Egypt. J. Agron. **Vol. 42**, No. 2, pp. 163-170 (2020)

Egyptian Journal of Agronomy
http://agro.journals.ekb.eg/

Cadmium Toxicity Alleviates by Seed Priming with Proline or Glycine Betaine in Cowpea (*Vigna unguiculata* (L.) Walp.)

Omid Sadeghipour
Department of Agronomy, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University, Tehran 18155/144, Iran.

CADMIUM (Cd) as a non-essential metal is a soil pollutant that inhibits plant growth and development. Proline (Pro) and Glycine betaine (GB) are two important compatible solutes that accumulate in response to abiotic stresses in different plant species. Here, a pot experiment was carried out to assess the role of exogenously applied Pro and GB in improving Cd toxicity tolerance in cowpea plants. Seeds were soaked in 0, 25 and 50mM Pro or GB, and then were sown in plastic pots that were irrigated with 0 or 100µM cadmium chloride. Results showed that Cd stress markedly decreased shoot length, shoot biomass, leaf area, chlorophyll value, stomatal conductance and relative water content (RWC) but increased root and shoot Cd levels, malondialdehyde (MDA) content and antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX). Nevertheless, seed treatment with Pro or GB reduced Cd toxicity and improved growth attributes via reducing Cd uptake and translocation, increasing the chlorophyll value, stomatal conductance, and RWC as well as more enhancing the activities of antioxidant enzymes and lowering the MDA level. Although both Pro and GB were effective, pre-treatment of seeds with Pro improved Cd stress tolerance better than GB. Thus, seed treatment with Pro (especially 50mM) could be used as an effective method for protecting cowpea plants against Cd toxicity.

Keywords: Cadmium, Glycine betaine, Proline, Seed treatment, Oxidative stress.

Introduction

Although the cadmium (Cd) level is very low in the earth's crust, various anthropogenic activities such as mining and smelting, application of sewage sludge, compost, phosphate fertilizer, pesticide, and insecticide have led to an increase in the Cd concentration in agricultural soils. Cd as a non-essential heavy metal element is easily taken up by plants due to its high mobility within the soil-plant system. Cd accumulation in the plant can cause biochemical, molecular, physiological and morphological disorders, such as induction of oxidative stress, impairment the activity of various enzymes, reduction of chlorophyll level, stomata opening, photosynthesis and transpiration, disturbance in uptake, transport and use of minerals, water imbalance, leaf rolling, chlorosis and necrosis, root browning, and finally inhibition of growth and productivity (Nagajyoti et al., 2010; Asgher et al., 2015; Shahid et al., 2016).

One of the most common biochemical responses in higher plants under environmental stress conditions is the accumulation of compatible organic solutes such as amino acid proline (Pro) and quaternary ammonium compound glycine betaine (GB). These compounds are low molecular weight, highly soluble and nontoxic at high cellular concentrations. These osmoprotectants protect plants against the stress through various ways, including osmotic adjustment, scavenging reactive oxygen species (ROS), maintenance of membrane integrity, and stabilization of enzymes and proteins (Ashraf & Foolad, 2007). Heavy

Corresponding author email: osadeghipour@yahoo.com 0000-0002-6725-0704 Tel: +989122965044
Received 8/2/ 2020; Accepted 23/6/ 2020
DOI: 10.21608/agro.2020.23667.1204

metal stress up-regulates the enzymes involved in Pro and GB biosynthesis which contribute in improving stress tolerance (Hossain et al., 2010). However, in many crop plants, the natural accumulation of Pro or GB is not sufficient to protect them against the adverse effects of various environmental stresses such as Cd. Therefore, the exogenous application of Pro or GB may help to enhance Cd stress tolerance. Rasheed et al. (2014) found that foliar application of Pro and GB caused a noticeable alteration in growth and physiochemical traits of two wheat cultivars under Cd stress. Although both Pro and GB were effective, plants treated with Pro had greater shoot and root biomass, leaf phenolics, lower degradation of chlorophylls, and accumulation of malondialdehyde (MDA) and H_2O_2 contents under Cd stress. Islam et al. (2009) also reported that both exogenous Pro and GB enhanced tolerance to Cd stress in cultured tobacco BY-2 cells through protecting cellular components, increasing antioxidant enzyme activities and intracellular contents of Pro and GB. Furthermore, Pro was more effective than GB. Application of Pro and GB through irrigation water alleviated the harmful effects of lead (Pb) stress on olive trees by reducing Pb content and increased the enzymatic and non-enzymatic antioxidant systems. Pro had a better influence on reducing Pb toxicity than GB (Zouari et al., 2018).

