wastewaters, electro-plating, tanning and mining (Ali et al., 2015; Medda and Mondal, 2017; Hassan et al., 2021). The pernicious consequences of Cr stress on plants have been widely documented by many researchers (Hayat et al., 2012; Ahmad et al., 2020). Chromium toxicity in plants decreased germination, growth, synthesis of photosynthetic pigments and biomass production, induced chlorosis, altered enzymatic activities, and caused ultra-structural changes in the plant cell membrane and chloroplasts (Ghani and Ghani, 2011; Yin et al., 2021). Moreover, Cr toxicity in plants also alters mineral nutrition and reduces the process of photosynthesis, and thereby leads to serious reduction in crop yield (Mushtaq et al., 2021). Additionally, Cr also induced ROS production in plants, which reduced the plant’s performance by the oxidation of important molecules i.e., proteins and lipids (Pandey et al., 2012). Plants possess excellent antioxidant system consisting of antioxidants including ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) in order to get protection from oxidative stress (Sofo et al., 2015). The response of antioxidants in plants depends upon plant species and stress faced by the plants. Nonetheless, Cr stress reduced the activity of different antioxidant enzymes and led to a serious reduction in crops productivity (Adrees et al., 2015; Gautam et al., 2020).

The current increase in global population demands a substantial increase in the crop in the scenarios of rapid climate change and different abiotic stress (Hassan et al., 2017, 2020a, b; Rasheed et al., 2020). Pea (*Pisum sativum* L.) is an imperative legume crop of tropics and subtropics and is an imperious source of protein, minerals, carbohydrates, vitamins, and antioxidants (Dahl et al., 2012). In Pakistan, pea crop is cultivated in the winter season and is used as food and fodder crop. Pakistan is facing severe problems of decreasing agricultural land due to the increasing human population, and it is estimated that Pakistan’s population would double by 2040 (Anjum et al., 2020). Therefore, it is necessary to increase crop production to meet the demands of the booming population. Phytoextraction of Cr using hyper-accumulator plant species is not a viable option for the remediation of Cr contaminated soils, especially for countries like Pakistan which is already facing population and food pressures. Thereby, the productive soils must be used for the cultivation of food crops to meet the needs of the population, whilst alternative measures should be adopted to reduce Cr toxicity and its accumulation in food crops.

Glycinebetaine (GB) is a non-toxic, water-soluble, and environmentally friendly agent. Elevated concentrations of GB have been reported to accumulate in plants under different stress conditions (Hassan et al., 2019; Ahmad et al., 2020). Nonetheless, GB accumulated by plants under various stresses is not enough to protect them from stresses. Surprisingly, exogenously applied GB has the potential to reduce harmful effects of these stresses on the plant (Hossain et al., 2010). Likewise, foliar-applied GB improved the drought and salinity stress tolerance in wheat (*Triticum aestivum*), rice (*Oryza sativa*) and maize (*Zea mays*) by increasing antioxidant activities, photosynthetic efficiency and decreasing oxidative stress through ROS scavenging (Sofy et al., 2020; Dustgeer et al., 2021). Exogenously applied GB increased wheat growth, photosynthetic pigments and decreased Cr toxicity and its accumulation by improving antioxidant activities (Ali et al., 2015). Likewise, in another study, Jabeen et al. (2016) noticed that GB application enhanced the Cr tolerance in mungbean (*Vigna radiata*) by reducing its uptake and enhancing antioxidant enzyme activities. However, no study has been performed where the significance of GB regarding the alleviation of Cr toxicity in pea has been reported. Therefore, we postulated that exogenously applied GB can reduce the Cr toxicity in pea plants by reducing Cr uptake and improving the activity of different antioxidant enzymes. This study investigated the beneficial role of exogenously applied GB on pea growth, morphology, photosynthetic pigments, Cr uptake and antioxidant activities grown in Cr stress conditions.

Materials and methods

Experimental site

The pot study was performed in the greenhouse area of the Faculty of Agriculture, University of Agriculture, Faisalabad in 2017. The soil was collected from the horticultural farm area from a depth of 0-20 cm and analyzed to determine the different physio-chemical properties. The pots with a diameter of 28 cm and a depth of 31 cm were filled with 8 kg of soil and 5 seeds of pea (Pea-2009) were sown in each pot. The soil physicochemical analysis revealed that the soil had sandy loam texture with organic matter (OM) 0.81%, pH, 7.7, nitrogen (N) 0.03%, phosphorus (P) 6.30 mg kg^{-1} and potassium (K) 177 mg kg^{-1}.

Experimental details

Three different levels of Cr stress i.e., 0 mM, 0.25 mM, and 0.50 mM were applied via utilizing $K_2Cr_2O_7$, while two levels of foliar-applied GB i.e., 0 and 50 mM were applied via dissolving GB in 0.1% tween-20 solutions. These levels of treatment were chosen after conducting a series of experiments to determine the toxic levels of Cr and to optimize the best level of GB application. $K_2Cr_2O_7$ were purchased from local market of Faisalabad. Chromium stress was applied with irrigation water after 20 days of plant emergence. Later, foliar application of GB was applied using a handheld sprayer according to treatments after 7 days of Cr stress. Moreover, a completely randomized design with factorial arrangements having three replications was used to perform the study. Experimental pots were regularly visited and carefully watered as per crop requirements. The pots were once fertilized with 160 mg of N and 240 mg of each P and K in the form of urea (46% N), single superphosphate (21% P) and sulfate of potash (50% K). The weeds grown in pots were manually uprooted and no attack of insects and diseases was noted during the study.

