ISSN: 0973-4929, Vol. 20, No. (**2**) 2025, Pg. 578-588

Current World Environment

www.cwejournal.org

Mitigation of Chromium-Induced Phytotoxicity in Citric Acid Supplemented *Vigna radiata* (L.) Seedlings by Modulation of Oxidative Biomarkers and Antioxidant System

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Abstract

Soil and water contamination by heavy metals, such as chromium, poses a significant food safety concern. Scientists are suggesting various remedial strategies to address this burning problem worldwide. Chromium-VI causes detrimental morphological, physiological and metabolic impacts on plant system. In this research, we examined how citric acid influences the reduction of stress caused by Cr-VI stress in mung bean (*Vigna radiata*) seedlings. Results showed that Cr exposure led to diminished plant growth, decrease in the photosynthetic pigment levels and antioxidant enzyme activity. Addition of citric acid with chromium improved shoot and root length, total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, anthocyanin, total sugar and reducing sugar contents, phenol and flavonoid levels in plants. Furthermore citric acid supplementation increases ROS scavenging enzyme activities while proline, MDA and H_2O_2 levels declined. These results concluded that citric acid boosted the antioxidant system of test seedlings to diminish the toxicity of Chromium-VI. Hence, application of exogenous citric acid might be a useful strategy for alleviating Cr-VI toxicity in mung bean seedlings in Cr contaminated field.

Article History
Received: 09 June 2025
Accepted: 25 July 2025

Keywords
Amelioration;
Chromium;
Citric acid;
Heavy metal toxicity;
ROS;
Vigna radiata.

Introduction

Heavy metal contamination in soil has become a menace to mankind. Exposure to escalated amount of heavy metals can lead to phytotoxicity due to bonding of heavy metals to sulfhydryl and generation of reactive oxygen species.[1] As a result of its significant toxicity and prevalence, chromium has become top target for the Environmental Protection Agency (EPA).[2] It is considered as one of the fourteen most toxic heavy metals and

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Doi: http://dx.doi.org/10.12944/CWE.20.2.5

commonly detected in ground water at hazardous waste sites.[3] Major sources of Cr pollution are industries like mining, steel, tanneries, aluminum, textile and paper. High level of Cr was found to be present in the untreated waste water released from these industries.[4] Cr exists in the environment in two stable forms viz., hexavalent (VI) and trivalent (III). Hexavalent (VI) chromium is more bioavailable and soluble compared to its trivalent (III) form.[5] Since chromium (VI) is more stable and possesses carcinogenic properties it is considered to be highly toxic and causes asthma, bronchitis, necrosis, dermatitis in humans.[6]

Plants growing in Cr^{6+} contaminated soil accumulate Cr primarily in their root, while a small portion is translocated to the shoot.[7] After incorporation Cr is reported to hinder normal growth, physiology, metabolism of plants including legume species like mung bean,[8] broad bean[9] and chick pea.[10] In addition, it destroys plant physiological processes through generation of excess amount of ROS, impairing plasma membrane activity. Plants detoxify ROS through a complex and coordinated action of various antioxidant enzymes such as ascorbate peroxidase, catalase, superoxide dismutase and peroxidase.[11] In India, chromium is a major cause of aquatic contamination encompassing states like Tamil Nadu, Uttar Pradesh and West Bengal.[12] A number of remedial strategies including biological and physio-chemical, have been proposed to combat with Cr contamination from time to time, but due to their lack of efficacy and environmental risks they are proven ineffective. Hence, it is crucial to build up a cost effective and eco-friendly strategy to trim down the negative effect of heavy metal contamination on food production globally.

Mung bean (*Vigna radiata* L.) is a rapid-growing, warm climate crop, wellknown for its dietetic protein, high folate content and iron levels compared to other legume species.[13] Cultivation of mung bean in Cr contaminated soil possess a potential threat to human as well as animal health.[14] Hence, it is of utmost importance to reduce Cr uptake form soil by mung bean cultivated in Cr challenged soil. In certain Indian regional cuisines, mung beans are processed by removing their external coats to create mung dal.