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important grain and forage legume in the tropical and subtropical regions. In addition to being rich in proteins, vitamins, minerals, fiber, and micronutrients, cowpea increase soil fertility. However, the growth and productivity of this crop are negatively affected by environmental stresses such as Cd toxicity. Although some studies have shown that exogenously applied Pro and GB alleviate Cd toxicity in some plant species, there is little information to compare the role of these organic compounds in reducing Cd toxicity in cowpea. Therefore, the current experiment aimed to evaluate and compare the role of Pro and GB in enhancing Cd toxicity tolerance in cowpea plants by measuring some biochemical, physiological and growth attributes.

Materials and Methods

Growth conditions, plant material, and treatments

This pot experiment was carried out in the research field of the Department of Agronomy, Yadegar-e-Imam Khomeini (RAH) Shahre Rey Branch, Islamic Azad University (35°35′ N; 51°28′ E and 1000 m altitude), Tehran, Iran, during spring and summer seasons of 2017, in a completely randomized design (CRD) with six treatments and four replicates under ambient air and temperature conditions. The meteorological data of the region for the growing season are presented in Table 1.

The plastic pots (50cm in diameter and height) were filled with 15kg soil containing an equal mixture of peat, decomposed manure, and farm soil. The texture of the soil was loam with pH 7.45, EC 1.90dS m^{-1}, organic carbon 2.82 %, N 0.28%, P, K and Cd 18.33, 425.30 and 0.44mg kg^{-1} soil, respectively. Cowpea seeds (cv. Kamran) were obtained from Pakan Bazr Corporation, Isfahan, Iran. Seeds without visible defect, insect damage or malformation were surface sterilized using 5 % sodium hypochlorite solution for five min and then rinsed three times with sterile distilled water. After sterilization, as a pre-sowing treatment, seeds were soaked for 8 h in different concentrations (0, 25 and 50mM) of Pro or GB. Then, 15 treated seeds were sown at a depth of three cm in each plastic pot on May 10, 2017. After seeding irrigation of the pots was done regularly using tap water. After thinning at the 2-leafy stage, six uniform seedlings were retained per pot and irrigation was done with 0 or 100µM of chloride cadmium as Cd stress treatments.

TABLE 1. Monthly meteorological data for the growing season of cowpea (2017).

Parameters	Mean Temperature (°C)	Precipitation (mm)	Mean Humidity (%)	Sunshine Duration (h)
April	17.6	20.5	37.3	247.4
May	24.9	10.1	25.2	302.4
June	28.7	0	16.3	328.7
July	31.5	0	21.0	356.8
August	30.0	0	16.0	358.9

Physiological traits assessment

At the early flowering stage, some physiological and biochemical traits were estimated. The chlorophyll value of the youngest fully expanded leaves was measured using a chlorophyll meter (Chlorophyll Content Meter, CL-01, Hansatech Instruments Ltd. England). Stomatal conductance was measured on a sunny day between 10:00 and 11:00hrs on the youngest fully expanded leaves using a Portable Leaf Porometer, SC-1, Decagon Devices, USA. To determine the relative water content (RWC); 10 disks (1cm in diameter) from the middle portion of the youngest fully expanded leaves were collected, immediately weighed to record the fresh weight (FW), then, rehydrated in petri dishes containing distilled water for 24h under dim light and room temperature to get the turgid weight (TW) and subsequently the discs were oven-dried at 70°C for 48hrs to record the dry weight (DW). RWC was calculated as: RWC (%)= (FW - DW) / (TW - DW) × 100.

