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Research Article

Potential role of L-glutamic acid in mitigating cadmium toxicity in lentil (*Lens culinaris* Medik.) through modulating the antioxidant defence system and nutrient homeostasis

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Abstract

Using phosphate fertilizers and wastewater as a source of irrigation and residuals from industries have considerably increased the level of cadmium (Cd) in soil which severely reduced the growth and yield of crop. L-glutamic acid (L-Glu), an amino acid, plays key roles in plant stress tolerance. Hence, the current study was conducted to determine the potential role of L-Glu pre-treatment in alleviating Cd-induced toxicity in lentil (*Lens culinaris* Medik.). Lentil seedlings were exposed to two doses of Cd (1 and 2 mM $CdCl_2$) with or without 10 mM L-Glu pre-treatment. The results suggested that a high dose of Cd negatively affected the shoot dry weight, root dry weight, and photosynthetic pigments (chlorophylls and carotenoids). Furthermore, Cd stress induced severe oxidative damage, a reduction in catalase (CAT) activity and ascorbate (AsA) content, and accumulation of Cd in both the roots and shoots. Adding L-Glu protected the photosynthetic pigments of the lentil seedlings and thus improved the growth of the seedlings. In addition, L-Glu pre-treatment enhanced the ascorbate (AsA) content; increased the activity of enzymes such as catalase, ascorbate peroxidase, monodehydroascorbate reductase, and glutathione peroxidase. L-Glu was also reduced Cd uptake and translocation, which in turn alleviated the oxidative damage in the Cd-stressed seedlings indicated the potential role of this chemical. Results suggest that pre-treatment with L-Glu reduces Cd toxicity in lentil seedlings by inhibiting Cd accumulation and by reducing oxidative damage.

Keywords: amino acid; cadmium stress; cadmium uptake; enzyme activities; oxidative damage; ROS

Introduction

Cadmium (Cd) has been considered as a highly toxic pollutant amongst other toxic metals that contaminate soil through injudicious use of phosphate fertilizers and pesticides, and disposal of sewage sludge into the environment (He *et al.*, 2016; Khan *et al.*, 2017). Cadmium serves no biological function in plants, so the growth and productivity of plants growing in Cd-contaminated soil are severely affected. Even at low concentrations (5-10 μg g^{-1} dry weight), Cd disrupts the physiological and biochemical processes of plants (Tran and Popova, 2013; Qadir *et al.*, 2014; Khan *et al.*, 2017). The noticeable damage caused by toxic Cd in plants includes growth reduction, photosynthesis and respiration restriction, and leaf chlorosis (Bayçu *et al.*, 2018; Rizwan *et al.*, 2018; Yotsova *et al.*, 2018; Song *et al.*, 2019; Xin *et al.*, 2019). Furthermore, Cd can easily

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be transported to other edible portions of plants, which poses a great risk to human health (Ismael *et al.*, 2019; Mishra *et al.*, 2019).

Overproduction of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\cdot-}$), singlet oxygen ($^1O_2$), and hydroxyl radical ($OH^{\cdot}$) is the well-established response of plants under abiotic stress conditions including Cd stress (Kapoor *et al.*, 2019). Plants have an innate capacity to balance ROS homeostasis by maintaining the antioxidant defense system composed of non-enzymatic antioxidants such as ascorbate (AsA) and reduced glutathione (GSH) and enzymatic antioxidants including ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and glutathione *S*-transferase (G*S*T) (Shanying *et al.*, 2017; Li *et al.*, 2019; Małecka *et al.*, 2019). Increased ROS caused by an increasing level of toxic Cd leads to oxidative stress (Muneer *et al.*, 2014; Latef *et al.*, 2018). To scavenge the overproduced ROS in plants under Cd stress, inducing the antioxidant defense system could be an effective approach compared with other approaches such as remediating Cd-contaminated soil and developing Cd stress-tolerant varieties, which are expensive and time consuming (Huybrechts *et al.*, 2019; Shah *et al.*, 2019). Therefore, researchers are looking for eco-friendly and cost-effective approaches to handle Cd toxicity in plants. Several reports indicate that using a variety of chemicals could be a feasible way of attenuating the deleterious effect of abiotic stress including Cd stress in plants (Savvides *et al.*, 2016; Kaya *et al.*, 2020). For example, exogenous application of chemicals such as auxin, ethylene, salicylic acid, and silicon reduce Cd toxicity in barley, mustard, maize, and wheat (Krantev *et al.*, 2008; Bočová *et al.*, 2013; Asgher *et al.*, 2014; Wu *et al.*, 2019). Along with those chemicals priming with amino acids have also provided signalling effect in reducing biotic and abiotic stresses in different crops (Nephali *et al.*, 2020). Among them, previous results suggested that an amino acid, glutamate (Glu) acts as a signalling molecule to induce many plants physiological processes including seed germination (Kong *et al.*, 2015) and root architecture (Forde, 2014). In addition, L-glutamic acid (L-Glu) can modulate the defense mechanism of plants to withstand the injurious effects of salinity (Sh Sadak *et al.*,2015; Fardus *et al.*, 2021) and drought stress (La *et al.*, 2020). Therefore, L-Glu is also considered as the eco-friendly chemical because it remains as the precursor of the synthesis of different polypeptides and proteins, which seems very essential for plant cell growth simulation (Qiu *et al.*, 2020). As L-glutamic acid is an amino acid it can be easily metabolized by living organisms in the soil and also by the plants (Kan *et al.*, 2017). However, the role of L-Glu in mitigating Cd toxicity has not yet been investigated in lentil.

Lentil is a principal crop among other pulse crops cultivated in many countries including Bangladesh, India, and Canada. It is a beneficial crop because of its high protein content and N_2-fixing ability (Andrews and Andrews, 2017; Foti *et al.*, 2019). However, compared with rice, wheat, and maize, limited research has focused on the Cd stress-tolerance mechanism in lentil.

Therefore, the aims of our current study were (1) to examine the L-Glu-induced effects on the physiological and biochemical parameters of lentil seedlings, (2) to determine whether L-Glu alleviates Cd-caused oxidative stress and growth reduction, and (3) to investigate whether L-Glu upregulates the antioxidant systems. Accordingly, we investigated different growth attributes, oxidative damage markers, the response of the antioxidant defense system, and the uptake of Cd in the roots, shoots, and leaves of lentil seedlings. To the best of our knowledge, this report is the first showing a positive role of L-Glu in mitigating Cd toxicity in lentil seedlings.

Materials and Methods

Plant materials, growth conditions, and treatments

Healthy lentil (*Lens culinaris* Medik cv. 'BARI Masur-7') seeds were surface sterilized by soaking them in 70% ethanol for 5 min. The disinfected seeds were then washed and soaked in distilled water for 24 h. The next day, the soaked seeds were washed again with distilled water and kept in a dark condition for 72 h for gemination in Petri dishes containing six layers of wetted paper towels. Forty germinated seedlings were kept

in each Petri dish and placed in a cultivation chamber under continuous illumination at 350 μmol m^{-2} s^{-1} photon flux density and 25 ± 1 °C. Hyponex (Tokyo, Japan) nutrient solution with the concentration of 0.2 mL L^{-1} was supplied to the seedlings with or without 10 mM l-Glu on the following day and left for another 48 h. The dose of L-Glu was selected based on the previous reports of Fardus *et al.* (2021) and Kan *et al.* (2017). The seedlings from four sets of Petri dishes with or without L-Glu were then exposed to 1- and 2-mM cadmium chloride ($CdCl_2$). The doses of Cd were chosen based on a preliminary trial testing a series of Cd concentrations (0.3-3 mM) (Supplementary Figure 1a-c). Stress treatments were continued for five days and changed on alternate days. The 9-day-old seedlings were then used to determine the physiological and biochemical attributes. Three replications were used for each treatment.

Growth parameters and water content determination

The shoots and roots of randomly selected lentil seedlings were separated, and their shoot fresh weight (SFW) and root fresh weight (RFW) were measured by removing additional moisture with paper towels. The detached portion of the seedlings were then dried at 80 °C in a dryer until a stable weight was obtained. The dried samples were then weighed to determine the dry weight of the shoots (SDW) and roots (RDW). The formula [WC (%) = {(FW − DW)/DW} × 100] was used to calculate the water content (WC).

Chlorophyll and carotenoid content measurement

The contents of chlorophyll (Chls) and carotenoid (Car) were extracted from the leaf tissue (0.1 g) of individual samples by heating with 10 mL DMSO (Dimethylsulfoxide, a useful extractant of chlorophyll in plants which extract chlorophyll without any hydration) in a water bath for 1 h at 65 °C. The absorbance of those supernatants was then measured at 645 and 663 nm wavelength to analyse the Chls content according to the formula of Wellburn (1994) and expressed as mg g^{-1} FW. The amount of Car was analysed from the outcome of the absorbance at 470 nm wavelength and expressed as mg g^{-1} FW (Wellburn, 1994).