The bioavailability and mobility of heavy metals is influenced by chelation reaction that alters the chemical form of heavy metals. Compared to synthetic chelating agents, organic acids like citric acid (CA) are comparatively more biodegradable and possess less chance of leaching.[15] Previous reports documented the chelating capacity and plant growth promoting nature of citric acid (CA) under different heavy metal stress conditions such as Cr,[16] Cadmium[17] and lead.[18] Citric acid (CA) generally contains three carboxyl groups which behaveas ligand for heavy metals, binding to them, altering theirredox nature by converting them into non-toxic compound or inhibiting their uptake.[19] Most heavy metals are attached to chelators like citric acid (CA) within the vacuole to minimize the risk.[20] Following chelation, heavy metals are transported to shoot through xylem vessel in the form of non-toxic CA-chelate complexes. On the other hand, root exudates comprising of high levels of citric acid (CA) makesHM-citrate complex thus preventingthe entry of heavy metals through root.[19]

The purpose of the study is to look at the detrimental effects due to chromium toxicity on *Vigna radiata* seedlings as well as the potential role of citric acid in reducing stress. There is still a study gap and no work has been done on the effect of CA on Cr stressed *Vigna radiata* seedlings till date. In the current study, it was hypothesized that CA may alleviate Cr toxicity in *Vigna radiata* seedlings by enhancing antioxidant machinery as well as boosting plant stress tolerance. The results of this investigation will offer new knowledge about CA-induced tolerance in *Vigna radiata* seedlings under Cr stress.

Materials and Methods

Growth Conditions and Stress Treatments

Mung bean (*Vigna radiata* L.) seeds were sterilized and germinated in petridishes. Three concentrations of chromium (100, 300 and 600 μM) in the form of potassium dichromate ($K_2Cr_2O_7$)with or without 2.5 mM citric acid were applied to the germinated seedlings for 21 days. Solutions were changed every other day. The seedlings were harvested after 21 days, rinsed, weighed equally and stored for biochemical studies.

Biochemical Analysis

The pigment content, estimation of sugar, estimation of total phenols and flavonoids, assay of enzymatic antioxidants-SOD and CAT, assay of stress markers, H2O2 content, malondialdehyde content (lipid peroxidation), proline content, protein estimation were done using standard protocols.[21-32]

Statistical Analyses

Three completely randomized designs were used for the studies, with two replications for each treatment. The significance of chromium with and without citric acid was compared utilizing data analysis using one-way analysis of variance (ANOVA).

Results

Table 1: Effect of citric acid (2.5 mM) and Cr stress (150, 300, and 600 µM) on various parameters in root of 21 days old mung bean seedlings. Values are expressed as mean ± SE of three independent replicates.