Biochemical traits assessment

Lipid peroxidation was estimated in terms of MDA content according to the method of Heath & Packer (1968). Fresh leaf samples (500mg) were homogenized in 10mL of 0.1% (w/v) trichloroacetic acid (TCA), and the homogenate solution was centrifuged at 10000rpm for 10min. Then, 1mL of the supernatant was mixed with 4mL of 0.5% (w/v) thiobarbituric acid containing 20% (w/v) TCA. The mixture was heated at 95°C for 30min, cooled rapidly in an ice bath, and then centrifuged at 10000rpm for 10min. The absorbance of the supernatant was recorded at 532nm. The value for non-specific absorption at 600nm was subtracted. The MDA content was calculated using the extinction coefficient of $155mM^{-1}$ cm^{-1} and expressed as nmol g^{-1} fresh weight.

For preparing the crude enzyme extract, fresh leaf samples (500mg) were homogenized in 5mL of potassium phosphate buffer (100mM, pH 7.0), containing 1mM EDTA and 1% (w/v) polyvinylpyrrolidone (PVP), with the addition of 2mM ascorbate in the case of ascorbate peroxidase assay. The homogenate was centrifuged at 15000rpm for 20min at 4°C and the supernatant was used for the following enzyme assays. Protein concentration was determined by the method of Bradford (1976) using bovine serum albumin as a standard.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560nm following the method of Beauchamp & Fridovich (1971). Catalase (CAT, EC 1.11.1.6) activity was determined by monitoring the decomposition of H_2O_2 (extinction coefficient $39.4mM^{-1}$ cm^{-1}) at 240nm using the procedure of Aebi (1984). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was assayed according to Nakano & Asada (1981) by the decrease in absorbance of ascorbate (extinction coefficient $2.8mM^{-1}$ cm^{-1}) at 290 nm.

Growth traits assessment

At the early flowering stage, shoot length was recorded. The leaf area of plants was also calculated using a leaf area meter (CI-202, CID Bio-Science, USA). Moreover, the above ground plant parts were oven-dried at 80°C for 48hrs and shoot biomass was calculated.

Cd content assessment

The oven-dried samples were grounded into a fine powder. Cd concentrations in the root and shoot were estimated after digesting the samples in HNO_3-$HClO_4$ (3:1, v/v) using an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan).

Statistical analysis

All data were analyzed by one-way ANOVA, using MSTAT-C statistical software and the means were compared by the least significant difference (LSD) test at $P \leq 0.05$.

Results

Cd content

Cd treatment significantly increased Cd accumulation in the root and shoot of cowpea plants as compared with the control. Cd content was greater in the root than in the shoot. An increase by 32 and 23 folds in Cd content comparing to control plants were found in the root and shoot, respectively, under Cd stress. However, seed treatment with Pro or GB, especially 50mM Pro, significantly reduced the accumulation of Cd in the root and shoot (Table 2).

TABLE 2. Effects of different concentrations of proline (Pro) and glycine betaine (GB) on root and shoot Cd content, malondialdehyde (MDA) level and antioxidant enzyme activities of cowpea plants under cadmium (Cd) stress.

Treatments	Root Cd (μg g^{-1} D.W.)	Shoot Cd (μg g^{-1} D.W.)	MDA (nmol g^{-1} F.W.)	SOD (Unit mg^{-1} pro.)	CAT (Unit mg^{-1} pro.)	APX (Unit mg^{-1} pro.)
Control	1.43 e	0.63 e	12.24 d	16.32 d	20.21 d	12.17 e
Cd	45.77 a	14.38 a	18.84 a	19.95 c	25.26 c	14.30 d
Cd + Pro (25mM)	18.93 c	8.98 c	16.54 b	31.60 ab	41.44 b	21.76 b
Cd + Pro (50mM)	15.51 d	7.48 d	15.59 c	33.98 a	45.84 a	22.91 a
Cd + GB (25mM)	23.75 b	10.47 b	16.85 b	29.50 b	38.78 b	20.39 c
Cd + GB (50mM)	20.63 c	9.73 bc	17.24 b	30.95 b	40.02 b	21.30 bc
LSD	1.85	0.88	0.92	2.80	3.31	1.02

Means within a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ level using LSD. Data are mean ± SE (*n*= 4).