Electrolyte leakage and proline content determination

Electrolyte leakage (EL) was measured according to Dionisio-Sese and Tobita (1998). Proline content (Pro) was measured following the method of Bates *et al.* (1973).

Malondialdehyde, other aldehyde, and hydrogen peroxide estimation

Lentil shoots (0.5 g) from each sample were homogenized and centrifuged with 3 mL of 5% TCA at 11500×g for 15 min. Supernatants (1 mL) were then heated in a water bath for 30 min after mixing with 4 mL TBA (thiobarbituric acid) and centrifuged again. Then the supernatant taken from that centrifugation was used to determine malondialdehyde (MDA) and other aldehyde at 532, 600, and 455 nm absorbance according to Heath and Packer (1968) and Keramat *et al.* (2010). To calculate MDA and other aldehyde, we used 155 mM^{-1} cm^{-1} and 0.457×105 M^{-1} cm^{-1} coefficients, respectively. The method of Yang *et al.* (2007) was used to measure hydrogen peroxide (H_2O_2) at 390 nm absorbance.

Reduced ascorbate, reduced glutathione, and oxidized glutathione content estimation

Lentil shoots (0.5 g) were homogenized in 5% TCA (3 mL). Reduced ascorbate (AsA), total glutathione (TG), and oxidized glutathione (GSSG) were determined from the supernatant after centrifugation according to Noctor *et al.* (2016).

Soluble protein estimation

Bovine serum albumin (BSA) was used as a protein standard to measure the total soluble protein concentration (Bradford, 1976).

Enzyme activity determination

A mortar and pestle were used to homogenize the lentil shoots along with 50 mL buffer solution containing ascorbic acid (1 mM), KCl (100 mM), β-mercaptoethanol (5 mM), and glycerol (10% w/v). The homogenate sample was then centrifuged at 11500×g for 12 min. The collected supernatant from the centrifuged sample was then used to determine the concentration of the soluble protein and activities of the different enzymes.

The method of Noctor *et al.* (2016) was used to assay catalase (CAT, EC:1.11.1.6) activity and expressed as μmol min^{-1} g^{-1} protein. In following this method, extracted enzyme was placed in a cuvette and absorbance was measured at 240 nm. The absorbance was calculated by using an extinction coefficient of 40 mM^{-1} cm^{-1}.

Ascorbate peroxide (APX, EC:1.11.1.11) activity was determined according to the method of Noctor *et al.* (2016) with absorbance measured at 290 nm using 50 mM K-P buffer (pH 7.0), 0.5 mM AsA, 0.1 mM H_2O_2, 0.1 mM EDTA, and enzyme.

Monodehydroascorbate reductase (MDHAR, EC:1.6.5.4) activity was assayed by measuring absorbance at 290 nm using Tris-HCl buffer at pH 7.5 (50 mM), AsA (2.5 mM), NADPH (0.2 mM), and enzyme, and expressed as nmol min^{-1} mg^{-1} protein (Noctor *et al.*, 2016).

Dehydroascorbate reductase (DHAR, EC:1.8.5.1) was determined following the method of Noctor *et al.* (2016): 50 mM K-P buffer (pH 7.0), 2.5 mM GSH, and DHA with enzyme in a cuvette were used to measure the absorbance of DHAR from that extracted enzyme.

Glutathione reductase (GR, EC:1.6.4.2) activity was determined by using K-P buffer (pH 7.0), EDTA, GSSG, and NADPH with extracted enzyme, and measuring spectrophotometry absorbance at 340 nm (Noctor *et al.*, 2016).

The activity of glutathione *S*-transferase (G*S*T, EC:2.5.1.18) at 340 nm absorbance was determined according to the method of Nahar *et al.* (2016) by using 250 mM K-P buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB), and extracted enzyme.

The activity of glutathione peroxidase (GPX, EC:1.11.1.9) was assayed by following the method of Noctor *et al.* (2016) and expressed as nmol min^{-1} mg^{-1} protein.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA) of three replications and XLSTAT v.2020 software. Fisher's least significant difference (LSD) with a probability of 5% was used to assay the mean difference of those replications.

Results

Involvement of L-Glu in improving the phenotypic appearance of lentil seedlings under the influence of Cd toxicity

Exposing the lentil seedlings to the liquid solution of Cd (1 and 2 mM $CdCl_2$) clearly affected the phenotypic appearance of the seedlings, including growth reduction and leaf chlorosis, compared with the 0.3–0.7 mM $CdCl_2$-treated seedlings (Figure 1; Supplementary Figure 1a–c). Conversely, compared with the Cd-stressed plants, exogenous pre-treatment with 10 mM L-Glu reversed the phytotoxic effects of Cd by reviving the leaves and improving the phenotypic appearance of the lentil seedlings (Figure 1). Furthermore, the seedlings under Cd stress also exhibited better phenotypic appearance with pre-treatment by 10 mM L-Glu compared with other amino acids such as L-glutamine, L-glycine, L-aspartic acid, L-phenylalanine, L-methionine, and L-cysteine at the same concentration (10 mM) (Supplementary Figure 2a–d).

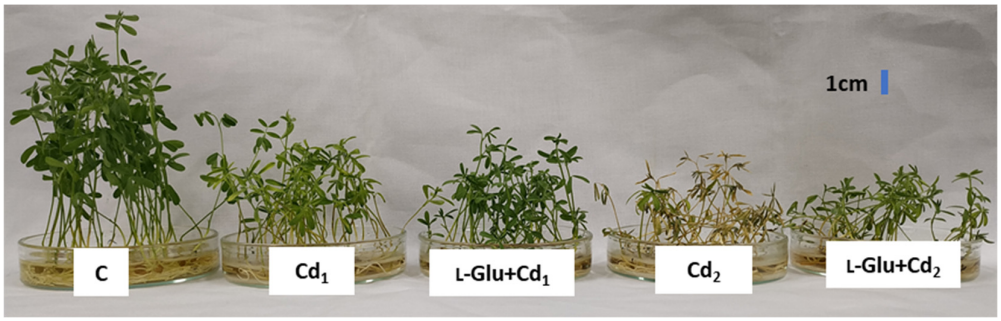

Figure 1. Effect of L-glutamic acid on the phenotypic appearance of the lentil seedlings under Cd stress
The treatments were control (C), 1 mM $CdCl_2$ (Cd_1), 10 mM L-glutamic acid + 1 mM $CdCl_2$ (L-Glu+Cd_1), 2 mM $CdCl_2$ (Cd_2), and 10 mM L-glutamic acid + 2 mM $CdCl_2$ (L-Glu+Cd_2).

L-Glu alleviated Cd-induced inhibition of plant growth and proline content and improved water content in the lentil seedlings

The growth of the lentil seedlings was dramatically reduced by the Cd treatments. The results indicated that, in comparison with control, the fresh weight of the Cd (1 and 2 mM) treated shoots declined by 42% and 63% (Table 1). Fresh weight of Cd treated roots also decreased by 9 and 19%, compared to control seedlings under toxic Cd condition (1 and 2 mM) (Table 1). Similarly, the dry weight of the shoots and roots was also significantly reduced by 10 and 18%, and 20 and 48%, with increasing toxic Cd concentration, respectively, compared with control (Table 1). However, L-Glu pre-treatment diminished the negative effect of toxic Cd (1 and 2 mM) on the fresh weight of the shoots and roots by 41 and 61%, and 40 and 19%, respectively (Table 1). Adding L-Glu also increased the dry weight of the shoots (10 and 19%) and roots (20 and 48%) significantly in response to both levels of Cd compared with the Cd-stressed seedlings (Table 1). Furthermore, under Cd stress, L-Glu supplementation increased the water content (5 and 9%) and reduced the proline content (20 and 25%) in comparison with the Cd-treated seedlings (1 and 2 mM) (Table 1). The decline in water content (6 and 13%) and increase in proline content (169 and 286%) occurred with the treatment of 1 and 2 mM $CdCl_2$ compared with control (Table 1).