Parameters	Concentrations							
	Control	150 µM Cr	300 µM Cr	600 µM Cr	CA 2.5mM	150 µM Cr + CA	300 µM Cr + CA	600 µM Cr +CA
Length (cm)	10.96± 0.46a	9.16±0 .34bc	8.30± 0.20cd	5.36± 0.12e	11.16± 0.46a	10.26± 0.40ab	8.76± 0.29bc	6.96± 0.17de
Total sugar (mg g^{-1} fw)	25.53± 1.24ab	21.40±0 .93bc	16.26± 0.60d	6.80± 0.12e	26.02± 1.30a	23.60± 1.02abc	19.45± 0.71cd	11.02± 0.31e
Red sugar (mg g^{-1} fw)	19.77± 0.95a	17.41±0 .72ab	13.67± 0.49c	6.16± 0.15d	20.12± 0.99a	18.63± 0.80ab	16.17± 0.58bc	9.39± 0.25d
Total Phenol (mg g^{-1} fw)	40.79± 2.05a	35.50±1 .58ab	28.58± 1.05bc	14.35± 0.44d	41.03± 1.99a	40.23± 1.81a	31.62± 1.21b	21.47± 0.65cd
Flavonoid (mg g^{-1} fw)	80.98± 3.63a	72.40±2 .56abc	60.94± 1.87cd	32.49± 0.81e	84.02± 3.78a	76.21± 3.00ab	67.57± 2.01bc	48.88± 1.17d
SOD (EU mg^{-1} protein)	9.76± 0.24e	11.20±0 .33de	15.18± 0.65bc	17.53± 0.84ab	10.14± 0.30e	13.25± 0.48cd	16.76± 0.71ab	19.57± 0.95a
Catalase (µM H_2O_2 decomposed min^{-1} mg^{-1} protein)	12.81± 0.60a	10.37±0 .42bc	7.08± 0.22de	3.60± 0.09f	13.07± 0.62a	11.53± 0.47ab	8.64± 0.27cd	5.78± 0.15e
H_2O_2 (nmol g^{-1} fw)	17.57± 0.54e	20.04±0 .72cde	24.38± 1.06abc	28.95± 1.42a	16.39± 0.49e	18.68± 0.70de	22.51± 0.95bcd	26.59± 1.18ab
MDA (nmol g^{-1} fw)	26.46± 0.80de	29.60±1. 12cde	35.13± 1.58bc	42.23± 2.10a	25.13± 0.83e	27.93± 1.06de	32.43± 1.41cd	39.53± 1.99ab
Proline (µmol g^{-1} fw)	35.5± 1.06e	42.0±1. 70cde	50.5± 2.19bc	63.2± 3.20a	34.3± 1.15e	38.7± 1.50de	46.8± 2.10cd	57.3± 2.56ab

Influence on Growth

Root and shoot length was of the test seedlings decreased under Cr treatments and revealed appreciable variations among different Cr treatments (Fig. 1A). Root length decreased by about 17, 25, and 51 percent in root at 150 µM Cr, 300 µM Cr, and 600 µM Cr doses over control. Similarly, shoot length decreased by about 11, 22, and 47 percent under the similar Cr concentrations (Tables1 & 2). Exogenous CA combined with identical Cr treatments narrowed down the decrease in growth in contrast with the only Cr-challenged sets by about 6, 20, 37 percent in root and 4, 14, 33 percent in shoot, respectively (Tables1 & 2)

Table 2: Effect of citric acid (2.5 mM) and Cr stress (150, 300, and 600 µM) on various parameters in shoot of 21 days old mung bean seedlings. Values are expressed as mean ± SE of three independent replicates.