MDA content

The MDA content as an indicator of membrane lipid peroxidation severely elevated during Cd stress by 54 % as compared to non-stress conditions. Nonetheless, seed soaking in Pro or GB alleviated the adverse effect of Cd on membrane lipid peroxidation and consequently reduced the MDA content. Among the seed treatments, 50 mM Pro was the most effective (Table 2).

Antioxidant enzyme activities

Cd stress markedly raised SOD, CAT and APX activities by 22.2, 25 and 17.5%, respectively, compared with the control. On the other hand, seed priming with Pro or GB, especially 50mM Pro, further increased the activities of these antioxidant enzymes under Cd stress (Table 2).

Chlorophyll value

Cd stress caused a significant decrease in the chlorophyll value by 42% as compared with the control. Nevertheless, the treatment of seeds with Pro or GB enhanced the chlorophyll value under Cd stress. The highest improvement in the chlorophyll value was achieved at 50mM Pro (Table 3).

Stomatal conductance

Upon exposure to Cd stress, the stomatal conductance of cowpea leaves decreased noticeably by 39.3% compared to the control. However, seed treatment with Pro or GB, especially 50 mM Pro, improved the stomatal conductance under Cd stress (Table 3).

RWC

Cd toxicity caused a significant reduction in the RWC of cowpea leaves by 34.1% in comparison with the control. Nonetheless, seed priming with Pro or GB elevated the RWC under Cd stress conditions. Among the seed treatments, 50mM Pro was the most effective (Table 3).

Growth traits

Cowpea growth attributes including shoot length, shoot biomass and leaf area markedly decreased due to Cd stress by 39.8, 65 and 51.3%, respectively, compared with the control. On the other hand, seed soaking in Pro or GB significantly enhanced the above characteristics under Cd stress conditions. The maximum improvement in terms of the growth traits was recorded at 50mM Pro (Table 3).

Discussion

In the current experiment, Cd toxicity markedly reduced the growth attributes of cowpea plants, including shoot length, shoot biomass and leaf area. These results are in conformity with the previous findings on different plant species (Jhanji et al., 2012; Perveen et al., 2016; Sun et al., 2016; Chen et al., 2018). Nonetheless, seed priming with Pro or GB, especially 50mM Pro, improved all measured growth parameters under Cd stress. Similarly, the positive role of exogenously applied Pro and GB on the growth traits of various plants has been reported under Cd (Rasheed et al., 2014), Pb (Zouari et al., 2018) and Ni (Shahbaz et al., 2019) stress conditions.

TABLE 3. Effects of different concentrations of proline (Pro) and glycine betaine (GB) on some physiological and growth traits of cowpea plants under cadmium (Cd) stress.

Treatments	Chlorophyll value (Spad unit)	Stomatal conductance (mmol m^{-2} s^{-1})	RWC (%)	Shoot length (cm)	Shoot biomass (g $plant^{-1}$)	Leaf area (cm^2 $plant^{-1}$)
Control	38.25 a	13.51 a	92.48 a	76.02 a	110.47 a	824.54 a
Cd	22.19 d	8.20 d	60.75 d	45.75 d	38.61 d	401.39 d
Cd + Pro (25mM)	27.24 bc	10.00 bc	71.67 bc	56.76 bc	62.62 bc	574.16 bc
Cd + Pro (50mM)	29.29 b	10.65 b	77.91 b	60.43 b	71.71 b	611.11 b
Cd + GB (25mM)	26.00 c	9.61 c	68.59 c	55.68 c	57.13 c	530.48 c
Cd + GB (50mM)	26.66 bc	9.89 bc	70.84 bc	56.54 bc	62.11 bc	562.39 bc
LSD	3.10	0.86	7.11	4.60	14.24	79.39

Means within a column followed by the same letter (s) are not significantly different at $P \leq 0.05$ level using LSD. Data are mean ± SE (*n*= 4).