Table 1. Effect of L-glutamic acid on shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), water content (WC), and proline content (Pro) of the lentil seedlings under Cd stress

Treatment	SFW (mg shoot^{-1})	SDW (mg shoot^{-1})	RFW (mg root^{-1})	RDW (mg root^{-1})	WC (%)	Pro (µmol g^{-1} DW)
C	67.7±1.2a	9.2±0.1a	46.2±1.9a	3.5±0.1a	86.4±0.3a	113.4±7.4d
Cd_1	39.1±2.1c	7.4±0.3c	22.2±0.5c	1.8±0.1c	80.7±0.5b	304.6±8.8b
L-Glu+Cd_1	55.2±0.9b	8.8±0.2b	30.4±0.6b	2.2±0.1b	85.2±0.2a	245.1±4.1c
Cd_2	25.1±1.5d	6.2±0.3d	17.8±0.3d	1.1±0.1d	75.3±0.3c	437.2±27a
L-Glu+Cd_2	40.3±0.7c	7.3±0.1c	21.3±0.1c	1.6±0.1c	81.8±0.6b	289.7±13b

The means (±SE) were calculated from three replications. Values with different letters indicate statistically significant differences at $P \leq 0.05$ (Fisher's LSD test).

L-Glu pre-treatment relieved the chlorosis of the lentil leaves

The lentil seedlings showed severe leaf chlorosis when treated with $CdCl_2$, and the symptoms increased dramatically with increasing Cd concentration. The contents of chlorophyll a (Chl a, 34 and 71%), chlorophyll b (Chl b, 34 and 73%), chlorophyll (a+b) (Chls (a+b), 39 and 71%), and carotenoid (Car, 81 and 57%) decreased compared with control under Cd-stress conditions (Figure 2a–d). However, compared with both the Cd-alone treatments, L-Glu pre-treatment increased Chl a (52 and 218%), Chl b (29 and 158%), Chls (a+b) (48 and 146%), and Car (183 and 622%) in response to the 1 and 2 mM $CdCl_2$ concentrations (Figure 2a–d).

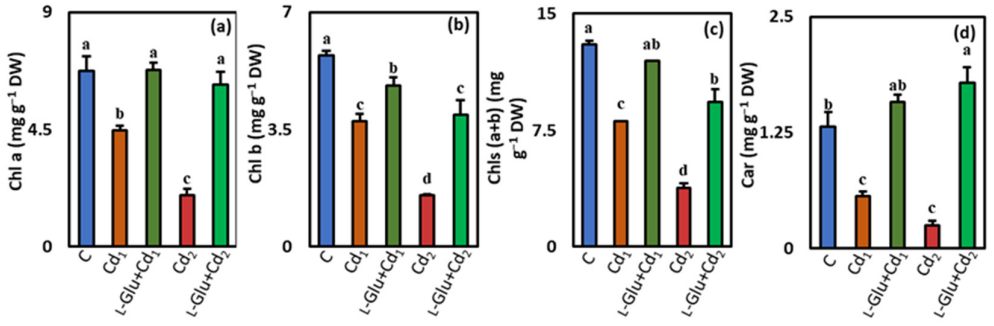

Figure 2. Effect of L-glutamic acid on the contents of the photosynthetic pigments a) chlorophyll a (Chl a), b) chlorophyll b (Chl b), c) chlorophyll (a+b) (Chls (a+b)), and d) carotenoid (Car) in the lentil seedlings under Cd stress

The above means (±SE) were calculated from three replications. Values with different letters indicate statistically significant differences at P≤0.05 (Fisher's LSD test).

L-Glu alleviated the Cd-induced oxidative stress (malondialdehyde, hydrogen peroxide, and percentage of electrolyte leakage)

The consequence of Cd stress and L-Glu pre-treatment on membrane lipid peroxidation was determined by measuring the amount of MDA and other aldehyde content in the lentil leaves. In comparison with control, a marked increase in MDA (63 and 106%) and other aldehyde (78 and 173%) was detected in the 1- and 2-mM Cd-stressed seedlings (Figure 3a, b). Conversely, L-Glu pre-treatment reduced the contents of MDA and other aldehyde by 37 and 29%, and 23 and 15%, respectively, compared with the Cd-stressed seedlings (Figure 3a, b). Moreover, our results also showed that the induction of ROS led to an increase in H_2O_2 content and EL percentage under the same stresses. In our experiment, H_2O_2 content and EL percentage increased by 73 and 106%, and 58 and 221%, respectively, in comparison to control (Figure 3c, d). However, L-Glu supplementation lowered the H_2O_2 content (34 and 27%) and EL percentage (15, 42%) compared with the Cd-stressed lentil seedlings (Figure 3c, d).

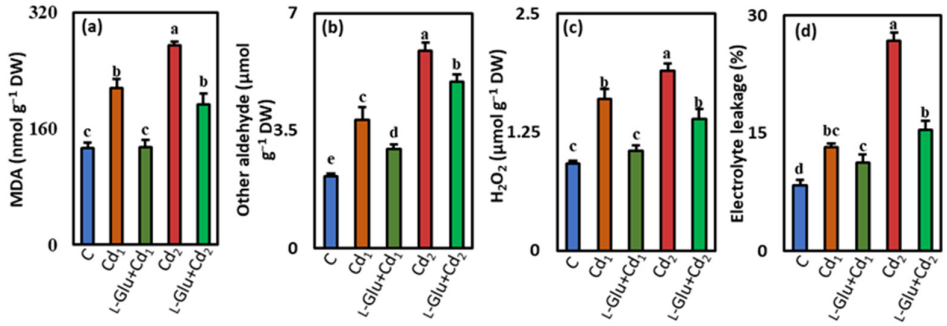

Figure 3. Effect of L-glutamic acid on a) malondialdehyde (MDA), b) other aldehyde, c) hydrogen peroxide (H_2O_2), and d) electrolyte leakage of the lentil seedlings under Cd stress

The above means (±SD) were calculated from three replications. Values with different letters indicate statistically significant differences at P≤0.05 (Fisher's LSD test).

L-Glu application modulates the activities of non-enzymatic and enzymatic antioxidants in the lentil seedlings

The key factor of the cellular system of plants is to scavenge the continuously produced excess ROS. To reduce the excess ROS, plants possess an antioxidant system comprising non-enzymatic antioxidants such as AsA, GSH, and GSSG, and enzymatic antioxidants such as CAT, APX, MDHAR, DHAR, GR, G*S*T, and GPX (Figure 4a–k). Our results showed increased activities of a few of the above-mentioned enzymes in the

control and l-Glu-pre-treated seedlings (Figure 4a–k). In comparison with the $CdCl_2$-treated seedlings, the level of AsA (140 and 218%) significantly increased with pre-treatment by L-Glu (Figure 4a), whereas the content of GSH (27 and 17%) and GSSG (33 and 24%) decreased with pre-treatment by L-Glu (Figure 4b,c). Furthermore, the GSH/GSSG ratio was significantly similar in the lentil seedlings with or without application of L-Glu under both levels of Cd stress (Figure 4d) The activities of the enzymes CAT, APX, MDHAR, and GPX also increased by 14 and 128%, 36 and 90%, 19 and 59%, and 28 and 62%, respectively, with the application of L-Glu when exposed to 1 and 2 mM Cd stress (Figure 4e–g,k). Supplementation with L-Glu reduced the activities of the other enzymes, DHAR, GR, and G*S*T, by 24 and 28%, 28 and 33%, and 19 and 35%, respectively, compared with the $CdCl_2$-treated seedlings (1 and 2 mM) (Figure 4h–j). However, the Cd-stressed seedlings exhibited decreasing levels of AsA (61 and 78%) and increasing levels of GSH (46 and 133%) and GSSG (122 and 130%) compared with control (Figure 4 a–c). Moreover, the activities of APX, MDHAR, DHAR, GR, G*S*T, and GPX increased, and CAT activity decreased with the application of 1 and 2 mM $CdCl_2$ in comparison with control (Figure 4e–k).