Parameters	**Concentrations**							
	Control	**150 µM Cr**	**300 µM Cr**	**600 µM Cr**	**CA 2.5mM**	**150 µM Cr + CA**	**300 µM Cr + CA**	**600 µM Cr +CA**
Length (cm)	21.56± 1.04a	19.26± 0.80ab	16.86± 0.57bc	11.50± 0.26d	21.96± 1.04a	20.76± 0.86a	18.50± 0.60ab	14.56± 0.40cd
Total chl (mg g^{-1}fw)	2.80± 0.14a	2.43±0 .10ab	1.98±0. 07bc	1.34± 0.04d	2.94± 0.15a	2.65± 0.12a	2.12±0 .08bc	1.71±0 .05cd
Chl a (mg g^{-1} fw)	1.95±0 .08ab	1.73±0 .07bc	1.46±0 .05cd	0.99± 0.02e	2.12± 0.10a	1.86±0 .08ab	1.51±0 .05cd	1.29± 0.04de
Chl b (mg g^{-1} fw)	0.85± 0.04a	0.70±0 .02bc	0.52±0 .01de	0.35± 0.01f	0.89± 0.04ab	0.79± 0.03ab	0.61± 0.02cd	0.42±f 0.01e
Carotenoid (mg g^{-1}fw)	1.35± 0.06a	1.18±0. 05abc	0.97±0 .03cd	0.71± 0.02e	1.40± 0.06a	1.29±0 .05ab	1.10±0 .04bcd	0.89±0 .02de
Anthocyanin (µM g^{-1} fw)	12.6± 0.60a	11.3±0 .46abc	9.4±0. 29cd	6.3± 0.14e	12.9± 0.57a	12.1± 0.46ab	10.5±0 .34bc	7.8±0 .17de
Total sugar (mg g^{-1} fw)	37.60± 1.87a	33.25± 1.47ab	26.26± 0.99c	11.72± 0.33e	38.42± 1.94a	35.04± 1.52ab	29.19± 1.12bc	18.36± 0.55d
Red sugar (mg g^{-1} fw)	28.52± 1.41a	25.66± 1.12ab	20.59± 0.74cd	10.87± 0.31e	29.07± 1.47a	26.96± 1.17ab	22.93± 0.87bc	15.63± 0.48de
Total Phenol (µg g^{-1} fw)	35.30± 3.05a	31.68± 2.47ab	26.24± 1.77bc	13.98± 0.76d	35.92± 3.12a	32.96± 2.57a	29.62± 1.95ab	21.51± 1.17
Flavonoid (µg g^{-1} fw)	74.46± 3.21ab	68.52±2 .56abc	59.64± 1.85c	35.02± 0.90d	77.38± 3.25a	70.79± 2.59abc	63.99± 1.94bc	43.83± 1.03d
SOD (EU mg^{-1} protein)	8.20±0 .22e	9.13±0 .33de	12.22± 0.50bc	14.00± 0.66ab	8.94±0 .26de	11.11± 0.39cd	13.17±0 .55abc	15.10± 0.71a
Catalase (µM H_2O_2 decomposed min^{-1} mg^{-1} protein)	11.45± 0.56a	9.55±0 .37bc	6.91± 0.22de	3.69± 0.09f	12.11± 0.56a	10.63± 0.43ab	8.41±0 .27cd	5.18±0 .13ef
H_2O_2 (nmol g^{-1} fw)	12.67± 0.36de	14.05±0 .49cde	16.56± 0.71bc	20.09± 0.99a	11.66± 0.32e	12.98± 0.45de	15.79± 0.65bcd	18.11± 0.88ab
MDA (nmol g^{-1} fw)	21.76± 0.75d	23.83± 0.89cd	27.56± 1.21abc	32.93± 1.64a	20.13± 0.66d	22.33± 0.83cd	25.60± 1.09bcd	29.33± 1.47ab
Proline (µmol g^{-1} fw)	29.5± 0.95d	33.2±1 .29cd	39.8±1 .79bc	50.1± 2.56a	27.8± 0.89d	30.3± 1.15d	35.7±1 .58cd	46.5±2 .33ab

Influence on Pigment Content

Total chlorophyll contents of mung bean seedlings reduced by 13, 29, and 52 percent under 150 µM, 300 µM, and 600 µM chromium treatments, respectively over control (Table 2). On the other hand, 150 µM+CA, 300 µM + CA, and 600 µM + CA treatments reduced thedecrease in total chlorophyll by about5, 24, and 38 percent compared to control (Table 2)

Chromium application led to a reduction inboth chlorophyll a and chlorophyll b contents in the test seedlings. Chlorophyll a levelsdecreasedby about 11, 25 and 49 percent and chlorophyll b levels decrease by about 17, 39, 59 percent under 150 µM, 300 µM and 600 µM chromium treatments, respectively (Table 2).Application of CA in combination with chromium there was a decline in chlorophyll a by about 5, 23, and 34 percent, whereas chlorophyll b decreased by 7, 28, and 51 percent under 150 Cr µM + CA, 300 µM Cr + CA, and 600 µM Cr + CA treatments, respectively, compared to control. However, the levels improved compared to treatments with chromiumalone (Table 2)

Carotenoid levels decreased by 13, 28, and 47 percent at chromium concentrations of 150 µM, 300 µM, and 600 µMrespectively, whereas anthocyanin contents decreased by 10, 25, and 50 percent at same chromium treatments over control (Table 2). However, when treated with chromium combined with CA significant improvement in both these contents was noted (Table 2).