Observations

Growth parameters

Three plants were marked and manually uprooted from pots 10 days post GB application their roots and shoots were separated and their lengths were measured and averaged. Likewise, harvested plant roots and shoots were weighed separately on the balance to determine the root and shoot fresh weights. The cumulative length of roots was taken for the determination of root length. Moreover, three plants were selected in each pot and leaves were counted and plant height was measured.

Estimation of photosynthetic pigments

For the measurements of chlorophyll and carotenoid contents, plant samples were homogenized in an 80% solution of acetone and absorbance was noted with a spectrophotometer **(**PerkinElmer AAnalyst™ 800**)** at three different wavelengths (663, 645 and 480 nm) and chlorophyll and carotenoid contents were determined by the methods of Arnon (1949).

Appraisal of the antioxidant enzyme activities

Peroxidase (POD) activities were determined by the standard procedures of Zhang (1992). Plant samples (0.5 g) were homogenized in 5 ml of potassium buffer (50 mM) and centrifuged at 15,000 rpm and 4 °C and absorbance was noted at 470 nm. For SOD determination the 1 mL reaction mixture constituted 10 mM pyrogallol, 50 mL extract of the enzyme, 10 mM EDTA, and 50 mM sodium-phosphate buffer having a pH of 7.8. SOD activity in the reaction mixture was measured at 420 nm (Roth and Gilbert, 1984). Catalase (CAT) and ascorbate peroxidase (APX) activities were determined by the standard methods of Aebi (1984) and Nakano and Asada (1981). For CAT determination, we took 3 mL assay mixture that contained 100 μL of enzyme extract, 100 μL H_2O_2 (300 mM), 2.8 mL phosphate buffer (50 mM) with 2 mM CA having a 7.0 pH, afterwards CAT activity was noted at 240 nm. For APX determination the mixture consisted of 100 μL enzyme extracts, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL potassium buffer (25 mM) 2 mM CA having a 7.0 pH. The activities of APX enzyme were measured with variations in wavelength at 290 nm.

Assessment of H_2O_2 and MDA contents

Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were determined by the standard methods of Velikova et al. (2000) and Dhindsa et al. (1981). For H_2O_2 determination, 0.5 g of plant samples were taken and homogenized on an ice bath in 1 mL of 0.1% TCA. After this supernatant (0.5 mL) was reacted with 0.5 mL of 50 mM PP buffer (pH = 7.5) and 1 mL potassium iodide (IM). The spectrophotometer was used to measure the absorbance at 390 nm wavelength. For MDA determination 1 g of plant sample was taken and homogenized in 10%, 8 ml trichloroacetic acid and centrifuged for 20 min at 4000 rpm. After that 2 ml of thiobutaric acid (60%) were mixed in the plant extracts and heated for 20 min at 100 °C and then cooled quickly with ice cubes for 20 min and centrifuged for 10 min at 10,000 ×*g*. Then the spectrometer was used to measure the absorbance at different wavelengths (450, 532, 600 nm).

Determination of Cr concentrations in shoots and roots of pea plant

Root and shoot samples were initially air-dried and stored and later oven dried. After oven drying, 0.5 g of each plant part was digested on hot plate by adding a mixture of HNO_3 and $HClO_4$ in 2:1 ratio (Jones and Case, 1990). Afterwards Cr concentration in plant parts was determined by atomic absorption spectrometer.

Statistical analysis

Fisher’s analysis of variance was done for all the recorded data using CoStat6.2 (Monterey, USA) to analyze the date and least significant difference (LSD) test was utilized for comparing significant differences among the treatment means at $p \leq 0.05$ (Steel et al., 1996).

Results

Growth attributes

Chromium stress had deleterious effects on the growth and morphological traits of pea. Plant height and number of leaves were significantly decreased with increasing Cr concentrations. However, foliar-applied GB (50 mM) considerably increased the plant height and the number of leaves in normal as well as Cr stressed plants (*Table 1*). A considerable reduction in leaf length, leaf width, and root length was observed in pea plants under Cr stress. Foliar spray of GB increased the leaf length by 17%, leaf width 30%, and root length 11% under Cr stress (50 mM), compared to control. The results depicted that Cr considerably decreased the root and shoot fresh weights as compared to control treatment. However, in the case of foliar-applied GB, we noted a significant increase in root (67%, 56%) and shoot fresh weight (36%, 37%) under Cr stress (25 mM, 50 mM) as compared to control (*Table 1*).