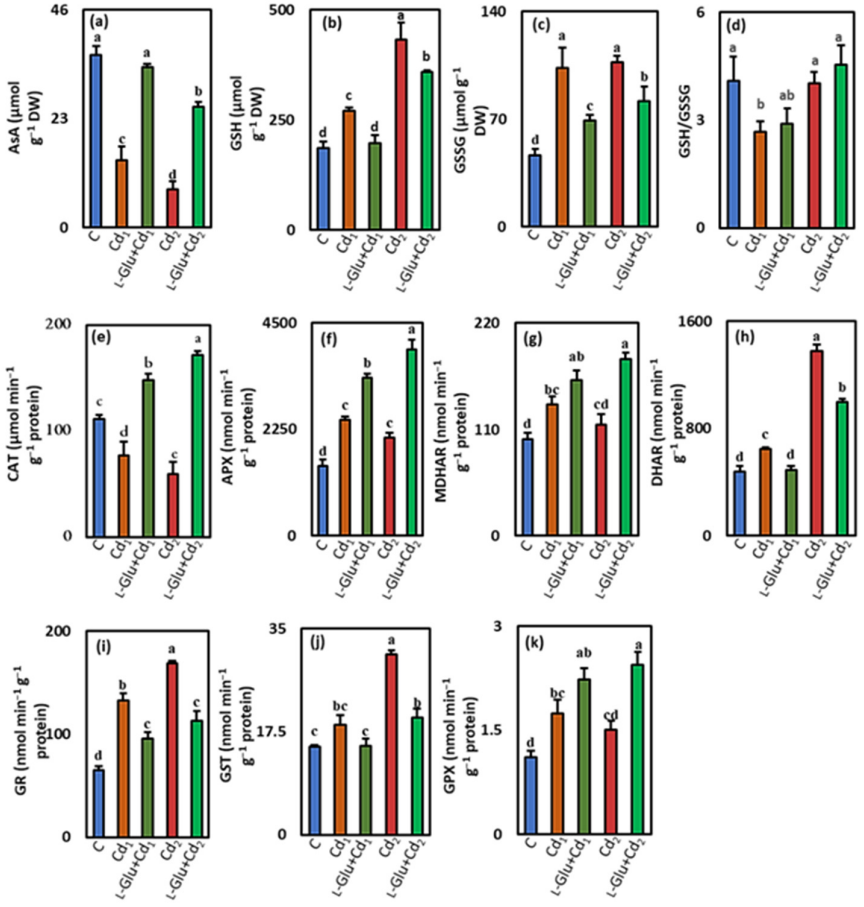

Figure 4. Effect of L-glutamic acid on the contents of the non-enzymatic antioxidants, a) ascorbate (AsA) b) reduced glutathione (GSH), c) oxidized glutathione (GSSG), and d) GSH/GSSG; and the activity of the enzymatic antioxidants, e) catalase (CAT), f) ascorbate peroxidase (APX), g) monodehydroascorbate reductase (MDHAR), h) dehydroascorbate reductase (DHAR), i) glutathione reductase (GR), j) glutathione peroxidase (GPX), and k) glutathione *S*-transferase (G*S*T), in the lentil seedlings under Cd stress

The above means (±SE) were calculated from three replications. Values with different letters indicate statistically significant differences at P≤0.05 (Fisher's LSD test).

Involvement of L-Glu in inhibiting the accumulation of Cd under Cd stress

No accumulation of Cd was recorded in the leaves, shoots, and roots of the control seedlings (Figure 5a–c). In contrast, Cd accumulation in the roots increased by 159 and 326%, respectively, with the increasing concentration of Cd, which was then translocated to the aboveground part of the lentil seedlings, namely, the shoots (101 and 256%) and leaves (9 and 30%) (Figure 5a–c). Conversely, L-Glu pre-treatment reduced the accumulation of Cd in the roots (30 and 23%) and also impeded the translocation of Cd to the shoots (59 and 38%) and leaves (89 and 60%) under 1 and 2 mM $CdCl_2$ stress (Figure 5a–c).

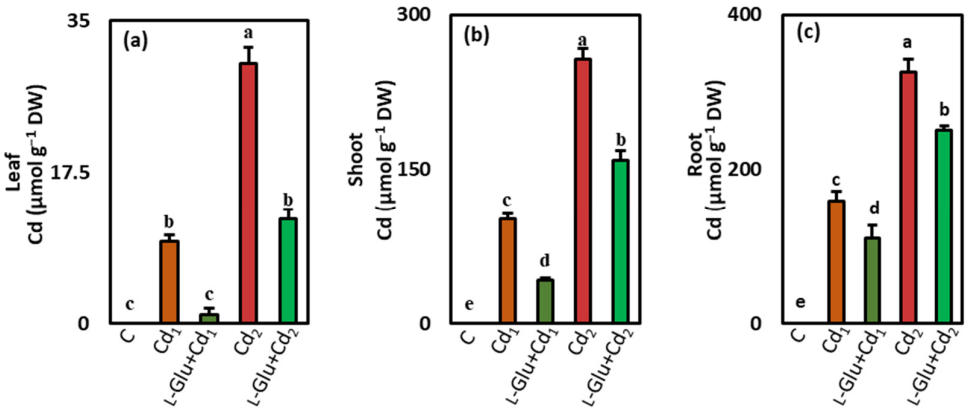

Figure 5. Effect of L-glutamic acid on the levels of a) leaf Cd, b) shoot Cd, and c) root Cd in the lentil seedlings under Cd stress

The above means (±SE) were calculated from three replications. Values with different letters indicate statistically significant differences at P≤0.05 (Fisher's LSD test).

L-Glu application improved ion homeostasis of the lentil seedlings under Cd stress

Upon exposure to 1 and 2 mM of $CdCl_2$, the uptake of Ca^{2+} and Mg^{2+} through the roots dramatically decreased in comparison with control (Supplementary Figure 3). The leaves and shoots of the lentil seedlings also exhibited lower amounts of Ca^{2+} and Mg^{2+} under both levels of Cd stress (Supplementary Figure 3). However, the uptake and translocation of Ca^{2+} and Mg^{2+} increased in the L-Glu-pre-treated seedling roots (25 and 93%, 123 and 151%), shoots (81 and 206%, 21 and 69%), and leaves (168 and 216%, 33 and 40%) in response to $CdCl_2$ (1 and 2 mM) in comparison with the Cd-treated seedlings (Supplementary Figure 4). Furthermore, the uptake of K^+ through the roots was 233 and 147 µmol g^{-1} DW under 1 and 2 mM $CdCl_2$, respectively, and remained 41 and 63% lower than the control seedlings (Supplementary Figure 4). In response to the Cd stress, the seedlings also translocated reduced amounts of K^+ to the shoots (50 and 13%) and leaves (34 and 60%) compared with control (Supplementary Figure 3). In comparing the seedlings exposed to both levels of Cd stress, adding L-Glu significantly increased the amount of K^+ uptake by the roots of around 52 and 100%, respectively (Supplementary Figure 3). Translocation of K^+ from the roots to the shoots to leaves also increased with the application of L-Glu under both levels of Cd stress (Supplementary Figure 3).

Correlation analysis

Correlation analysis was performed to determine the actual relationship between different factors and application of Cd (1 and 2 mM) and L-Glu in the lentil seedlings (Supplementary Figure 4). Generation of the oxidative stress markers, MDA, H_2O_2, other aldehyde, and EL, correlated positively with both concentrations of Cd, while the growth parameters of biomass production and photosynthetic pigment contents. The ion accumulation (K^+, Ca^{2+}, and Mg^{2+}) correlated negatively with Cd concentration (Supplementary Figure 4). Conversely, L-Glu concentration correlated positively with the growth attributes (biomass production, photosynthetic pigment contents) and ion accumulation (K^+, Ca^{2+}, and Mg^{2+}), but correlated negatively with

the oxidative stress markers (MDA, H_2O_2, other aldehyde, and EL) according to 1 and 2 mM $CdCl_2$ concentration (Supplementary Figure 4). The antioxidant defense components, especially AsA, CAT, APX, and MDHAR, showed a negative correlation with the generation of the oxidative stress markers under both Cd concentrations (1 and 2 mM) with or without L-Glu supplementation (Supplementary Figure 4).

Discussion

Heavy metals, particularly Cd (a non-essential element), can easily enter plant cells and negatively interfere with plant physiological processes (Cuypers *et al.*, 2010; Jia-Wen *et al.*, 2013). As a result of excess Cd accumulation, plants suffer from oxidative damage due to ROS generation, which ultimately reduces the survivability of plants by restricting growth and biomass (Gill and Tuteja, 2010). Therefore, inhibiting Cd uptake and reducing Cd-induced toxicity in plants is an important objective for plant scientists. Recently, researchers have been trying to use effective and inexpensive technologies, including external application of chemical, to modulate ingression and accumulation of Cd in plants, especially those used as food for people (Corpas and Palma, 2020; Khan *et al.*, 2020). Even though some chemicals have shown a promising protective role, scientists are still looking for inexpensive and eco-friendly chemicals. Evidence suggests that L-Glu is involved in plant growth and development, and also plays a role in protecting plants under different adverse conditions (La *et al.*, 2020; Toyota *et al.*, 2018; Zheng *et al.*, 2018; Kong *et al.*, 2015). Our results suggested a protective role of L-Glu in mitigating damage induced by Cd in lentil seedlings.