Influence on Sugar Contents

In root, total sugar decreased 16, 36 and 73 percent under 150 µM, 300 µM and 600 µM chromium treatments, respectively (Table 1) while reducing sugar contents decreased by about 12, 31 and 69 percent under similar treatments (Table 1). Total sugar decreased by about12, 30 and 68 percent under similar chromium concentrations in shoot. Reducing sugar levels in shoot decreased by about 10, 28, and 62 percent under three Cr treatments (Table2).Total sugar level of root decreased to8, 24 and 57 percent and in shoot the decline was 14, 36 and 45 percent under 150 µM Cr + CA, 300 µM Cr + CA, and 600 µM Cr + CA treatments, respectively (Tables 1 & 2). Suppression in reducing sugar was 8, 18, and 53 percent in root and 5, 20, and 45 percent in shoot under said concentrations (Tables1 & 2)

Influence on Total Phenolics and Flavonoid Contents

Phenolic contents decreased by about 13, 30, and 65 percent in root at 150 µM Cr, 300 µM Cr, and 600 µM Cr treatments, respectively (Table 1). In shoot, the said level decreased by about 10, 26, and 60 percent under the similar concentrations (Table 2). Exogenous CA, when combined with the same Cr treatments reduced the decrease in phenol contents compared to Cr treated sets alone and were approximately 8, 22 and 47 percent in root and 4, 16, and 39 percent in shoot over control (Tables 1 & 2).Flavonoid contents decreased by about 11, 25, and 60 percent in root and in shoot it wasabout 8, 20 and 53 percent under three doses of Cr treatments in comparison to control (Tables 1 & 2). CA administration along with Cr also decreased flavonoid contents but was less than Cr treatments alone under identical concentrations (Tables1 & 2)

Influence on Superoxide Dismutase and Catalase Activity

Superoxide dismutase activity amplified in both root as well as shoot under different Cr concentrations. The activity showed a noticeable increase by about 15, 56, and 80 percent in root and 12, 49, and 71 percent in shoot, in response to three doses of Cr treatments, respectively, over control (Tables 1 & 2). Inclusion of CA in Cr further enhanced activity of SOD compared to those receiving no CA treatments (Table. 1 & 2). Catalase activity decreased appreciably with reduction of 19, 45, and 72 percent in root and by about 17, 40, and 68 percent in shoot under 150 µM Cr, 300 µM Cr, and 600 µM Cr treatments, respectively, overcontrol. When CA was applied jointly with same Cr doses, the decrease in enzyme activity was less pronounced, with reductions of 10, 33, and 55 percent in root and by about 7, 27, and 54 percent in shoot (Tables1 & 2)

Influence on H_2O_2 and MDA Contents

Root showed an increase in H2O2 contents by about 14, 39, and 65 percent while in shoot the increase was recorded to be about 11, 31, and 59 percent under three Cr treatments, from control (Table. 1 & 2). CA supplementation with different concentrations of Cr reduced the H_2O_2 content in both root and shoot of test seedlings over only Cr exposed seedlings. (Tables1 & 2).

MDA levels in the test seedlings increasedby12, 33, and 59 percent in root under 150 µMCr, 300 µMCr and 600 µM Cr treatments, respectively. In shoots, the increase was approximately18, 42, and 85 percent under the same concentrations. Joint application of Cr and CA, however, reduced the increase in MDA levels compared to stressed seedlings without CA treatment (Tables1 & 2)

Influence on Proline Contents

Level of proline increased approximately 18, 42, and 78 percent in root and by about 13, 35, and 70 percent in shoot at 150 µM Cr, 300 µM Cr and 600 µM Cr treatments respectively (Tables1 & 2). When CA was added along with different doses of Cr, increase in proline contents was noticed in all Cr stressed seedlings over control. Though, these levels were lesser than those observed in Cr treatment alone (Tables 1 & 2)