Table 1. *Effect of GB application on plant height, leaf length, leaf width, fresh weight of shoot and root, number of leaves and root length of pea under different levels of Cr stress*

Cr stress (mM)	GB (mM)	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Shoot FW (g)	Root FW (g)	Leaves per plant	Root length (cm)
0	0	36.3 ± 12.1b	5.80 ± 1.90b	2.90 ± 1.67cd	7.63 ± 2.54b	0.34 ± 0.11b	35.0 ± 0.4c	27.0 ± 9.01b
	50	42.6 ± 0.40a	6.30 ± 0.08a	4.10 ± 2.36a	7.91 ± 0.01a	0.58 ± 0.01a	63.3 ± 1.2 a	31.3 ± 1.22a
25	0	26.3 ± 1.20c	2.60 ± 7.50e	1.63 ± 0.94e	3.27 ± 7.24e	0.19 ± 8.26e	32.6 ± 2.3cd	20.0 ± 1.85d
	50	35.0 ± 1.63b	4.36 ± 0.12c	2.43 ± 1.40d	5.43 ± 0.01c	0.26 ± 0.01c	35.0 ± 0.4c	23.3 ± 0.4 c
50	0	23.0 ± 9.01c	2.13 ± 15.9 f	2.60 ± 1.50cd	3.21 ± 15.5e	0.16 ± 16.6e	29.3 ± 7.2e	17.0 ± 11.1e
	50	27.0 ± 0.81c	3.50 ± 0.04d	3.40 ± 1.96b	5.02 ± 0.01d	0.22 ± 0.01d	31.0 ± 0.4de	19.0 ± 0.81ed
LSD ≤ 0.05 P		4.47	0.222	0.415	0.101	0.209	2.54	2.17

FW: Fresh weight, GB: glycinebetaine, Cr: chromium, different letters showing the significant differences at 0.05 P level according to LSD test

Photosynthetic pigments

Chromium stress significantly reduced the photosynthetic pigments as compared to control (*Fig. 1*). However, chlorophyll (a, b and a/b) and carotenoid contents were significantly increased under normal and Cr stressed conditions with GB application as compared to Cr stress without foliar-applied GB. The maximum chlorophyll and carotenoid contents were recorded in normal conditions (no Cr stress) with foliar application of GB (50 mM) and minimum chlorophyll and carotenoid contents were recorded in Cd stress (50 mM) without GB application (*Fig. 1*).

MDA and H_2O_2 contents

Higher concentrations of H_2O_2 and MDA were recorded in pea plants subjected to higher Cr concentrations (*Fig. 2*). Foliar application of GB (50 mM) significantly reduced the H_2O_2 by 51% and 18% at 25- and 50-mM Cr stress whereas foliar applied GB (50 mM) reduced MDA contents by 42% and 23% under Cr stress of 25 and 50 mM, compared to control (*Fig. 2*).

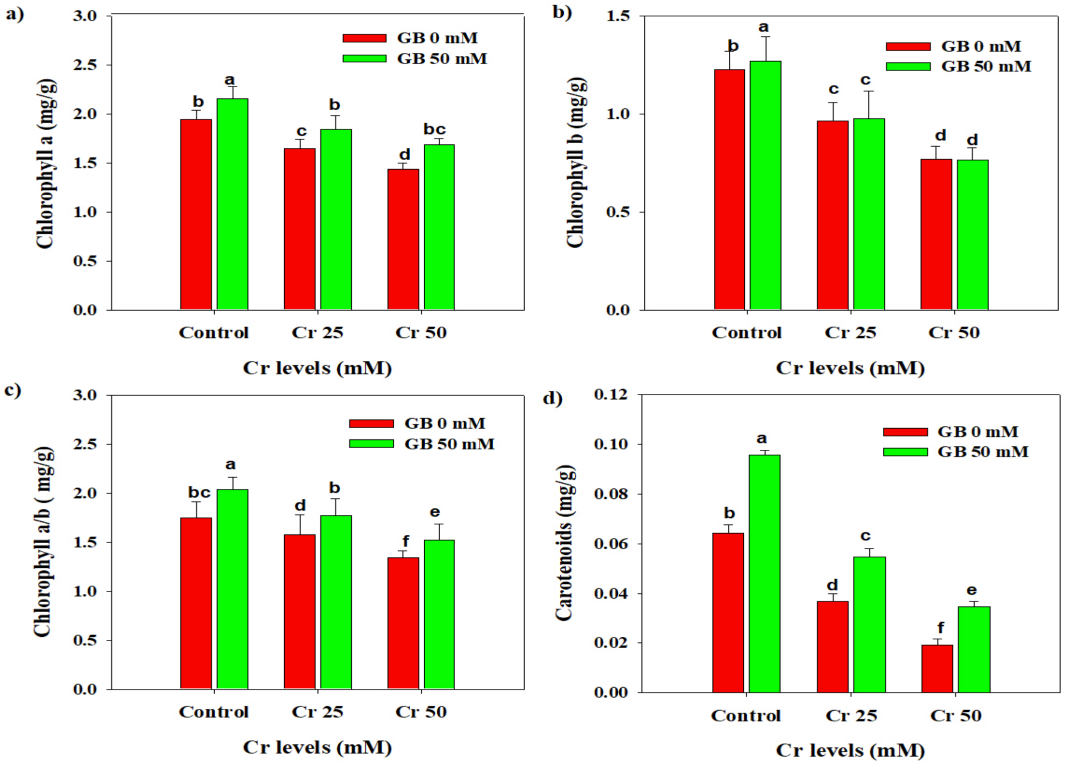

Figure 1. *Chlorophyll (a, b, a/b) and carotenoid contents in pea leaves grown in different concentrations of Cr stress with and without exogenously applied GB. Vertical bars are three replicates mean have ± S.E. and different letters indicate significant difference at 0.05 P level according to LSD test*