In the present study, when cowpea plants were exposed to Cd, the accumulation of Cd in the root was much higher than in the shoot. This result is in accordance with the previous reports on different plant species (Gaballah & Rady, 2012; Mostofa et al., 2015; Ahmad et al., 2016). Higher Cd content in the root as compared to the shoot is one of the most important defense mechanisms of plants against Cd toxicity (Ahmad et al., 2016; Rahman et al., 2017), because of Cd accumulated in the root is immobilized by the cell wall and extracellular carbohydrates (Ahmad et al., 2016). Nevertheless, in the current experiment, seed priming with Pro or GB not only reduced Cd accumulation in the root but also markedly decreased its translocation from the root to shoot. Reduction of Cd uptake and translocation in cowpea plants by exogenous application of Pro or GB in this research is in concurrence with the earlier researches (Islam et al., 2009; Zouari et al., 2016). According to these findings, Pro and GB play an important role in reducing Cd toxicity in cowpea plants by reduction of Cd uptake and translocation to the above-ground parts.

MDA, an end product of membrane lipid peroxidation is a proper marker for oxidative stress by heavy metal toxicity. In the current experiment, an elevated level of MDA in cowpea leaves was observed in Cd treated plants, which is conformity with the other findings (Shamsi et al., 2008; Ahmad et al., 2016; Chen et al., 2018). However, in this study, seed priming with Pro or GB significantly reduced the MDA level under Cd toxicity. Similarly, reduction of MDA content by exogenous application of Pro or GB under Cd stress conditions have been reported by other authors (Islam et al., 2009; Hossain et al., 2010; Rasheed et al., 2014). The positive effect of Pro and GB on preventing the degradation of biological membranes due to oxidative damage is attributed to their potential role in membrane stability, scavenging ROS, and the activation of antioxidant enzymes (Ashraf & Foolad, 2007; Hasanuzzaman et al., 2014).

Although Cd is not a redox-active metal and unable to produce ROS directly via Fenton and Haber-Weiss reactions, indirect ROS production is common in Cd-exposed plants (Shahid et al., 2016). At high concentrations, ROS can react with proteins, lipids, and nucleic acids, causing alteration or loss of structure and function which leads to lipid peroxidation and membrane leakage, enzyme inactivity, and DNA breakage and mutation (Andresen & Kupper, 2013). To scavenging excessive ROS, plants have developed antioxidant defense systems. ROS are efficiently scavenged by enzymatic (SOD; CAT; APX; GR; MDHAR; DHAR; GOPX and GST) and non-enzymatic (ascorbate, glutathione, phenolic compounds, alkaloids, non-protein amino acids and α-tocopherols), antioxidant systems, which protect them against oxidative damage. The enzymes SOD and CAT are involved in the detoxification of O_2•- and H_2O_2, respectively, thereby preventing the formation of OH• radicals, whereas, APX and GR, as well as ascorbate (AsA) and glutathione (GSH), are important components of the ascorbate-glutathione cycle responsible for the removal of H_2O_2 in different cellular compartments (Gill et al., 2013). In the present work, the activities of antioxidant enzymes such as SOD, CAT and APX, were enhanced in cowpea plants by Cd stress, and these activities were further increased by seed treatment with Pro or GB under Cd stress conditions. These results suggest that the enhanced activities of these enzymes are beneficial for cowpea response to Cd stress and that Pro and GB can stimulate the activity of antioxidant enzymes, which further strengthens the cowpea plants to withstand Cd toxicity. Similarly, several studies have reported Cd-induced activation of antioxidant enzymes, possibly via modulation of gene expression or Cd-induced restriction of enzyme inhibitors (Mostofa et al., 2015; Rady & Hemida, 2015; Rahman et al., 2017). In accordance with the present findings, exogenous application of Pro or GB enhanced antioxidant enzyme activities in mung bean (Hossain et al., 2010), and tobacco (Islam et al., 2009) under Cd stress.

