Calcium induces phytochelatin accumulation to cope with chromium toxicity in rice (Oryza sativa L.)

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ABSTRACT

Heavy metal chromium (Cr) is considered to be a serious environmental contaminant due to its toxic effect on living organisms. To mitigate and reduce the negative impacts of Cr in rice plant, the effect of exogenous supplementary calcium was evaluated as it functions as a signaling molecule at cellular level. In this study, growth parameters, protein content, and membrane stability were found to be restored due to calcium under Cr stress. Further, Atomic absorption spectrophotometric analysis revealed that calcium inhibits Cr translocation from root to shoot in rice seedlings. This event was addressed by the enhanced accumulation of phytochelatin that leads to vacuolar sequestration of Cr in roots. Furthermore, increased activity of Catalase, Peroxidase, and Glutathione reductase along with elevated glutathione also assures that calcium enhances antioxidant defense mechanism to cope with Cr toxicity.

Abbreviations: Chromium: Cr; Iron: Fe; Calcium: Ca2+; PC: Phytochelatin; ROS: Reactive oxygen species; CAT: Catalase; POD: Peroxidase; SOD: Superoxide dismutase; GR: Glutathion reductase

Introduction

Chromium toxicity has become a serious problem for plants and animals over the past few decades (Shanker et al. 2005; Gill et al. 2015) and received highlighted attention. The stable forms of Cr are the trivalent Cr(III) and the hexavalent Cr (VI), although there are various other valence states which are unstable in biological systems. Among these two forms, Cr(VI) is considered the most toxic, which usually forms chromate (CrO42−) or dichromate (Cr2O72−) oxyanions with oxygen. Stunting plant growth, chlorosis in new leaves, wilting of tips, impaired photosynthesis, damage of roots, and finally plant death are the common phenomenon of Cr toxicity (Sharma et al. 2003; Scoccianti et al. 2006). Moreover, reactive oxygen species (ROS) produced by Cr can harm the biomolecule (such as lipids, proteins, and nucleic acids) synthesis, thereby, interrupting both mitochondrial respiration and carbohydrate metabolism (Gill and Tuteja 2010; Gill et al. 2015).

To cope with the toxicity of heavy metal like Cr, plants make grow different mechanisms such as prevention of heavy metals (HM) uptake into root cells by confining HM ions to the apoplast, binding them to the cell wall, storage into the cell vacuoles, activation of oxidative stress defense mechanisms and the synthesis of stress-related proteins and signaling molecules.

The plasma membrane prevents the uptake of metals into the cell by active efflux pumping outside the cell. Active efflux systems are more vernacular and are used to control heavy metal accumulation inside the cell. This heavy metal efflux pumps in plants are the P1B-ATPases and the CDF families of transporters. P1B-type ATPases is a member of P-type ATPase super family and apply energy from ATP hydrolysis to translocate diverse metal cations across biological membranes (Axelsen and Palmgren 2001).

Several physiological studies pointed out the role of PCs in the homeostasis and detoxification of Cr and other metals in plants (Shanker et al. 2005; Singh et al. 2013). Plant removes the toxic effect of Cr by reducing Cr (VI) to Cr (III), followed by complexation of Cr (III) with PC and then this PC-Cr complexes transported to vacuoles (Wu et al. 2013). In addition to PC, metallothioneins (MTs), cysteine-rich low molecular weight proteins, also act in Cr detoxification in plants (Shanker et al. 2004; Panda and Choudhury 2005).

In order to remove the injurious effect of ROS induced oxidative stress, plants have developed a complex ROS scavenging enzymatic mechanism comprising of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and Glutathione reductase (GR) (Maiti et al. 2012; Pourrut et al. 2013). The activation or suppression of antioxidants in plants against metal-induced oxidative damage depends upon plant and ROS type (Shahid et al. 2014). Chromium-induced increase in CAT, POD, SOD, and GR activities has been reported in Gossypium hirsutum (Daud et al. 2014; Farooq et al. 2016). Moreover, lower molecular weight glutathione provides defense against Cr-mediated oxidative damage by taking part in various physiological and biochemical processes such as modulation of thiol-disulphide status, reduction of peroxides, and free radical scavenging (Foyer and Noctor 2005).