Discussion

Cr uptake into plants mostly depends on its oxidation state, concentration and nature of crop species.[26] Following absorption within the plant body at first Cr is found to be accumulated in root and then translocates to the aerial plant parts.[33] The usage of inorganic chelators is a helpful approach for the decontamination of heavy metals. These chelators are easily obtainable, immobilize metals, and decrease pH of soil resulting in diminished metal uptake in plants and promote plant growth.[34]

Citric Acid Enhances Growth in Cr Stressed Plants

Metal toxicity harms root morphology and growth that in turn inhibits water and nutrient absorption and impede shoot growth. The current study shows that, in comparison to the control, Cr-induced stress inhibits the growth of mung bean seedlings. Heavy metals contamination suppresses root and shoot length, dry and fresh weight of plants due to its harmful effects on photosynthetic machinery.[35] Suppression of plant growth is due to the disruptive nature of Cr, that intervene with cell division as well as cell extension and microtubule fabrication, ROS production and inhibiting water absorption.[36] Application of CA considerably augmented root and shoot growth in the test seedlings compared to only Cr treated counterparts. The enhanced development of mung bean seedlings treated with citric acid in chromium challenged environments can be attributed to its potentiality in escalating nutrient uptake and protection against abiotic and biotic stressors.[37]

Citric Acid Elevates Photosynthetic Pigments and Anthocyanins In Cr Stressed Plants

Various photosynthetic pigments are utilized to evaluate the toxicity impact of different stressors in plants.[38] In our study, a noticeable reduction in chlorophyll contents in the test seedlings was noted under Cr exposure. The negative effect of chromium on chlorophyll contents may be attributed to the inhibiting action of chromium on chlorophyll biosynthetic enzymes like protochlorophyllide reductase and δ-aminolevulinic acid dehydratase. Another reason might be the replacement of Mg^{2+} molecules by Cr which eventually boosts ROS generation and oxidative stress injury.[39] Conversely, co-application of Cr with CA resulted in an increase in photosynthetic pigment contents. According to[6] CA enhances absorption of essential mineral nutrients that lead to synthesis of chlorophyll. Our results are in line with those of,[40] who reported that exogenous CA application increased the chlorophyll content of *Phaseolus calcaratus* in Cr affected conditions.

Similarly, we observed suppression in anthocyanins in the test seedlings when Cr stress was imposed. However, CA+Cr treated plants showed an appreciable increase in anthocyanin levels compared to only Cr treatments. Anthocyanin possess metal chelating and ROS scavenging activities, that help plant to combat metal toxicity.[41] Therefore it is evident that upsurge in anthocyanin level upon CA administration is linked to the increase in tolerance potential of plant against Cr toxicity. Accretion in anthocyanin level in Cr+CA treated seedlings might be due to over-expression of chalcone synthase gene.[42]

Citric Acid Co-Application Improves Total Soluble Sugar and Reducing Sugar Under Cr Stress

During stress condition photosynthetic efficiency regulates soluble sugar concentration in plants.[43] As photosynthetic pigments decreased upon Cr exposure we also found lower levels of soluble sugar in mung bean seedlings after Cr stress imposition. However, CA application increased these parameters compared to the only Cr stressed sets that were comparable with the enhanced pigment contents (chlorophyll and carotenoids) upon CA administration in the test seedlings. These outcomes suggested that CA boosted photosynthetic efficiency by regulating enhanced levels of sugars that facilitates induction of energy as well as metabolites.