In the current research, it was found that Cd stress significantly reduced the chlorophyll value of cowpea leaves, while seed treatment with Pro or GB improved this physiological trait under Cd toxicity. The reduction in the chlorophyll content under Cd stress is well known (Moussa & El-Gamal, 2010; Gaballah & Rady, 2012; Jhanji et al., 2012). The reduction of chlorophyll value in cadmium-treated plants is attributed to inhibition of its biosynthesis and also lower concentrations of Mg and Fe in the leaves (Gill et al., 2013). The positive role of exogenously Pro or GB on the chlorophyll value might be due to improving its biosynthesis and reducing its degradation under Cd stress conditions. In agreement with the present findings, treatment with Pro or GB enhanced the chlorophyll content of wheat under Cd stress (Rasheed et al., 2014), and also rice under salinity stress (Hasanuzzaman et al., 2014).

In this study, Cd induced a reduction in stomatal conductance of cowpea leaves. On the other hand, pretreatment of cowpea seeds with Pro or GB improved stomatal conductance under Cd stress conditions. In conformity with these findings, there are several reports that Cd strongly induced closure of stomata in different plant species (Shamsi et al., 2008; Sun et al., 2016; Sadeghipour, 2018). Under Cd stress, stomata were closed independently regardless of the water status. Moreover, Cd led to stomatal closure as a result of Cd entry into the guard cells and it reduced the stomata number per unit area (Ismael et al., 2019). Pro application caused an increase in stomatal conductance by maintaining appropriate cellular turgor (Wani et al., 2019). De Freitas et al. (2019) found that exogenously Pro elevated K^+ and Ca^+ accumulation which likely acted in membrane stability and water content, thus regulated stomatal conductance of sorghum plants under salinity stress. Rady et al. (2018) also demonstrated that foliar application of GB as an osmoprotectant increased the stomatal conductance of salt-stressed onion plants via maintaining the endogenous available water.

RWC is an important indicator of water status in plants which is related to water uptake from the roots and transpiration rate from the leaves. In the present study, Cd treatment significantly decreased the RWC of cowpea leaves through inhibition of root growth and stomata closure. In accordance with these results, Cd treatment reduced the RWC of wheat (Moussa & El-Gamal, 2010), pea (Gaballah & Rady, 2012) and common bean (Sadeghipour, 2018). Cd via an effect on stomatal conductance, water transport, and cell wall elasticity could disturb the plant water balance (De Maria et al., 2013). Nonetheless, in this study, seed treatment with Pro or GB through increase the root growth, and stomatal conductance improved the water relations in terms of the RWC under Cd toxicity. In agreement with these findings, Hasanuzzaman et al. (2014) reported that exogenous application of Pro or GB to salt-treated rice seedlings enhanced the RWC which was due to the maintenance of water in their tissue.

Conclusion

Cd stress caused oxidative stress and led to a significant decrease in chlorophyll value, stomatal conductance, RWC and finally growth attributes of cowpea plants. Nonetheless, pre-treatment of seeds with Pro or GB reduced the harmful effects of Cd. Pro was more effective than GB in mitigating the detrimental effects of Cd stress by reducing Cd uptake and translocation, improving chlorophyll value, stomatal conductance, and RWC, as well as enhance antioxidant enzyme activities and preventing membrane lipid peroxidation.

References

Aebi, H. (1984) Catalase in vitro. *Methods in*

Enzymology, **105**, 121-126.

Ahmad, P., Abdel Latef, A.A., Abd_Allah, E.F., Hashem, A., Sarwat, M., Anjum, N.A., 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 in Plant Science,* **7**, 513.

Andresen, E., Kupper, H. (2013) Cadmium toxicity in plants. In: "*Cadmium: from Toxicity to Essentiality, Metal Ions in Life Sciences"*, Sigel, A., Sigel, H., Sigel, R.K.O. (Ed.), pp. 395-413. Springer Science + Business Media, Dordrecht, Germany.