Calcium ions play vital roles as a second messenger in coupling physiological responses to external and developmental signals (Reddy and Reddy 2004). Changes in cytosolic free calcium ion concentration are authenticated during transduction of abiotic stimulants including light, low and...
Materials and methods

Plant cultivation

Seeds of rice (BRRI 51) were washed thoroughly and then sterilized with 95% (v/v) ethanol for 10 min. Seeds were then germinated in Petri Dishes containing moist sterilized paper for 2–3 days in the dark at room temperature. Only uniform germinated seedlings were transferred to the hydroponic solution (Hogland and Arnon 1950) containing the following nutrient concentrations (µM): KNO3 (16000), Ca(NO3)2.4H2O (6000), NH4H2PO4 (4000), MgSO4.7H2O (2000), KCl (50), H3BO3 (25), Fe-EDTA (25), MnSO4.4H2O (2), ZnSO4 (2), Na2MoO4.2H2O (0.5), and CuSO4.5H2O (0.5). The nutrient solution was supplemented with 0 or 10 mM K2Cr2O7 and 0 or 100 µM CaCl2 as mentioned previously (Greger et al. 2016; Kabir 2016; Mahmud et al. 2018). The pH of the hydroponic nutrient was adjusted to 6.0. The nutrient media were continuously aerated and incubated in the growth chamber under 10-h light and 14-h dark (550 μmol m–2 s–1) and gas mixture system (Model No. AA-6800, Shimadzu).

Measurement of morphological features

To determine morphological growth parameters, such as root length, root dry weight, shoot height, and shoot dry weight roots and leaves of 1-week-old plants were separated manually and then dried in an oven at 80°C for 2 days before taking the dry weight.

Estimation of relative water content (RWC)

Leaf RWC was measured as described previously (Barrs and Weatherley 1962). Fresh roots and leaves were weighed as FW and then dried at 80°C for 48 h. After drying, again weighed as DW. RWC was calculated applying the formula RWC (%) = [(FW – DW)/(TW – DW)] × 100. (Here, FW = Fresh weight, DW = Dried weight).

Determination of chlorophyll and carotenoids concentration

Leaf Chlorophyll and carotenoid content were determined according to the well-established method described by Lichtenthaler and Wellburn (1985) using 90% (v/v) acetone and calculated as FW basis.

Determination of Cr and Fe by atomic absorption spectroscopy

After harvesting, roots and shoots were washed with CaSO4 and deionized water and then dried in oven at 80°C for 3 days. Then dried samples were digested in 3 ml HNO3 with 1 ml H2O2 and were heated at 109°C for 15 min. These digested samples were then analysed for Cr and Fe concentration by flame atomic absorption spectroscopy outfitted with ASC- 6100 autosampler and air–acetylene atomization gas mixture system (Model No. AA-6800, Shimadzu).

Determination of total soluble proteins

Total soluble proteins of root and shoot were extracted according to the procedure described by, Guy et al. (1992) with some modification. Amount of soluble proteins were determined according to Bradford (1976) method. A calibration curve prepared with different concentrations of bovine serum albumin (BSA) was used.

Measurement of electrolyte leakage

Electrolyte leakage was determined by an electrical conductivity meter as previously described, with some modifications (Lutts et al. 1996). Shortly, seedlings were washed with deionized water, weighed, and kept in individual vials containing 20 ml deionized water and then incubated at 25°C on a shaker (100 rpm) for 2 h. Electrical conductivity of the solution was then measured after incubation.

Estimation of lipid peroxidation

Malondialdehyde (MDA) content as a marker of lipid peroxidation was determined according to Heath and Packer (1968). Root samples (0.5 g) were homogenized in 5% (w/v) trichloroacetic acid (TCA), and then centrifuged at 11,500 × g for 15 min. A mixture of the supernatant with thiobarbituric acid (TBA) was heated at 95°C for 30 min in a water bath. Absorbance was read at 532 nm after cooling the supernatant. MDA content was calculated on FW basis by using extinction coefficient 155 mM−1 cm−1 and expressed as nmol of MDA mg−1 FW.