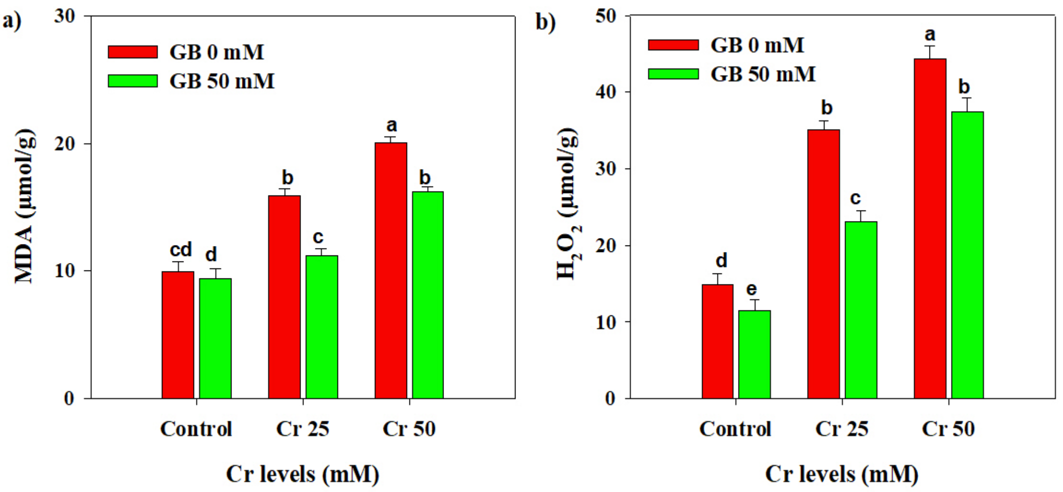

Figure 2. *The contents* ***of*** *MDA and H_2O_2 in pea leaves grown in different concentrations of Cr stress with and without exogenously applied GB. Vertical bars are three replicates mean have ± S.E. and different letters indicate significant difference at 0.05 P level according to LSD test*

Antioxidant enzymes

Antioxidant activities were significantly increased in lower and higher Cr stress conditions as compared to no Cr stress. Moreover, foliar application of GB has further increased in activities of APX and CAT under both levels of Cr stress and control. Likewise, POD and SOD activities were also increased under Cr stress which gives clear evidence that antioxidant activities considerably increased under Cr stress conditions. Moreover, GB has a positive effect on the activities of POD and SOD both under Cr stress and control (no Cr stress) conditions. However, the maximum improvement in POD and SOD activity was recorded under Cr stress (50 mM) with foliar application of GB (50 mM) as compared to control (no Cr stress) (*Fig. 3*).