To investigate the role of L-Glu in Cd-stress tolerance, we first tested the response of lentil seedlings under a series of Cd stresses from 0.3 to 3 mM Cd with or without 10 mM L-Glu. Severe phenotypic damage was observed at 2- and 3-mM Cd stress (Supplementary Figure 1a-c). We selected the doses 1- and 2-mM Cd for further investigation. The present study reveals that the Cd-stressed lentil seedlings showed reduced growth, reduced shoot and root fresh and dry weight, greyish leaves, and withered seedlings with increasing Cd concentration (Figure 1, Table 1). Similarly, arrested growth (reduction of shoot and root fresh and dry masses) of wheat (Hussain *et al.*, 2018), *Brassica juncea* (Kapoor *et al.*, 2019), and lentil (Feizi *et al.*, 2020) has been observed under different levels of Cd stress. However, adding L-Glu to Cd-stressed lentil seedlings was found to improve the phenotypic appearance of the lentils by restoring the green to leaves, and recovering the growth and biomass of seedlings, suggesting that L-Glu treatment could mitigate the detrimental effect of Cd. L-Glu application promotes the growth of lentil and *Brassica napus* L. under drought- and salt-stress conditions (La *et al.*, 2020; Fardus *et al.*, 2021). In comparison with other amino acids (L-glutamine, L-glycine, L-aspartic acid, L-phenylalanine, L-methionine, and L-cysteine), L-Glu-pre-treated lentil seedlings were phenotypically healthier under a Cd-stress conditions, suggesting that L-Glu has a specific role in mitigating Cd toxicity in the lentil seedlings (Figure 1, Supplementary Figure 2a–d).

Substantial water and nutrient uptake are required for maintaining the optimal growth of a plant (Nazar *et al.*, 2012). In our current study, we found a decreasing percentage of WC in the Cd-stressed lentil seedlings, indicating that Cd in the growing media reduced the uptake of water (Table 1). Under abiotic stress conditions, the common physiological response of plants to overcome water deficiency is accumulating different osmo-protectants including Pro (Kaur and Asthir, 2015). Therefore, in our investigation, the Cd-stressed seedlings accumulated a higher amount of Pro to adjust the osmotic condition in the cells (Table 1). Conversely, L-Glu pre-treatment restored the percentage of WC in the lentil seedlings, and thus Pro accumulation was lower under Cd stress compared with the Cd alone-stressed seedlings (Table 1). Our result is similar to that of Kaya *et al.* (2019), who observed that exogenous application of sodium nitroprusside and sodium hydrogen sulfide resulted in a lower level of Pro because there was a higher relative water content in the Cd-stressed wheat seedlings.

A higher concentration of Cd in the growing media negatively affects plant photosynthesis by distorting chloroplasts, damaging photosynthetic pigments, and deactivating the enzymes or proteins that are responsible

for photosynthesis (Xu *et al.*, 2015). Our current investigation revealed that the Cd-stressed seedlings exhibited significantly lower contents of Chls and Car, suggesting that toxic Cd reduces photosynthetic pigments, which results in impairment of growth of the lentil seedlings (Figure 2a–d, Table 1). Shahwar *et al.* (2019) also reported that Cd reduces the photosynthetic pigments Chl a, Chl b, and Car in lentils. However, L-Glu application reduces the loss of photosynthetic pigments under Cd stress, indicating that L-Glu improved the photosynthetic pigments in the leaf tissue, and consequently, seedling growth and biomass increased under Cd stress (Figure 2a–d; Table 1). The role of L-Glu in ameliorating the damage to the photosynthetic apparatus has also been noticed under drought- and salt-stress conditions (La *et al.*, 2020; Fardus *et al.*, 2021).

Excessive amounts of heavy metals including Cd can trigger the generation of ROS in plants directly or indirectly, which leads to lipid peroxidation (Küpper and Andresen, 2016; Rizwan *et al.*, 2017). In our experiment, Cd stress induced oxidative damage in the membranes and accumulation of ROS in the lentil seedlings, which is indicated by higher amounts of MDA, H_2O_2, and another aldehyde, and higher EL (Figure 3a–d). Bashri and Prasad (2016), Anjum *et al.* (2016), and Chen *et al.* (2019) also found increased MDA and EL in fenugreek, maize, and rice, respectively, in response to Cd stress. On the other hand, L-Glu pre-treatment considerably reduced the contents of MDA, H_2O_2, and other aldehyde, and reduced EL in the lentil seedlings, suggesting that L-Glu application alleviates the Cd-induced membrane damage and reduces the overaccumulation of ROS (Figure 3a–d). Our results are in line with La *et al.* (2020), who suggest that exogenous Glu application modulates the response to drought stress, especially the accumulation of H_2O_2.

The efficient functioning of the enzymatic and non-enzymatic components of the antioxidant defense system of plants regulates excessive ROS production and maintains a redox potential under adverse environmental conditions (Nazar *et al.*, 2012; Khademian *et al.*, 2019). Our current study investigated the response of different components of the antioxidant defense system in the lentil seedlings (Figure 4a–k). The non-enzymatic antioxidants AsA and GSH are an integral part of plants, act as a ROS scavenger, and also counteract the different stress-induced oxidative stresses (Foyer and Noctor, 2005; Halliwell, 2006). Our study revealed that L-Glu application increased the amount of AsA and the GSH/GSSG ratio but lowered the amount of GSH and GSSG under Cd stress (Figure 4a–d). These results indicated that L-Glu reduced the inhibitory effect of toxic Cd and maintained the redox balance of the plant cells with the help of AsA and GSH/GSSG.

The antioxidant enzymes CAT, APX, and GPX play an important role in converting H_2O_2 to H_2O (Suzuki *et al.*, 2012). DHAR and MDHAR regulate the pool of AsA (Wang *et al.*, 2018; Xia *et al.*, 2018). GR and G*S*T also play an important role in scavenging ROS. In our study, L-Glu supplementation in the lentil seedlings increased the activities of CAT, APX, GPX, and MDHAR and decreased DHAR, GR, and G*S*T under Cd stress (Figure 4e–k). These results mean that application of L-Glu stimulated some enzymes to protect the lentils from Cd-induced oxidative damage. On the other hand, the Cd-stressed seedlings exhibited reduced AsA content and CAT activity resulting in increased ROS (Figure 4a, e). Nahar *et al.* (2016) also reported that the activity of CAT and the level of AsA decreased in response to Cd stress in mungbean plants. A similar reduction in these enzymes was also found in pepper and strawberry plants in response to Cd stress (Kaya *et al.*, 2020, Wu *et al.*, 2021). Moreover, higher contents of GSH and GSSG, and higher activities of APX, MDHAR, GR, G*S*T, GPX, and DHAR were found in the Cd-stressed seedlings, indicating that the stressed seedlings increased their levels to manage the sustainability of the seedlings under stress conditions (Figure 4b–d,f–k). Increased enzyme activity and non-enzyme content were also found in lentil, rice, and maize plants with the application of Cd (Horemans *et al.*, 2015; Khodarahmi and Khoshgoftarmanesh, 2017; Per *et al.*, 2017; Lu *et al.*, 2019). The response of the antioxidant defense system might vary depending on several factors such as types of stress, duration of stress, experimental conditions, and plant species (Shanying *et al.*, 2017). Efficient functioning of one or two enzymes among the sets of enzymes might enhance stress tolerance (Abogadallah, 2010). Based on our results, we surmised that the combined action of CAT, APX, MDHAR, and AsA inhibited the overaccumulation of ROS in the lentil seedlings under Cd stress with pre-treatment by L-Glu.