Enzymatic analysis

CAT (EC. 1.11.1.6), POD (EC. 1.11.1.7), SOD (EC. 1.15.1.1), and GR (EC. 1.6.4.2) enzymes were extracted in the roots of one-week-old plants according to Goud and Kachole (2012) with slight modifications. Shortly, root tissues were ground in phosphate buffer (100 mM) and then centrifuged for 10 min at 13,000 × g. For CAT(EC. 1.11.1.6) analysis, the reaction mixture (2 ml) comprised of 100 mM potassium phosphate buffer (pH 7.0), 400 µl of 6% (v/v) H2O2, and 100 µl root extract. Once root extract was added, the decrease in absorbance was read at 240 nm (extinction coefficient of 0.036 mM−1 cm−1) in a UV spectrophotometer at 30-s intervals up to 1 min. CAT activity is expressed as mmol of H2O2 oxidized min−1 (mg protein−1). Reaction mixture (2 ml) of POD (EC. 1.11.1.7) analysis carried out in 100 mM potassium...
phosphate buffer (pH 6.5), 1 ml of 0.05 M pyrogallol solution, 400 μl of 200 mM H₂O₂, and 100 μl root extract. Similarly, the differences of absorbance were read at 430 nm (extinction coefficient 12 μM⁻¹ cm⁻¹) in a spectrophotometer from 30 s up to 1.5 min. The specific activity of POD is expressed as mmol pyrogallol oxidized min⁻¹ (mg protein⁻¹). Moreover, SOD (EC. 1.15.1.1) assay mixture comprised of 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine, and enzyme (Sun and Zigman 1978). Adrenochrome formation for 4 min was then recorded at 475 nm in a UV-Vis spectrophotometer once epinephrine is supplied. Per unit SOD activity is expressed as the amount of enzyme needed for 50% inhibition of epinephrine oxidation. For GR (EC. 1.6.4.2) analysis, the reaction mixture was prepared containing 1 ml of 0.2 M phosphate buffer (pH 7.0), 1 mM EDTA, 0.75 ml distilled water, 0.1 ml of 20 mM oxidized glutathione (GSSG), and 0.1 ml of 2 mM NADPH and 0.1 ml root extract. Oxidation of NADPH by GR was then recorded at 340 nm. The rate of GR activity (nmol min⁻¹) was then counted using the extinction coefficient of 6.12 mM⁻¹ cm⁻¹ (Halliwell and Foyer 1978).

The hydrogen peroxide

The hydrogenperoxide (H₂O₂) concentration was measured in roots as previously described (Alexieva et al. 2001). Briefly, tissues were grinded in 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10,000×g for 15 min. The supernatant was kept in dark for 1 h before adding K-phosphate buffer (10 mM, pH7.0) and potassium iodide (M). The absorbance of the mixture was read at 390 nm. The amount of hydrogen peroxide was calculated applying a standard curve prepared with known concentrations of H₂O₂.

Estimation of phytochelatin (PC) content

Phytochelatin content was determined by subtracting the total glutathione (GSH) content from the total non-protein thiol content. Ellman's assay mixture was used to measure non-protein thiol content after homogenizing leaves in 3% (w/v) sulfosalicylic acid and recorded spectrophotometrically at 412 nm (Ellman 1959).

Estimation of proline (pro) content

Leaf and root samples were homogenized in 3% (w/v) sulfosalicylic acid and centrifuged at 11,500×g for 12 min. Reaction mixture was prepared by using 100 μl of 3% (w/v) sulfosalicylic acid, 200 μl glacial acetic acid, 200 μl acidic ninhydrin and 100 μl supernatant of the plant extract and incubated the tubes at 96°C for 60 min and then cooled on ice. Readings were taken immediately at a wavelength of 520 nm in spectrophotometer (Bates et al. 1973). The standard curve was used to determine proline concentration and calculated on a FW basis.

Estimation of glutathione content

Glutathione extraction followed the protocol of Anderson et al. (1992). Total glutathione was measured after the reduction of GSSG to GSH. The GSSG reduction was performed by applying the root extract to a mixture consisting of 130 mM sodium phosphate buffer (pH 7.4) containing one unit of GR for 10 min. at 30°C. Thereafter, NADPH at 50 mM and sodium phosphate buffer at 7 mM (pH 6.8) containing 6 mM of 5,5'-dithiobis (2- nitrobenzoic acid) (DTNB) were added and the reaction mixture was maintained at 30°C for 10 min. The absorbance was read at 412 nm and GSH were estimated using GSH as a standard (Griffith 1980).