Citric Acid Alters Secondary Metabolite Production in Cr Stressed Plants

Total polyphenol contents and flavonoids escalate enzyme activities and play the role of antioxidants in stress.[44] In the current study, we noticed Cr induced decline in polyphenols and flavonoid contents in the test seedlings. Similar to our findings heavy metals have shown to reduce these parameters in tomato plants.[45] Conversely, seedlings treated with CA along with Cr exhibited an increase in total polyphenol compounds and flavonoid contents in the test seedlings. This increase in polyphenols after citric acid application may be due to decrease in pH of the cell, resulting in enhanced synthesis of total polyphenols that directly scavenge ROS.[46]

Integration of Citric acid Enhances the Antioxidant Defense System in Cr-Stressed Plants

The escalated generation of ROS due to Cr stress leads to oxidative stress injury in plants. To sustain normal metabolism in plants it is imperative to maintain ROS production. Antioxidant enzymes confer heavy metal resistance in plants by detoxifying ROS in plants under stress conditions. In our study, CAT activities decreased under Cr stress, consistent with findings in *Trigonella corniculata* where reduced CAT activities were observed under chromium stress.[47] In this study, the antioxidant enzyme activities in leaves and roots under the CA+Cd treatment were significantly higher compared to those under the Cr treatment. These results indicated the role of these enzymes in antioxidant defense against chromium toxicity.

Exogenous Citric Acid Application Reduces Oxidative Stress in Cr Stressed Plants

In our study, mung bean seedlings showed elevated levels of ROS (H2O2) and malondialdehyde upon Cr exposure which indicated upsurge in oxidative stress, lipid peroxidation and membrane injury. Earlier findings have also revealed increase in above mentioned parameters in Cr stressed chick peas.[10] Joint application of CA with Cr helped to balance ROS homeostasis by plummeting H_2O_2 build up, thereby mitigating oxidative stress damage. CA was also found to confer membrane stability, as evidenced by lower MDA contents. This may be attributed to ability of CA to lessen free radical generation and sustain metabolism through regulating water status. Similar to our result, a decline in ROS and MDA levels has been reported in Cr stressed *Solanum lycopersicum*[48] supplemented with CA.

To survive under water deficit caused by heavy metal toxicity, plants usually boost their concentration of osmoprotectants like proline.[49] The reason behind proline accumulation under Cr stress is not yet known. According to Zulfiqar *et al.*,[11] proteins are broken down into amino acids, which are then utilized for proline production. Our findings align with earlier research indicating that proline concentrations rise in response to combat toxicity due to Cr exposure in chickpea.[50] Contrarily, CA+Cr treatments reduced the accumulation of proline compared to only Cr treatments. This may be attributable to the effect of CA in regulating ROS generation, thereby lessening the need for excess proline production in plants.

Conclusion

Chromium toxicity reduced plant growth, photosynthetic pigment levels, total soluble sugar, phenol and flavonoid contents due to ROS generation and suppressed antioxidant defense potential in mung bean seedlings. However, exogenous citric acid application fosters these parameters by reducing ROS accumulation through up-regulating antioxidant defense machinery. Therefore, citric acid can be effectively administered to alleviate Cr induced stress in mung bean and to maintain product safety. However, the mechanism by which citric acid mitigates Cr toxicity is yet not clear. Furthermore, rigorous field trainings and investigations are necessary to comprehend the efficiency and reliability of citric acid application to overcome Cr toxicity in legumes. Moreover, the identification of vital biomarkers (viz., oxidative stress, antioxidant defense and osmoprotectants) concerned in the uptake, accumulation and mitigating Cr phytotoxicity should be focused for booming cultivation of mung bean in Cr-contaminated soils.

Acknowledgement

The authors are thankful to the Botany Department and Central Laboratory of Tamralipta Mahavidyalaya for instrumental (DST-FIST, New Delhi, India Sponsored) and administrative support.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

The manuscript incorporates all datasets produced or examined throughout this research study.

Ethics statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Permission to Reproduce Material from other Sources

Not Applicable

Author Contributions

- **Prabal Das:** conceived the idea, helped to design experiments.
- **Debashis Bandyopadhyay & Buddhadev Guria:** performed experiments, analysed data and drafted the manuscript.
- **Palin Sil:** performed statistical analysis.
- **Prabal Das:** analysed data and finalized the manuscript.

All authors equally approve the publication.

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