Accumulation of heavy metals in plants depends upon plant species and their particular parts in addition to the type of metal and their toxicity characteristics (Zhou *et al.*, 2018; Rohani *et al.*, 2019). Our present study showed that the Cd content in the leaves, shoots, and roots increased in the Cd-stressed lentil seedlings, where a higher amount of Cd was found in the root tissue (Figure 5a–c). Perhaps the transportation of Cd to the aerial parts from the roots is obstructed by the action of plant resistance. Bansal *et al.* (2021) also reported that under Cd stress, the uptake of Cd in lentil roots increased, which was then transferred to the shoots and leaves in lower amounts compared with the roots. However, L-Glu played an important role in maintaining Cd homeostasis by inhibiting the accumulation of Cd in the roots and translocation of Cd to the shoots and leaves (Figure 5a–c). It might be due to strengthening of lentil seedlings against Cd stress through increasing the other ion uptake by root and shoot ion transporter channels. Forde and Lea (2007) reported that, external application of Glu in soil increased the uptake of Ca^{2+} and K^+ by increasing the activity of responsive genes of glutamate-gated Ca^{2+} and K^+ channel under deficient condition of Ca^{2+} and K^+. Our current study also revealed that exogenous application of l-Glu improved the accumulation and translocation of K^+, Ca^{2+}, and Mg^{2+} in the leaves, shoots, and roots of the lentil seedlings under both Cd concentrations, indicating that L-Glu limits the accumulation of Cd (Supplementary Figure 3a-i). Perhaps different L-Glu responsive genes in the nutrient transport channel of lentil seedlings restricted the uptake and translocation of Cd which in turn increase nutrient availability to the seedling under toxic Cd stress condition. However, toxic Cd caused an imbalance in the uptake and transport of K^+, Ca^{2+}, and Mg^{2+} to the roots, shoots, and leaves of the lentil seedlings (Supplementary Figure 3a-i). Correlation analysis also showed a negative correlation between ionic homeostasis and Cd concentration (1 and 2 mM) in the lentil seedlings (Supplementary Figure 4). It might be, one toxic effect of Cd stress is that it competes with the accumulation of other nutrient elements such as K^+, Ca^{2+}, and Mg^{2+} in plants and causes an imbalance in ionic homeostasis (Liu *et al.*, 2016). According to the report of Kurtyka *et al.* (2008), uptake and translocation of K^+ become declined in response of toxic Cd which in turn failed to conduct chlorophyll and carotenoid biosynthesis of plant. In addition, Cd also compete with Ca^{2+}, and Mg^{2+} uptake and transportation through infiltrating their transportation channel due to having divalent properties of Cd^{2+} similar of Ca^{2+}, and Mg^{2+} (Yang *et al.*, 2021). Our results are similar to those of other studies, in which a reduction of the accumulation of K^+, Ca^{2+}, and Mg^{2+} by toxic Cd were found in mustard, pepper, tomato, and chickpea plants (Gratão *et al.*, 2015; Ahmad *et al.*, 2016; Wang *et al.*, 2018; Kaya *et al.*, 2020). Concerning this point of view, we can assume that L-Glu maintain nutrient homeostasis by reducing the uptake of Cd in lentil seedlings which in turn remains as the main cause of protection against toxic Cd.

Conclusions

Cadmium inhibited the growth of lentil seedlings, which was exacerbated by the increase in Cd concentration. The growth parameters and photosynthetic pigments of the lentil seedlings also decreased severely with exposure to Cd stress. However, L-Glu pre-treatment significantly improved the seedling growth and photosynthetic pigments in both the 1- and 2-mM Cd-stressed seedlings. The application of L-Glu to the lentil seedlings alleviated Cd toxicity by hindering the accumulation of Cd and transportation of the accumulated Cd to the shoots and leaves. In addition, L-Glu pre-treatment alleviated the damage caused by the Cd-induced oxidative stress through the efficient functioning of AsA, CAT, APX, MDHAR, and GPX in the lentil seedlings. Our findings suggest that exogenous L-Glu pre-treatment could be a potential candidate to alleviate the noxious effect of Cd stress. However, further investigation is needed to determine the long-term effects of L-Glu for stress tolerance and to understand the molecular mechanism of how L-Glu controls Cd uptake and the antioxidant responses.

Authors' Contributions

Conceptualization: JF and MSH; Formal analysis: MSH; Supervision: MF; Writing - original draft: JF; Writing - review and editing: MF and MSH. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Abogadallah GM (2010). Insights into the significance of antioxidative defense under salt stress. Plant Signaling and Behavior 5:369-374. *https://doi.org/10.4161/psb.5.4.10873*

Ahmad P, Abdel Latef AA, Abd Allah EF, Hashem A, Sarwat M, Anjum NA, Gucel S (2016). Calcium and potassium supplementation enhanced growth, osmolyte secondary metabolite production, and enzymatic antioxidant machinery in cadmium-exposed chickpea (*Cicer arietinum* L.). Frontiers and Plant Science 7:513. *https://doi.org/10.3389/fpls.2016.00513*

Andrews M, Andrews ME (2017). Specificity in legume-rhizobia symbioses. International Journal of Molecular Sciences 18:705. *https://doi.org/10.3390/ijms18040705*

Anjum SA, Tanveer M, Hussain S, Shahzad B, Ashraf U, Fahad S, ... Bajwa AA (2016). Osmoregulation and antioxidant production in maize under combined cadmium and arsenic stress. Environmental Science and Pollution Research 23:11864-11875. *https://doi.org/10.1007/s11356-016-6382-1*

Asgher M, Khan NA, Khan MIR, Fatma M, Masood A (2014). Ethylene production is associated with alleviation of cadmium-induced oxidative stress by sulfur in mustard types differing in ethylene sensitivity. Ecotoxicology and Environmental Safety 106:54-61. *https://doi.org/10.1016/j.ecoenv.2014.04.017*

Bansal R, Priya S, Dikshit HK, Jacob SR, Rao M, Bana RS, ... Siddique K (2021). Growth and antioxidant responses in iron-biofortified lentil under cadmium stress. Toxics 9:182. *https://doi.org/10.3390/toxics9080182*

Bashri G, Prasad SM (2016). Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in Cd stressed *Trigonella foenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate-glutathione cycle. Ecotoxicology and Environmental Safety 132:329-338. *https://doi.org/10.1016/j.ecoenv.2016.06.015*

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant and Soil 39:205-207. *https://doi.org/10.1007/BF00018060*

Bayçu G, Moustaka J, Gevrek N, Moustakas M (2018). Chlorophyll fluorescence imaging analysis for elucidating the mechanism of photosystem II acclimation to cadmium exposure in the hyperaccumulating plant *Noccaea caerulescens*. Materials 11:2580. *https://doi.org/10.3390/ma11122580*

Bočová B, Huttová J, Mistrík I, Tamás L (2013). Auxin signalling is involved in cadmium-induced glutathione-*S*-transferase activity in barley root. Acta Physiologiae Plantarum 35:2685-2690. *https://doi.org/10.1007/s11738-013-1300-3*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72:248-254. *https://doi.org/10.1016/0003-2697(76)90527-3*

Chen D, Chen D, Xue R, Long J, Lin X, Lin Y, ... Song Y (2019). Effects of boron, silicon and their interactions on cadmium accumulation and toxicity in rice plants. Journal of Hazardous Materials 367:447-455. *https://doi.org/10.1016/j.jhazmat.2018.12.111*

Corpas FJ, Palma JM (2020). H_2S signaling in plants and applications in agriculture. Journal of Advanced Research 24:131-137. *https://doi.org/10.1016/j.jare.2020.03.011*

Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, ... Nawrot T (2010). Cadmium stress: An oxidative challenge. BioMetals 23:927-940. *https://doi.org/10.1007/s10534-010-9329-x*

Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Science 135:1-9. *https://doi.org/10.1016/S0168-9452(98)00025-9*

Fardus J, Hossain M, Fujita M (2021). Modulation of the antioxidant defense system by exogenous L-glutamic acid application enhances salt tolerance in lentil (*Lens culinaris* Medik.). Biomolecules 11:587. *https://doi.org/10.3390/biom11040587*

Feizi H, Agheli N, Sahabi H (2020). Titanium dioxide nanoparticles alleviate cadmium toxicity in lentil (*Lens culinaris* Medic) seeds. Acta Agriculturae Slovenica 116:59-68. *http://dx.doi.org/10.14720/aas.2020.116.1.1116*

Forde BG, Lea PJ (2007). Glutamate in plants: metabolism, regulation, and signalling. Journal of Experimental Botany 58:2339-2358. *https://doi.org/10.1093/jxb/erm121*

Forde BG (2014). Glutamate signalling in roots. Journal of Experimental Botany 65:779-787. *https://doi.org/10.1093/jxb/ert335*

Foti C, Khah EM, Pavli OI (2019). Germination profiling of lentil genotypes subjected to salinity stress. Plant Biology 21:480-486. *https://doi.org/10.1111/plb.12714*

Foyer CH, Noctor G (2005). Redox homeostasis and antioxidant signaling: A metabolic interface between stress perception and physiological responses. The Plant Cell 17:1866-1875. *https://doi.org/10.1105/tpc.105.033589*

Gill SS, Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48:909-930. *https://doi.org/10.1016/j.plaphy.2010.08.016*

Gratão PL, Monteiro CC, Tezotto T, Carvalho RF, Alves LR, Peters LP, Azevedo RA (2015). Cadmium stress antioxidant responses and root-to-shoot communication in grafted tomato plants. BioMetals 28:803-816. *https://doi.org/10.1007/s10534-015-9867-3*

Halliwell B (2006). Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology 141:312-322. *https://doi.org/10.1104/pp.106.077073*

He F, Arce AL, Schmitz G, Koornneef M, Novikova P, Beyer A, De Meaux J (2016). The footprint of polygenic adaptation on stress-responsive cis-regulatory divergence in the *Arabidopsis* genus. Molecular Biology and Evolution 33:2088-2101. *https://doi.org/10.1093/molbev/msw096*