Statistical analysis

All experiments were performed in a completely randomized block design with four independent replications for each sample. Statistical significance was set at p ≤ .05 by one-way ANOVA followed by Duncan’s multiple range test (DMRT) using SPSS Statistics 20 Software. Moreover, the graphical presentation was developed using Graph Pad Prism 8.

Results

Morphological growth features

Root length, shoot height, root dry weight, and shoot dry weight significantly decreased under the treatment with Cr compared to non-treated plants. Application of calcium along with chromium in the culture medium, root length, shoot height, root dry weight, and shoot dry weight were significantly increased likened to chromium stressed plants. Addition of calcium chloride in the absence of chromium did not show any significant change in both root and shoot compared to control plants. However, no significant change in total chlorophyll content (A &B) was observed among these treatments. But carotenoid synthesis was significantly increased under chromium stress compared to control plants. In addition, the rate of water retention was reduced in both root and shoot of chromium treated plants compared to control. But the presence of calcium with chromium enhanced this rate in both root and shoot (Table 1).

Cr and Fe concentration

The concentration of chromium significantly increased in both roots and shoots in chromium stressed plants compared to non-treated plants. However, the application of calcium with chromium could reduce the amount of chromium significantly in the shoot but not in root. However, Cr concentration both in root and shoot was as like as that of non-treated control due to calcium treatment without Cr. Translocation rate of chromium was reduced from 41% to 26% due to treated with calcium under chromium stress compared with chromium treated plants (Figure 1).

Furthermore, Iron content in both root and shoot of rice grown under chromium stress is significantly higher than that of non-treated control plant (Figure 1). Due to the application of calcium along with and without chromium in media, iron content showed no significant change in both roots and shoots compared to Cr stressed plants.

Measurement of electrolyte leakage and total soluble protein

As the initial investigation reveals that calcium-mediated chromium detoxification is root based mechanism, Electrolyte
leakage and total soluble protein of root were examined. Electrolyte leakage was significantly increased under chromium stress compared to control plant. However, the addition of calcium chloride in the presence of chromium did significantly decrease compared to chromium stressed plant. Furthermore, chromium stress caused a significant reduction in total soluble protein content compared to non-treated control plants. However, it was significantly increased in root compared to chromium forced when calcium was applied along with chromium. Action of calcium in the absence of chromium was not considerable compared to the control plant (Figure 2).

**Lipid peroxidation**

Cr in growth medium enhanced the accumulation of MDA in root compared to the control. However, the application of calcium along with chromium CAT, POD, and GR activities increased significantly compared with chromium stressed plant. Moreover, the application of calcium solely GR activity was decreased significantly compared with non-treated control plants. But in the case of SOD activity, no significant differences were found among the treatments (Table 2). Furthermore, the amount of H2O2 in root raised under chromium stress. Supplementation of calcium with chromium reduced this amount significantly compared with non-treated control as well as chromium stressed plants.

**Changes in enzymatic activity**

CAT, POD, and GR enzyme activities in root were significantly decreased under chromium stress compared with control plants. However, the application of calcium along with chromium CAT, POD, and GR activities increased significantly compared with chromium stressed plant. Moreover, the application of calcium solely GR activity was decreased significantly compared with non-treated control plants. But in the case of SOD activity, no significant differences were found among the treatments (Table 2). Furthermore, the amount of H2O2 in root raised under chromium stress. Supplementation of calcium with chromium reduced this amount significantly compared with non-treated control as well as chromium stressed plants.

**Changes in phytochelatin, glutathione, and proline in root**

The level of glutathione in chromium stressed plants was similar to that of non-treated plants in roots. However, after the application of calcium under chromium stress, the

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**Table 1.** Root length, root dry weight, shoot height, shoot dry weight, level of photosynthetic pigment (chl a and chl b) in leaves and water retention rate of root and shoot of *Oryza sativa* seedlings. Means (±SD) were calculated from four replications (n = 4) for each treatment. Values with different letters are significantly different at ≤0.05 applying test.