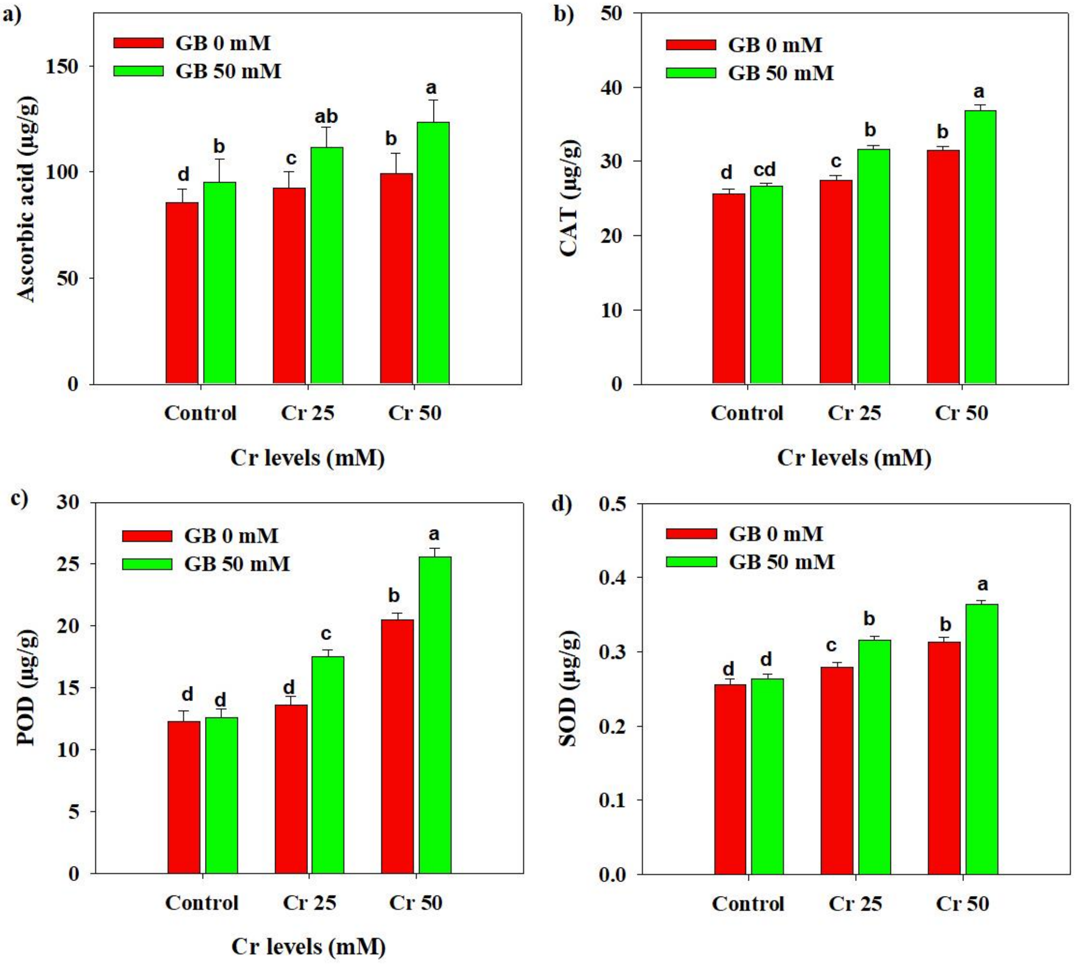

Figure 3. *Ascorbic acid contents, CAT, POD, and SOD activities in pea leaves grown in different concentrations of Cr stress with and without exogenously applied GB. Vertical bars are three replicates mean have ± S.E. and different letters indicate significant difference at 0.05 P level according to LSD test*

Chromium concentration in plant parts

The results indicated that Cr concentration in plant parts was increased under Cr stress conditions, however, it was dose-dependent; increase in Cr concentration in growing media significantly increased the Cr in plant root and shoot. The maximum Cr concentration was recorded in plant roots as compared to shoots (*Fig. 4*). Nonetheless, foliar-applied GB (50 mM) significantly reduced the Cr concentration in roots and shoots (*Fig. 4*).

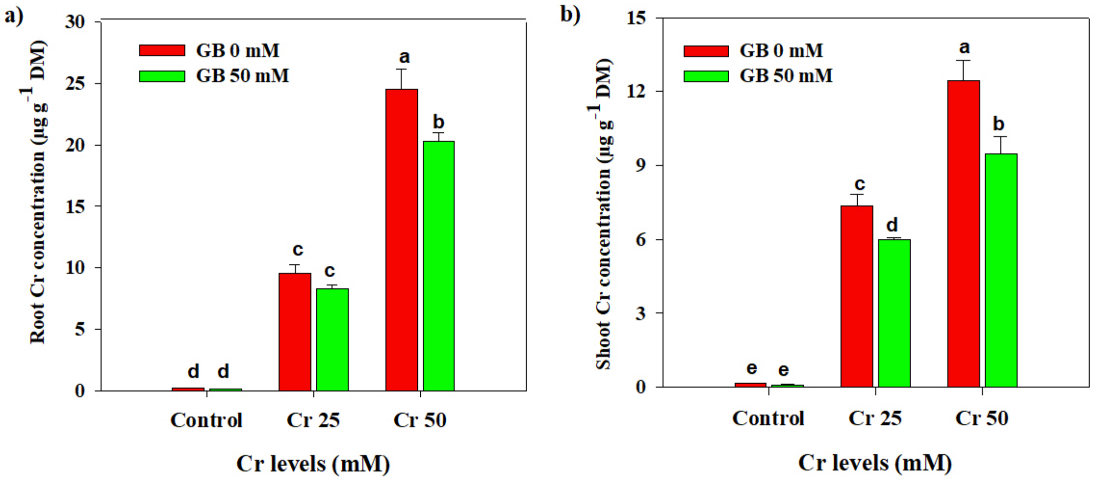

Figure 4. *The concentrations of Cr in roots and shoots of pea grown in different concentrations of Cr stress with and without exogenously applied GB. Vertical bars are three replicates mean have ± S.E. and different letters indicate significant difference at 0.05 P level according to LSD test*

Discussion

Chromium toxicity in soils has been continuously increasing and posing a serious threat to food production. We tested the impact of Cr stress on growth, physiological traits, and antioxidant enzymes and role of the GB in alleviating the deleterious impacts of Cr stress. We noticed that Cr stress had negative effects on plant growth and morphology and an increase in Cr concentration had more deleterious effects on plant growth and morphological characteristics (*Table 1*).

Reduction in plant growth due to Cr has been widely documented by different authors (Ali et al., 2013; Wakeel and Xu, 2020). Reduction in growth attributes might be due to a restricted supply of important nutrients (Aamer et al., 2018; Majhi and Samantaray, 2020) as Cr interferes with different nutrients and reduced their uptake and availability and therefore reduced plant growth (Guo et al., 2020). Cr stress reduced root length which can be due to Cr accumulation in pea roots or mutilation of root tip cells due to Cr stress (Gill et al., 2015).

Foliar applied GB featured a remarkable improvement in the growth and biomass production of pea plants. *Glycinebetaine* improves nutrient uptake and leaf gas exchange qualities and therefore, leads to a substantial increase in the growth and morphology under stress conditions (Shahbaz et al., 2012; Aamer et al., 2018). Moreover, GB-mediated improvement in plant growth was due to positive effect of GB on photosynthetic and transpiration rates and sub-stomatal CO_2 concentration (Jan et al., 2020). *Glycinebetaine* also increased gene expression for scavenging of ROS which, therefore, protects photosynthetic machinery, photosynthetic enzymes (rubisco and rubisco activase) and major molecules (proteins, DNA) from damaging effects of oxidative stress and therefore, increased the growth under stress conditions (Chen and Murata, 2011).