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Archives of Biochemistry and Biophysics 125:189-198. *https://doi.org/10.1016/0003-9861(68)90654-1*

Horemans N, Van Hees M, Van Hoeck A, Saenen E, De Meutter T, Nauts R, ... Vandenhove H (2015). Uranium and cadmium provoke different oxidative stress responses in *Lemna minor* L. Plant Biology 17:91-100. *https://doi.org/10.1111/plb.12222*

Hussain A, Ali S, Rizwan M, Zia-ur-Rehman MZ, Javed MR, Imran M, ... Nazir R (2018). Zinc oxide nanoparticles alter the wheat physiological response and reduce the cadmium uptake by plants. Environmental Pollution 242:1518-1526. *https://doi.org/10.1016/j.envpol.2018.08.036*

Huybrechts M, Cuypers A, Deckers J, Iven V, Vandionant S, Jozefczak M, Hendrix S (2019). Cadmium and plant development: An agony from seed to seed. International Journal of Molecular Sciences 20:3971. *https://doi.org/10.3390/ijms20163971*

Ismael MA, Elyamine AM, Moussa MG, Cai M, Zhao X, Hu C (2019). Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. Metallomics 11:255-277. *https://doi.org/10.1039/c8mt00247a*

Wu J-W, Shi Y, Zhu Y-X, Wang Y-C, Gong H-J (2013). Mechanisms of enhanced heavy metal tolerance in plants by silicon: A review. Pedosphere 23:815-825. *https://doi.org/10.1016/S1002-0160(13)60073-9*

Kan CC, Chung TY, Wu HY, Juo YA, Hsieh MH (2017). Exogenous glutamate rapidly induces the expression of genes involved in metabolism and defense responses in rice roots. BMC Genomics 18:186. *https://doi.org/10.1186/s12864-017-3588-7*

Kapoor D, Singh MP, Kaur S, Bhardwaj R, Zheng B, Sharma A (2019). Modulation of the functional components of growth, photosynthesis, and antioxidant stress markers in cadmium exposed *Brassica juncea* L. Plants 8:260. *https://doi.org/10.3390/plants8080260*

Kaur G, Asthir BJBP (2015). Proline: A key player in plant abiotic stress tolerance. Biologia Plantarum 59:609-619. *https://doi.org/10.1007/s10535-015-0549-3*

Kaya C, Ashraf M, Alyemeni MN, Ahmad P (2019). Responses of nitric oxide and hydrogen sulfide in regulating oxidative defence system in wheat plants grown under cadmium stress. Physiologia Plantarum 168:345-360. *https://doi.org/10.1111/ppl.13012*

Kaya C, Ashraf M, Alyemeni MN, Ahmad P (2020). The role of nitrate reductase in brassinosteroid-induced endogenous nitric oxide generation to improve cadmium stress tolerance of pepper plants by upregulating the ascorbate-glutathione cycle. Ecotoxicology and Environmental Safety 196:110483. *https://doi.org/10.1016/j.ecoenv.2020.110483*

Keramat B, Kalantari KM, Arvin MJ (2010). Effects of methyl jasmonate treatment on alleviation of cadmium damages in soybean. Journal of Plant Nutrition 33:1016-1025. *https://doi.org/10.1080/01904161003728685*

Khademian R, Asghari B, Sedaghati B, Yaghoubian Y (2019). Plant beneficial rhizospheric microorganisms (PBRMs) mitigate deleterious effects of salinity in sesame (*Sesamum indicum* L.): Physio-biochemical properties, fatty acids composition and secondary metabolites content. Industrial Crops and Products 136:129-139. *https://doi.org/10.1016/j.indcrop.2019.05.002*

Khan MA, Khan S, Khan A, Alam M (2017). Soil contamination with cadmium, consequences and remediation using organic amendments. Science of The Total Environment 601:1591-1605. *https://doi.org/10.1016/j.scitotenv.2017.06.030*

Khan MN, Al Solami MA, Basahi RA, Siddiqui MH, Al-Huqail AA, Abbas ZK, ... Khan F (2020). Nitric oxide is involved in nano-titanium dioxide-induced activation of antioxidant defense system and accumulation of osmolytes under water-deficit stress in *Vicia faba* L. Ecotoxicology and Environmental Safety 190:110152. *https://doi.org/10.1016/j.ecoenv.2019.110152*

Khodarahmi S, Khoshgoftarmanesh AH (2017). The effect of cadmium toxicity and silicon supplementation on the activity of antioxidative enzymes and the concentration of zinc and iron in hydroponically grown cucumber. Communications in Soil Science and Plant Analysis 48:51-62. *https://doi.org/10.1080/00103624.2016.1253720*

Kong D, Ju C, Parihar A, Kim S, Cho D, Kwak JM (2015). Arabidopsis glutamate receptor homolog3.5 modulates cytosolic Ca^{2+} level to counteract effect of abscisic acid in seed germination. Plant Physiology 167:1630-1642. *https://doi.org/10.1104/pp.114.251298*

Krantev A, Yordanova R, Janda T, Szalai G, Popova L (2008). Treatment with salicylic acid decreases the effect of cadmium on photosynthesis in maize plants. Journal of Plant Physiology 165:920-931. *https://doi.org/10.1016/j.jplph.2006.11.014*

Kurtyka R, Małkowski E, Kita A, Karcz W (2008). Effect of calcium and cadmium on growth and accumulation of cadmium, calcium, potassium and Sodium in Maize Seedlings. Polish Journal of Environmental Studies 17:51-56. *https://www.researchgate.net/publication/259852721*

Küpper H, Andresen E (2016). Mechanisms of metal toxicity in plants. Metallomics 8:269-285. *https://doi.org/10.1039/c5mt00244c*

La VH, Lee BR, Islam M, Mamun M, Park SH, Bae DW, Kim TH (2020). Characterization of glutamate-mediated hormonal regulatory pathway of the drought responses in relation to proline metabolism in *Brassica napus* L. Plants 9:512. *https://doi.org/10.3390/plants9040512*

Latef AA (2013). Growth and some physiological activities of pepper (*Capsicum annuum* L.) in response to cadmium stress and mycorrhizal symbiosis. Journal of Agricultural Science and Technology 15:1437-1448. *http://jast.modares.ac.ir/article-23-11530-en.html*

Li Q, Wang G, Wang Y, Yang D, Guan C, Ji J (2019). Foliar application of salicylic acid alleviates the cadmium toxicity by modulation the reactive oxygen species in potato. Ecotoxicology and Environmental Safety 172:317-325. *https://doi.org/10.1016/j.ecoenv.2019.01.078*

Liu Z, Ding Y, Wang F, Ye Y, Zhu C (2016). Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Reports 35:719-731. *https://doi.org/10.1007/s00299-015-1925-3*

Lu Y, Wang QF, Li J, Xiong J, Zhou LN, He SL, ... Liu H (2019). Effects of exogenous sulfur on alleviating cadmium stress in tartary buckwheat. Scientific Reports 9:1-12. *https://doi.org/10.1038/s41598-019-43901-4*

Małecka A, Konkolewska A, Hanć A, Barałkiewicz D, Ciszewska L, Ratajczak E, ... Jarmuszkiewicz W (2019). Insight into the phytoremediation capability of *Brassica juncea* (v. Malopolska): Metal accumulation and antioxidant enzyme activity. International Journal of Molecular Sciences 20:4355. *https://doi.org/10.3390/ijms20184355*

Mishra S, Bharagava RN, More N, Yadav A, Zainith S, Mani S, Chowdhary P (2019). Heavy metal contamination: an alarming threat to environment and human health. In Environmental biotechnology: For sustainable future; Springer: Singapore 103-125. *https://doi.org/10.1007/978-981-10-7284-0_5*

Muneer S, Kim TH, Choi BC, Lee BS, Lee JH (2014). Effect of CO, NO_x and SO_2 on ROS production, photosynthesis and ascorbate–glutathione pathway to induce *Fragaria× annasa* as a hyperaccumulator. Redox Biology 2:91-98. *https://doi.org/10.1016/j.redox.2013.12.006*

Nahar K, Hasanuzzaman M, Rahman A, Alam M, Mahmud JA, Suzuki T, Fujita M (2016). Polyamines confer salt tolerance in mung bean (*Vigna radiata* L.) by reducing sodium uptake, improving nutrient homeostasis, antioxidant defense, and methylglyoxal detoxification systems. Frontiers in Plant Science 7:1104. *https://doi.org/10.3389/fpls.2016.01104*

Nazar R, Iqbal N, Masood A, Khan MIR, Syeed S, Khan NA (2012). Cadmium toxicity in plants and role of mineral nutrients in its alleviation. American Journal of Plant Sciences 3:4. *https://doi.org/10.4236/ajps.2012.310178*