| Treatment          | Root length (cm) | Root dry weight (mg) | Shoot height (cm) | Shoot dry weight (mg) | total chl a and b (µg/gm FW) | Carotenoids (µg/gm FW) | Water retention rate root (%) | Water Retention rate shoot (%) |
|--------------------|------------------|----------------------|-------------------|-----------------------|----------------------------|------------------------|-----------------------------|-------------------------------|
| Control            | 1.55 ± .129b     | 1.7 ± .41b           | 3.88 ± .83b       | 3.6 ± .83b            | 49.69 ± 3.760a             | 111.46 ± 5.95a         | 79                          | 78                            |
| Cr+                | 0.975 ± .125a    | 1.03 ± .69a          | 2.73 ± .84b       | 2.8 ± .93b            | 55.66 ± 11.08a             | 146.49 ± 14.3b         | 64                          | 70                            |
| Cr+ + CaCl₂        | 1.425 ± .65b     | 1.85 ± .58b          | 3.9 ± .50b        | 3.72 ± .35b           | 51.10 ± 10.78a             | 99.68 ± 18.0a          | 71                          | 75                            |
| Cr− + CaCl₂        | 1.375 ± .55b     | 1.8 ± .32b           | 3.9 ± .87b        | 3.73 ± 1.24b          | 50.48 ± 19.89a             | 113.96 ± 7.95a         | 72                          | 75                            |

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Figure 1. Chromium and iron concentration in the roots and shoots and their translocation rate (%) from root to shoot of 7 days old rice plants grown under different growth conditions of Cr and Ca++. Different letters in each column indicates significant differences between means ± SD of treatments (n = 4) at a p < .05 significance level.
level of glutathione significantly increased. Further, Phytochelatin content in root was significantly increased due to calcium addition with chromium compared to treated and non-treated plants. Calcium without Cr also increased this level compared to control plants. In addition, proline content showed no significant changes in roots due to Ca+2 applications under Cr stress in comparison with Cr-stressed plants (Table 3).

Discussion

In plants, chromium toxicity is associated with the increaseable production of ROS and oxidative stress development as well as with inhibition of pigment synthesis and modification of virtually all cellular components (Farooq et al. 2016; Jabeen et al. 2016; Ahmad et al. 2017; Uliaña et al. 2018; Yang-ErChena et al. 2018). These metabolic changes result in seedling development, cell death, reduction of plant biomass and crop yield (Antoniadis et al. 2017; Yang-ErChena et al. 2018) reduction of soluble protein content (Singh et al. 2012; Das et al. 2014; Jabeen et al. 2016) and membrane stability (Begum et al. 2016). Plants subjected to chromium also showed reduced growth in this study. In the present experiment Ca+2 was applied alone as well as in combination with Cr to restore the altered plant growth in rice as Calcium protects plants from deleterious impacts of stress by acting in signaling pathways and regulating calmodulin like proteins to promote several growth mechanisms in plants (Sarwat et al. 2013). The present study also indicates that the presence of Ca+2 during cultivation with Cr restored the growth features.

Biochemical analyses also indicate that the supplementation of Ca+2 under Cr stress restored the growth features, membrane stability and the total soluble protein content. These findings are in agreement with the previous reports shown in mustard (Ahmad 2015; Ahmad et al. 2015), wheat, almond, and sunflower plants under cadmium stress (Elloumi et al. 2014; Abd Allah et al. 2015). The increased soluble proteins (SP) content might be due to the efficient working of gas exchange and photosystem of plants which was regulated by the addition of Ca+2 under Cr stress (Aderholt et al. 2017). Moreover, Carotenoids is the key pigment known to be involved in protecting plant organs from stresses and its production may be enhanced depending on the metal type, concentration, and the plant species (Sinha et al. 2003).

In this investigation, the concentration of carotenoid was significantly higher compared with non-treated control plant as well as plants were treated with calcium in combination with chromium indicating that Ca alleviate chromium stress in rice plant.

In the present study, we found that Cr and Fe concentration both in root and shoot increased significantly under

| Enzymes | Control | Cr | Cr+CaCl2 | Cr+CaCl2 | Cr+CaCl2 |
|---------|---------|----|----------|----------|----------|
| CAT (U min⁻¹mg⁻¹) | 0.547 ± 0.074b | 0.138 ± 0.084a | 0.636 ± 0.073c | 0.298 ± 0.073b |
| POD (U min⁻¹mg⁻¹) | 0.890 ± 0.020c | 0.541 ± 0.084a | 0.776 ± 0.003b | 0.740 ± 0.028b |
| SOD (U min⁻¹mg⁻¹) | 0.209 ± 0.005a | 0.170 ± 0.0563a | 0.218 ± 0.0558a | 0.184 ± 0.027a |
| GR [nmol.NADH.min⁻¹gm protein⁻¹] | 0.189 ± 0.038b | 0.109 ± 0.014a | 0.194 ± 0.014b | 0.105 ± 0.014a |
| H₂O₂ (µgg⁻¹ FW) | 5.3 ± 0.357b | 7.893 ± 0.328c | 4.351 ± 1.163a | 5.583 ± 0.608b |