Asgher, M., Iqbal, M., Khan, R., Anjum, N.A., Khan, N.A. (2015) Minimising toxicity of cadmium in plants—Role of plant growth regulators. *Protoplasma*, **252**(2), 399-413.

Ashraf, M., Foolad, M.R. (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany,* **59**, 206-216.

Beauchamp, C., Fridovich, I. (1971) Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry,* **44**, 276-287.

Bradford, M.M. (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.

Chen, W., Dong, Y., Hu, G., Bai, X. (2018) Effects of exogenous nitric oxide on cadmium toxicity and antioxidative system in perennial ryegrass. *Journal of Soil Science and Plant Nutrition,* **18**(1), 129-143.

De Freitas, P.A.F., De Carvalho, H.H., Costa, J.H., De Souza Miranda, R., Da Cruz Saraiva, K.D., De Oliveira, F.D.B., Coelho, D.G., Prisco, J.T., Gomez Filho, E. (2019) Salt acclimation in sorghum plants by exogenous proline: physiological and biochemical changes and regulation of proline metabolism. *Plant Cell Reports,* **38**(3), 403-416.

De Maria, S., Puschenreiter, M., Rivelli, A.R. (2013) Cadmium accumulation and physiological response of sunflower plants to Cd during the vegetative growing cycle. *Plant, Soil and Environment,* **59**, 254-261.

Gaballah, M.S., Rady, M.M. (2012) Salicylic acid mitigated cadmium toxicity by attenuating the oxidative stress in pea (*Pisum sativum* L.) plants. *International Journal of Biological, Ecological and Environmental Sciences,* **1**(4), 159-165.

Gill, S., Anjum, N., Gill, R., Hasanuzzaman, M., Sharma, P., Tuteja, N. (2013) Mechanism of cadmium toxicity and tolerance in crop plants. In: "*Crop Improvement under Adverse Conditions*", Tuteja, N., Gill, S. (Ed.), pp. 361-385. Springer Science + Business Media, New York, USA.

Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., Fujita, M. (2014) Exogenous proline and glycine betaine mediated up-regulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed Research International,* Article ID 757219, 17 pages.

Heath R.L., Packer, L. (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics,* **125**, 189-198.

Hossain, M.A., Hasanuzzaman, M., Fujita, M. (2010) Up-regulation of antioxidant and glyoxalase systems by exogenous glycine betaine and proline in mung bean confer tolerance to cadmium stress. *Physiology and Molecular Biology of Plants,* **16**(3), 259-272.

Islam, M.M., Hoque, M.A., Okuma, E., Banu, M.N.A., Shimoishi, Y., Nakamura, Y., Murata, Y. (2009) Exogenous proline and glycine betaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *Journal of Plant Physiology,* **166**, 1587-1597.

Ismael, M.A., Elyamine, A.M., Moussa, M.G., Cai, M., Zhao, X., Hu, C. (2019) Cadmium in plants: Uptake, toxicity, and its interactions with selenium fertilizers. *Metallomics,* **11**, 255-277.

Jhanji, S., Setia, R.C., Kaur, N., Kaur, P., Setia, N. (2012) Role of nitric oxide in cadmium-induced stress on growth, photosynthetic components and yield of *Brassica napus* L. *Journal of Environmental Biology,* **33**, 1027-1032.

Mostofa, M.G., Rahman, A., Ansary, M.M.U., Watanabe, A., Fujita, M., Tran, L.S.P. (2015)

Hydrogen sulfide modulates cadmium-induced physiological and biochemical responses to alleviate cadmium toxicity in rice. *Scientific Reports,* **5**, 14078.

Moussa, H.R., El-Gamal, S.M. (2010) Effect of salicylic acid pretreatment on cadmium toxicity in wheat. *Biologia Plantarum,* **54**(2), 315-320.

Nagajyoti, P.C., Lee, K.D., Sreekanth, T.V.M. (2010) Heavy metals, occurrence and toxicity for plants: A review. *Environmental Chemistry Letters,* **8**, 199-216.