Nephali L, Piater LA, Dubery IA, Patterson V, Huyser J, Burgess K, Tugizimana F (2020). Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. Metabolites 10:505. *https://doi.org/10.3390/metabo10120505*

Noctor G, Mhamdi A, Foyer CH (2016). Oxidative stress and antioxidative systems: recipes for successful data collection and interpretation. Plant, Cell and Environment 39:1140-1160. *https://doi.org/10.1111/pce.12726*

Per TS, Masood A, Khan NA (2017). Nitric oxide improves *S*-assimilation and GSH production to prevent inhibitory effects of cadmium stress on photosynthesis in mustard (*Brassica juncea* L.). Nitric Oxide 68:111-124. *https://doi.org/10.1016/j.niox.2016.12.012*

Qadir S, Jamshieed S, Rasool S, Ashraf M, Akram NA, Ahmad P (2014). Modulation of plant growth and metabolism in cadmium-enriched environments. Reviews of Environmental Contamination and Toxicology 51-88. *https://doi.org/10.1007/978-3-319-03777-6_4*

Qiu XM, Sun YY, Ye XY, Li ZG (2020). Signaling role of glutamate in plants. Frontiers in Plant Science 10:1743. *https://doi.org/10.3389/fpls.2019.01743*

Rizwan M, Ali S, Adrees M, Ibrahim M, Tsang DC, Zia-ur-Rehman M, ... Ok YS (2017). A critical review on effects, tolerance mechanisms and management of cadmium in vegetables. Chemosphere 182:90-105. *https://doi.org/10.1016/j.chemosphere.2017.05.013*

Rizwan M, Ali S, Zia-ur-Rehman MZ, Rinklebe J, Tsang DC, ... Ok YS (2018). Cadmium phytoremediation potential of *Brassica* crop species: A review. Science of The Total Environment 631:1175-1191. *https://doi.org/10.1016/j.scitotenv.2018.03.104*

Rohani N, Daneshmand F, Vaziri A, Mahmoudi M, Saber-Mahani F (2019). Growth and some physiological characteristics of *Pistacia vera* L. cv Ahmad Aghaei in response to cadmium stress and *Glomus mosseae* symbiosis. South African Journal of Botany124:499-507. *https://doi.org/10.1016/j.sajb.2019.06.001*

Savvides A, Ali S, Tester M, Fotopoulos V (2016). Chemical priming of plants against multiple abiotic stresses: mission possible? Trends in Plant Science 21:329-340. *https://doi.org/10.1016/j.tplants.2015.11.003*

Sh Sadak M, Abdelhamid MT, Schmidhalter U (2015). Effect of foliar application of amino acids on plant yield and some physiological parameters in bean plants irrigated with seawater. Acta Biológica Colombiana 20:141-152. *https://doi.org/10.15446/abc.v20n1.42865*

Shah AA, Ahmed S, Yasin NA (2019). 24-epibrassinolide triggers cadmium stress mitigation in *Cucumis sativus* through intonation of antioxidant system. South African Journal of Botany 127:349-360. *https://doi.org/10.1016/j.sajb.2019.11.003*

Shahwar D, Ansari MYK, Choudhary S (2019). Induction of phenotypic diversity in mutagenized population of lentil (*Lens culinaris* Medik) by using heavy metal. Heliyon 5. *https://doi.org/10.1016/j.heliyon.2019.e01722*

Shanying HE, Xiaoe YANG, Zhenli HE, Baligar VC (2017). Morphological and physiological responses of plants to cadmium toxicity: A review. Pedosphere 27:421-438. *https://doi.org/10.1016/S1002-0160(17)60339-4*

Song X, Yue X, Chen W, Jiang H, Han Y, Li X (2019). Detection of cadmium risk to the photosynthetic performance of Hybrid *Pennisetum*. Frontiers in Plant Science 10:798. *https://doi.org/10.3389/fpls.2019.00798*

Sun H, Wang X, Shang L, Zhou Z, Wang R (2017). Cadmium accumulation and its effects on nutrient uptake and photosynthetic performance in cucumber (*Cucumis sativus* L.). Philippine Agricultural Scientist 100:263-270. *https://www.researchgate.net/publication/320735530*

Suzuki N, Koussevitzky SHAI, Mittler RON, Miller GAD (2012). ROS and redox signaling in the response of plants to abiotic stress. Plant, Cell and Environment 35:259-270. *https://doi.org/10.1111/j.1365-3040.2011.02336.x*

Toyota M, Spencer D, Sawai-Toyota S, Jiaqi W, Zhang T, Koo AJ, Howe GA, Gilroy S (2018). Glutamate triggers long-distance, calcium-based plant defense signaling. Science 361:1112-1115. *https://doi.org/10.1126/science.aat7744*

Tran TA, Popova LP (2013). Functions and toxicity of cadmium in plants: Recent advances and future prospects. Turkish Journal of Botany 37:1-13. *https://doi.org/10.3906/bot-1112-16*

Wang J, Anderson CW, Xing Y, Fan Y, Xia J, Shaheen SM, ... Feng X (2018). Thiosulphate-induced phytoextraction of mercury in *Brassica juncea*: Spectroscopic investigations to define a mechanism for Hg uptake. Environmental Pollution 242:986-993. *https://doi.org/10.1016/j.envpol.2018.07.065*

Wellburn AR (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144:307-313. *https://doi.org/10.1016/S0176-1617(11)81192-2*

Wu J, Mock HP, Giehl RF, Pitann B, Mühling KH (2019). Silicon decreases cadmium concentrations by modulating root endodermal suberin development in wheat plants. Journal of Hazardous Materials 364:581-590. *https://doi.org/10.1016/j.jhazmat.2018.10.052*

Wu S, Wang Y, Zhang J, Gong X, Zhang Z, Sun J, Chen X, Wang Y (2021). Exogenous melatonin improves physiological characteristics and promotes growth of strawberry seedlings under cadmium stress. Horticultural Plant Journal 7:13-22. *https://doi.org/10.1016/j.hpj.2020.06.002*

Xia H, Zhao X, Ni Z, Liang D (2018). Enzyme's activities analysis involved in AsA-GSH cycle of yellow-flesh kiwifruit genotypes. In IOP Conference Series: Materials Science and Engineering 392:052019. IOP Publishing. *https://doi.org/10.1088/1757-899X/392/5/052019*

Xin J, Zhao XH, Tan QL, Sun XC, Zhao YY, Hu CX (2019). Effects of cadmium exposure on the growth, photosynthesis, and antioxidant defense system in two radish (*Raphanus sativus* L.) cultivars. Photosynthetica 57:967-973. *https://doi.org/10.32615/ps.2019.076*

Xu LL, Fan ZY, Dong YJ, Kong J, Bai XY (2015). Effects of exogenous salicylic acid and nitric oxide on physiological characteristics of two peanut cultivars under cadmium stress. Biologia Plantarum 59:171-182. *https://doi.org/10.1007/s10535-014-0475-9*

Yang J, Sun H, Qin J, Wang X, Chen W (2021). Impacts of Cd on temporal dynamics of nutrient distribution pattern of *Bletilla striata*, a traditional Chinese medicine plant. Agriculture 11:594. *https://doi.org/10.3390/agriculture11070594*

Yang SH, Wang LJ, Li SH (2007). Ultraviolet-B irradiation-induced freezing tolerance in relation to antioxidant system in winter wheat (*Triticum aestivum* L.) leaves. Environmental and Experimental Botany 60:300-307. *https://doi.org/10.1016/j.envexpbot.2006.12.003*

Yotsova EK, Dobrikova AG, Stefanov MA, Kouzmanova M, Apostolova EL (2018). Improvement of the rice photosynthetic apparatus defence under cadmium stress modulated by salicylic acid supply to roots. Theoretical and Experimental Plant Physiology 30:57-70. *https://doi.org/10.1007/s40626-018-0102-9*

Zheng Y, Luo L, Wei J, Chen Q, Yang Y, Hu X, Kong X (2018). The glutamate receptors AtGLR1. 2 and AtGLR1. 3 increase cold tolerance by regulating jasmonate signaling in *Arabidopsis thaliana*. Biochemical and Biophysical Research Communications 506:895-900. *https://doi.org/10.1016/j.bbrc.2018.10.153*

Zhou J, Hao M, Liu Y, Huang G, Fu Q, Zhu J, Hu H (2018). Effects of exogenous sulfur on growth and Cd uptake in Chinese cabbage (*Brassica campestris* spp. *pekinensis*) in Cd-contaminated soil. Environmental Science and Pollution Research 25:15823-15829. *https://doi.org/10.1007/s11356-018-1712-0*