Table 2. Enzymatic activities and hydrogen peroxide content in roots of rice seedlings, cultivated for 7 days in nutrient medium with calcium in presence or absence of chromium. Different letters in each column indicated significant differences between means ± SD of treatments (n = 4) at p ≤ .05 significance level.
chromium stress indicating that Cr uptake is associated with iron transportation system which was established in rice previously (Kabir 2016). Calcium treatment also enhances Fe uptake and its translocation. However, in case of chromium, calcium could not inhibit its uptake but restrict its translocation from root to shoot. This accumulating evidence indicates the compartmentation of excess Cr in the root which is a mechanism of metal detoxification in plants (Dragisic Maksimovic et al. 2007; Adrees et al. 2015). Plant cell vacuoles are pivotal organelle functioning in the storage of metabolites, mineral nutrients, and toxics in higher plants. This Vacuolar sequestration primarily controlled by cytosolic metal chelators and tonoplast-localized transporters, or the interaction between them under HM stress (Mendoza-Cozatl et al. 2011; Peng and Gong 2014). To do that plants often increase the synthesis of PC and metallothioneins (Lee et al. 2004; Roo-sens et al. 2004; Peng and Gong 2014; Nahar et al. 2016; Mahmud et al. 2018).

In this study, phytochelatin accumulation was found to enhance due to calcium treatment under chromium stress that was involved with vacuolar sequestration of Cr. Increased PC inside cells due to varying levels of HMs, binds to HM via sulphhydryl and carboxyl groups (Gobbett 2000; Enamverdian et al. 2015). However, PC found to facilitate Cd storage in wheat, which varies with tissue type (Marentes and Rauser 2007).

In addition, GSH is an antioxidant known to play critical roles in scavenging ROS (Gobbett and Goldsbrough 2002) as well as precursor of PC were increased when Ca \(^{2+}\) was applied along with Cr. These findings suggest that Ca \(^{2+}\) interact with cellular mechanisms associated with vacuolar sequestration upon Cr exposure through PC synthesis regulation in the roots of rice plants to withstand Cr toxicity. Similar type of result was also found in rice after applying silicon and salicylic acid under chromium stress (Huda et al. 2016, 2017).

In the present investigation, CAT, POD, and GR enzyme activities were found to be increased and H2O2 to be reduced significantly due to Ca \(^{2+}\) supplementation with Cr, pointing that Ca \(^{2+}\) interacts with ROS signal pathway for scavenging Cr-induced oxidative stress in rice plants. Previous investigation also supports the interaction of Ca \(^{2+}\) with ROS signal pathway and induce defense mechanisms by keeping the H\(_2\)O\(_2\) and O\(_2\) at a constant level (Thounaojam et al. 2012). Similarly, Cd-induced oxidative damage was minimized by modifying the antioxidant defense system in sesame due to Ca \(^{2+}\) applications (Abd_Allah et al. 2017).

Furthermore, outcomes of this study established that S-containing metabolite, proline showed no significant increase in roots due to Ca \(^{2+}\) application under Cr stress in comparison with Cr-stressed plants, suggesting that metabolites having antioxidant properties (Yadav 2010; Nahar et al. 2016) are not linked with Ca \(^{2+}\) mediated tolerance to Cr stress in rice plants.

**Conclusion**

In the present study, morpho-physiological findings suggest that the exogenous supplementation of Ca alleviates the detrimental effects of Cr in rice. This mitigation is due to effectively stores excess Cr in roots through phytochelatin mediated vacuolar sequestration leading to reduced translocation in shoots. Moreover, regulation of antioxidant defense by modulating CAT, POD, GR as well as glutathione in roots provides partial protection from Cr induced oxidative damage. Findings of this study not only advance our understanding of chromium stress tolerance in rice plant but also point to potential areas of improvement for the alleviation of heavy metal toxicity. These improvements ultimately diminish the Cr contamination in crops and food materials.

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**Disclosure statement**

No potential conflict of interest was reported by the authors.

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