Chromium stress also induced a significant reduction in chlorophyll (a,b and a/b) and carotenoids and increase in Cr concentration linearly deceased the photosynthetic pigments (*Fig. 1*). Cr stress decreased the photosynthetic pigments owing to alterations in chloroplast structures and reduction in synthesis of photosynthestic pigments and carotenoids due to substantial increase in activities of chlorophyllase (Hegedüs et al., 2001) and production of Cr induced ROS (Zewail et al., 2020). A significant increase in photosynthetic pigments was observed with GB application under control and Cr stress conditions (*Fig. 1*). Accumulation of GB under stress conditions protects the chlorophyll structure and improves the photosynthetic efficiency and stomatal conductance and therefore leads to significant improvement in chlorophyll and carotenoid contents under stress conditions (Wang et al., 2010). GB also reduced the Cr uptake and increased the antioxidant activities (*Fig. 3*) which in turn increased the concentration of photosynthetic pigments under stress conditions (Wang et al., 2010).

It was noticed Cr stress induced a significant increase in H_2O_2 production and MDA contents (*Fig. 2*), interestingly activities of the antioxidant enzymes were significantly increased under both levels of Cr stress (*Fig. 3*). The increase in activities of antioxidant under stress conditions has been reported in spinach and maize (Aamer et al., 2018; Dustgeer et al., 2021). The increase in activities of antioxidant enzymes gives evidence about the role of antioxidants in scavenging ROS and providing protection to plants under stress. In current investigation, foliar-applied GB considerably improved antioxidant activities under Cr stress. Glycinebetaine-induced increase in antioxidant activities reduced the Cr uptake and ROS production. Therefore, exogenous GB contributes toward the detoxification of ROS and leads to significant improvement in plant tolerance against the Cr stress (Ali et al., 2015). Exogenously applied GB also reduced the H_2O_2 and MDA contents under stress conditions owing to a marked increase in activities of antioxidant enzymes (*Fig. 3*) (Bharwana et al., 2014; Jabeen et al., 2016).

Chromium concentration was increased in plant roots and shoots under Cr contaminated soil (*Fig. 4*). Likewise, Ali et al. (2015) also noticed a significant increase in Cr accumulation plant parts with increasing Cr concentration. Nonetheless, in this study, foliar-applied GB remarkably reduced the Cr in plant roots and shoots (*Fig. 4*). GB protected the cell membranes and reduced Cr entrance in the cytoplasm and consequently lead to a reduction in Cr uptake and accumulation in plant parts (Giri, 2011; Daud et al., 2021). Another possible reason for the reduction in Cr uptake by foliar-applied GB might be due to Cr competition with other nutrients, as exogenously applied GB increased the uptake of nutrients that compete with the Cr and therefore reduce Cr uptake (Shahbaz and Zia, 2011; Castro-Duque et al., 2020).

Conclusions

In conclusion, chromium stress significantly reduced plant growth, photosynthetic pigments, and antioxidant activities, while enhanced Cr accumulation in different plant parts. However, exogenous applied GB alleviated the detrimental effects of Cr toxicity and increased plant growth, production of biomass and concentration of photosynthetic pigments. The GB-induced alleviation of Cr toxicity in pea was associated with improved antioxidant activities and reduced Cr uptake. However, further studies should be conducted to understand the molecular and cellular mechanisms of alleviation of Cr toxicity by exogenously applied GB.

REFERENCES

[1] Aamer, M., Muhammad, U. H., Li, Z., Abid, A., Su, Q., Liu, Y., Huang, G. (2018): Foliar application of glycinebetaine (gb) alleviates the cadmium (cd) toxicity in spinach through reducing cd uptake and improving the activity of anti-oxidant system. – Applied Ecology Environment Research 16: 7575-7583.

[2] Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, M., Farid, M., Bharwana, S. A. (2015): The effect of excess copper on growth and physiology of important food crops: a review. – Environmental Science and Pollution Research 22: 8148-8162.

[3] Aebi, H: (1984): Catalase in vitro. – Methods in Enzymology 105: 121-126.

[4] Ahmad, R., Ali, S., Abid, M., Rizwan, M., Ali, B., Tanveer, A., Ghani, M. A. (2020): Glycinebetaine alleviates the chromium toxicity in *Brassica oleracea* L. by suppressing oxidative stress and modulating the plant morphology and photosynthetic attributes. – Environmental Science and Pollution Research 27: 1101-1111.

[5] Ali, S., Farooq, M. A., Yasmeen, T., Hussain, S., Arif, M. S., Zhang, G. (2013): The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. – Ecotoxicology and Environmental Safety 89: 66-72.

[6] Ali, S., Chaudhary, A., Rizwan, M., Anwar, H. T., Adrees, M., Farid, M., Anjum, S. A. (2015): Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). – Environmental Science and Pollution Research 22: 10669-10678.

[7] Anjum, Q. S., Amjad, M., Ziaf, K., Habib, A. (2020): Seed treatment with salicylic acid improves growth, biochemical attributes and quality seed production of pea. – Pakistan Journal of Agricultural Science 57: 1370-1379.