Nakano, Y., Asada, K. (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology,* **22**, 867-880.

Perveen, S., Shahbaz, M., Iqbal, M., Akram, M.S., Parveen, A., Ali, H.M.M. (2016) induction of cadmium stress tolerance in *Triticum aestivum* L. by alfalfa leaf extract. *Applied Ecology and Environmental Research,* **14**(5), 121-136.

Rady, M.M., Hemida, K.A. (2015) Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. *Ecotoxicology and Environmental Safety,* **119**, 178-185.

Rady, M.O.A., Semida, W.M., Abd El-Mageed, T.A., Hemida, K.A., Rady, M.M. (2018) Up-regulation of antioxidative defense systems by glycine betaine foliar application in onion plants confer tolerance to salinity stress. *Scientia Horticulturae,* **240**, 614-622.

Rahman, M.F., Ghosal, A., Alam, M.F., Kabir, A.H. (2017) Remediation of cadmium toxicity in field peas (*Pisum sativum* L.) through exogenous silicon. *Ecotoxicology and Environmental Safety,* **135**, 165-172.

Rasheed, R., Ashraf, M.A., Hussain, I., Haider, M.Z., Kanwal, U., Iqbal, M. (2014) Exogenous proline and glycine betaine mitigate cadmium stress in two genetically different spring wheat (*Triticum aestivum* L.) cultivars. *Brazilian Journal of Botany*, **37**(4), 399-406.

Sadeghipour, O. (2018) Enhancing cadmium tolerance in common bean plants by potassium application. *The Philippine Agricultural Scientist,* **101**(2), 167-175.

Shahbaz, A.K., Ramzani, P.M.A., Saeed, R., Turan, V., Iqbal, M., Lewińska, K., Abbas, F., Saqib, M., Tauqeer, H.M., Iqbal, M., Fatima, M., Rahman, M. (2019) Effects of biochar and zeolite soil amendments with foliar proline spray on nickel immobilization, nutritional quality and nickel concentrations in wheat. *Ecotoxicology and Environmental Safety,* **173**, 182-191.

Shahid, M., Dumat, C., Khalid, S., Niazi, N.K., Antunes, P.M.C. (2016) Cadmium bioavailability, uptake, toxicity and detoxification in soil-plant system. In: "*Reviews of environmental contamination and toxicology*". Gunther, F.A., De Voogt, P. (Ed.), pp. 73-137. Springer International Publishing, Switzerland.

Shamsi, I.H., Jilani, G., Guo-Ping, Z., Kang, W. (2008) Cadmium stress tolerance through potassium nutrition in soybean. *Asian Journal of Chemistry,* **20**(2), 1099-1108.

Sun, H., Wang, X., Wang, Y., Wei, Y., Wang, G. (2016) Alleviation of cadmium toxicity in cucumber (*Cucumis sativus*) seedlings by the application of selenium. *Spanish Journal of Agricultural Research,* **14**(4), e1105, 12 pages.

Wani, A.S., Ahmad, A., Hayat, S., Tahir, I. (2019) Epibrassinolide and proline alleviate the photosynthetic and yield inhibition under salt stress by acting on antioxidant system in mustard. *Plant Physiology and Biochemistry,* **135**, 385-394.

Zouari, M., Ben Ahmed, C., Zorrig, W., Elloumi, N., Rabhi, M., Delmail, D., Ben Rouina, B., Labrousse, P., Ben Abdallah, F. (2016) Exogenous proline mediates alleviation of cadmium stress by promoting photosynthetic activity, water status and antioxidative enzymes activities of young date palm (*Phoenix dactylifera* L.). *Ecotoxicology and Environmental Safety,* **128**, 100-108.

Zouari, M., Elloumi, N., Labrousse, P., Ben Rouina, B., Ben Abdallah, F., Ben Ahmed, C. (2018) Olive trees response to lead stress: exogenous proline provided better tolerance than glycine betaine. *South African Journal of Botany,* **118**, 158-165.