[8] Arnon, D. (1949): Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. – Plant Physiology 24: 1.

[9] Bharwana, S. A., Ali, S., Farooq, M. A., Iqbal, N., Hameed, A., Abbas, F., Ahmad, M. S. A. (2014): Glycine betaine-induced lead toxicity tolerance related to elevated photosynthesis, antioxidant enzymes suppressed lead uptake and oxidative stress in cotton. – Turkish Journal of Botany 38: 281-292.

[10] Castro-Duque, N. E., Chávez-Arias, C. C., Restrepo-Díaz, H. (2020): Foliar glycine betaine or hydrogen peroxide sprays ameliorate waterlogging stress in cape gooseberry. – Plant 9: 644.

[11] Chen, T. H. H., Murata, N. (2011): Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. – Plant Cell Environment 34: 1-20.

[12] Dahl, W. J., Foster, L. M., Tyler, R. T. (2012): Review of the health benefits of peas (*Pisum sativum* L.). – The British Journal of Nutrition 108: 3-10.

[13] Daud, M. K., Ali, S., Variath, M. T., Khan, M., Jamil, M., Ahmad, M., Zhu, S. J. (2021): Chromium (VI)-Induced leaf-based differential physiological, metabolic and microstructural changes in two transgenic cotton cultivars (J208, Z905) and their hybrid line (ZD14). – Journal of Plant Growth Regulation 1-13.

[14] Dhindsa, R. S., Plumb-Dhindsa, P., Thorpe, T. A. (1981): Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. – Journal of Experimental Botany 32: 93-101.

[15] Dustgeer, Z., Seleiman, M. F., Imran, K., Chattha, M. U., Alhammad, B. A., Hassan, M. U. (2021): Glycine-betaine induced salinity tolerance in maize by regulating the physiological attributes, antioxidant defense system and ionic homeostasis. – Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49: 12248-12248.

[16] El-Baz, A. A., Hendy, I., Dohdoh, A. M., Srour, M. I. (2021): Adsorption of high chromium concentrations from industrial wastewater using different agricultural residuals. – Journal of Environmental Treatment Techniques 9: 122-138.

[17] Gautam, V., Sharma, P., Bakshi, P., Arora, S., Bhardwaj, R., Paray, B. A., Ahmad, P. (2020): Effect of Rhododendron arboreum leaf extract on the antioxidant defense system against chromium (VI) stress in *Vigna radiata* plants. – Plant 9: 164.

[18] Ghani, A., Ghani, A. (2011): Effect of chromium toxicity on growth, chlorophyll and some mineral nutrients of *Brassica juncea* L. – Egyptian Academic Journal of Biological Sciences 2: 9-15.

[19] Gill, R. A., Zang, L., Ali, B., Farooq, M. A., Cui, P., Yang, S., Ali, S., Zhou, W. (2015): Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. – Chemosphere 120: 154-164.

[20] Giri, J. (2011): Glycinebetaine and abiotic stress tolerance in plants. – Plant Signaling and Behavior 6: 1746-1751.

[21] Guo, X., Ji, Q., Rizwan, M., Li, H., Li, D., Chen, G. (2020): Effects of biochar and foliar application of selenium on the uptake and subcellular distribution of chromium in *Ipomoea aquatica* in chromium-polluted soils. – Ecotoxicology Environment Safety 206: 111184.

[22] Hassan, M. U., Aamer, M., Chattha, M. U., Ullah, M. A., Sulaman, S., Guoqin, H. (2017): The role of potassium in plants under drought stress: mini review. – Journal of Basic & Applied Sciences 13: 268-271.

[23] Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Aamer, M., Khan, T. A. (2019): Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities-a review. – Environmental Science and Pollution Research 26: 12673-12688.

[24] Hassan, U., Aamer, M., Chattha, M. U., Haiying, T., Shahzad, B., Guoqin, H. (2020a): The critical role of zinc in plants facing the drought stress. – Agriculture 10: 396.

[25] Hassan, M. U., Chattha, M. U., Khan, I., Chattha, M. B., Barbanti, L., Aslam, M. T. (2020b): Heat stress in cultivated plants: nature, impact, mechanisms, and mitigation strategies-a review. – Plant Biosystems 155: 211-234.

[26] Hassan, M. U., Aamer, M., Chattha, M. U., Haiying, T., Khan, I., Afzal, A. (2021): Sugarcane distillery spent wash (DSW) as a bio-nutrient supplement: a win-win option for sustainable crop production. – Agronomy 11: 183.

[27] Hayat, S., Khalique, G., Irfan, M., Wani, A. S., Tripathi, B. N., Ahmad, A. (2012): Physiological changes induced by chromium stress in plants: an overview. – Protoplasma 249: 599-611.

[28] Hegedüs, A., Erdei, S., Horváth, G (2001): Comparative studies of H_2O_2 detoxifying enzymes in green and greening barley seedlings under cadmium stress. – Plant Science 160: 1085-1093.

[29] Hossain, M. A., Hasanuzzaman, M., Fujita, M. (2010): Up-regulation of antioxidant and glyoxalase systems by exogenous glycinebetaine and proline in mung bean confer tolerance to cadmium stress. – Physiology and Molecular Biology of Plant 16: 259-272.

[30] Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M., Jabbar, A., Abbas, F. (2016): Glycinebetaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. – Archives of Agronomy and Soil Science 62: 648-662.

[31] Jan, S., Noman, A., Kaya, C., Ashraf, M., Alyemeni, M. N., Ahmad, P. (2020): 24-Epibrassinolide alleviates the injurious effects of Cr (VI) toxicity in tomato plants: insights into growth, physio-biochemical attributes, antioxidant activity and regulation of Ascorbate–glutathione and Glyoxalase cycles. – Journal of Plant Growth Regulation 39: 1587-1604.

[32] Jones, J. B., Case, W. W. (1990): Sampling, handling and analyzing plant tissue samples. – Sampling Handling and Analyzing Plant Tissue Samples 1990: 389-427.

[33] Majhi, P., Samantaray, S. M. (2020): Effect of hexavalent chromium on paddy crops (Oryza sativa). – Journal of Pharmacognosy Phytochemistry 9: 1301-1305.

[34] Medda, S., Mondal, K. K. (2017): Chromium toxicity and ultrastructural deformation of *Cicer arietinum* with special reference of root elongation and coleoptile growth. – Annals of Agrarian Science 15: 396-401.

[35] Mushtaq, Z., Asghar, H. N., Zahir, Z. A. (2021): Comparative growth analysis of okra (*Abelmoschus esculentus*) in the presence of PGPR and press mud in chromium contaminated soil. – Chemosphere 262: 127865.

[36] Nakano, Y., Asada, E. (1981): Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. – Plant cell Physiology 22: 867-880.

[37] Pandey, V., Dixit, V., Shyam, R. (2009): Chromium effect on ROS generation and detoxification in pea (*Pisum sativum*) leaf chloroplasts. – Protoplasma 236: 85-95.

[38] Rasheed, A., Hassan, M. U., Aamer, M., Batool, M., Sheng, F., Ziming, W., Huijie, L (2020): A critical review on the improvement of drought stress tolerance in rice (*Oryza sativa* L.). – Notulae Botanicae Horti Agrobotanici Cluj-Napoca 48: 1756-1788.

[39] Roth, E. F., Gilbert, P. S. (1984): The pyrogallol assay for superoxide dismutase: absence of a glutathione artifact. – Annuals of Biochemistry 137: 50-53.

[40] Samantaray, S., Das, S., Mohanty, R. C., Mohanty, M., Pradhan, C. (2015): Altered germination index and chlorophyll biosynthesis in seedlings of wild rice cultivars in response to hexavalent chromium stress. – Discovery 27: 27-35.

[41] Shahbaz, M., Zia, B. (2011): Does exogenous application of glycinebetaine through rooting medium alter rice (*Oryza sativa* L.) mineral nutrient status under saline conditions. – Journal of Applied Botany and Food Quality 84: 54-60.

[42] Shahbaz, M., Masood, Y., Perveen, S., Ashraf, M. (2012): Is foliar-applied glycinebetaine effective in mitigating the adverse effects of drought stress on wheat (*Triticum aestivum* L.). – Journal of Applied Botany and Food Quality 84: 192-198.

[43] Singh, P. K., Gautam, S. (2013): Role of salicylic acid on physiological and biochemical mechanism of salinity stress tolerance in plants. – Acta Physiologiae Plant 35: 2345-2353.

[44] Sofo, A., Scopa, A., Nuzzaci, M., Vitti, A. (2015): Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. – International Journal of Molecular Sciences 16: 13561-13578.

[45] Sofy, M. R., Elhawat, N., Alshaal, T (2020): Glycine betaine counters salinity stress by maintaining high K+/Na+ ratio and antioxidant defense via limiting Na+ uptake in common bean (*Phaseolus vulgaris* L.). – Ecotoxicology Environmental Safety 200: 110732.

[46] Steel, R. G. D., Torrie, J. H., Dickey, D. A. (1996): Principles and Procedures of Statistics: A Biometric Approach. 3rd Ed. – McGraw-Hill Book Company, Inc., New York.

[47] Velikova, V. I., Yordanov, A., Edreva, A (2000): Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. – Plant Science 151: 59-66.

[48] Wakeel, A., Xu, M. (2020): Chromium morpho-phytotoxicity. – Plant 9: 564.

[49] Wang, G. P., Li, J., Zhang, M. R., Zhao, Z., Hui, W., Wang, W. (2010): Overaccumulation of glycine betaine enhances tolerance of the photosynthetic apparatus to drought and heat stress in wheat. – Photosynthetica 48: 30-41.

[50] Yin, J., Wang, L., Wang, L., Huang, T., Zhang, X. (2021): Pretreatment with selenium prevented the accumulation of hexavalent chromium in rainbow trout (*Oncorhynchus mykiss*) and reduced the potential health risk of fish consumption. – Food Control 122: 107817.

[51] Zewail, R. M., El-Desoukey, H. S., Islam, K. R. (2020): Chromium stress alleviation by salicylic acid in Malabar spinach (*Basella alba*). – Journal of Plant Nutrition 43: 1268-1285.

[52] Zhang, X. Z. (1992): The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. – Research Methodology Crop Physiology Agriculture Press Beijing 208-211.