ISSN: 2347-4688, Vol. 11, No.(1) 2023, pg. 167-176

Current Agriculture Research Jsournal

www.agriculturejournal.org

Ascorbic Acid and Calcium Silicate Improve Morpho-Physiological Characteristics of Cadmium Stressed Mung Bean Crop

GAGANDEEP KAUR and KAMAL JIT SINGH*

Plant Physiology and Biochemistry Laboratory, Department of Botany, Panjab University, Chandigarh.

Abstract

A rise in heavy metal contamination especially in the rhizosphere affecting the growth and yield of crops is a major concern. We aimed to study the influence of using calcium silicate (CS) and ascorbic acid (AsA) supplements on lowering the impact of cadmium-induced toxicity in mung bean. Both the supplements alone or in combination improved growth characteristics of cadmium (Cd) stressed mung bean plants like root-shoot length and fresh-dry weight. Leaf pigments like chlorophyll and carotenoids were also restored. A significant improvement in the relative leaf water content (RLWC) and low electrolyte leakage (EL) at the membrane was recorded. Results were more promising when combinations of CS and AsA treatments were used against the lower concentration of cadmium. Hence, both CS and AsA interact synergistically to alleviate Cd induced metal toxicity in mung bean plants.

Article History

Received: 26 October 2022

Accepted: 24 February 2023

Keywords

Abiotic Stress;
Antioxidants;
Legumes;
Water Deficit.

Introduction

About 53 elements among the natural components of the earth's crust fall under heavy metals.[1] Although cadmium (Cd) is a non-essential element with no biological significance, it is considered highly toxic being soluble and mobile in a plant-soil system.[2] A longer half-life and non-biodegradability allow Cd to accumulate and persist in the agricultural food chain. Anthropogenic activities are the major cause of Cd contamination.[3] Its chemical similarity with minerals like zinc (Zn), iron (Fe), calcium (Ca), and manganese (Mn), makes its absorption easier at the root system, altering morphological, biochemical, and physiological characteristics of a plant. Among many cascading effects of cadmium, necrosis, chlorosis, and low tolerance index leading to plant death.[4] Cadmium hampers the photosynthetic activity including chloroplast organization, pigments, membrane integrity, stomatal conductance, and water balance.[5] Cadmium is a redox-inactive metal that promotes the formation of ROS through indirect mechanisms inhibiting antioxidative enzymes or stimulating NADPH oxidase, a ROS-producing enzyme.[6] The release of cytotoxic elements like superoxide ($O2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) oxidize membrane lipids

CONTACT Kamal Jit Singh kamal@pu.ac.in Plant Physiology and Biochemistry laboratory, Department of Botany, Panjab University, Chandigarh.

© 2023 The Author(s). Published by Enviro Research Publishers.
This is an Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).

Doi: http://dx.doi.org/10.12944/CARJ.11.1.14

to buildup malondialdehyde (MDA) leading to protein denaturation with altered enzyme functions, osmolyte production, and C/N metabolism.

[7]Ascorbic acid (AsA), a water-soluble organic compound, is an essential antioxidant in a plant system participating in metabolic activities. It is present in all plant tissues, usually more in photosynthetic cells, meristem, and fruits.[8] AsA, a non-enzymatic antioxidant acts as a redox buffer and ROS scavenger under stress conditions. Its application enhances photosynthesis, preserves chlorophyll contents, and maintains the integrity of cell membranes during stress.[10] Calcium has been reported as an essential plant macronutrient for the growth, cell wall stabilization, cell membrane integrity, ion transportation, photosynthesis, water relations, and enzyme activation.[9] Calcium silicate (CS) used in agriculture for liming also regulates physical, chemical, and biological properties of the soil, and has proved beneficial for plant growth by alleviating stress.[11] CS restricts the availability of Cd to a plant hence promoting the growth profile of the paddy crop.[12] Generation of ROS during stress involves an exchange of signal between ROS, Ca^{2+}, and Ca^{2+}- binding proteins, such as calmodulin and silicon.[13] Si, the 2nd most abundant element on the earth is also considered essential for plant growth and development.[14] Silicon improves water relations of the crop with enhanced photosynthetic activity during stress. We hypothesized that AsA and CS would play a key role in crop improvement, therefore, investigated various growth parameters focusing on the underlying mechanisms of Cd stressed plants.

Material and Methods

Seeds of mung bean (*Vigna radiata* (L.) R. Wilczek var. MH-421) were procured from CCS Haryana Agricultural University, Hisar, Haryana. Healthy seeds were surface sterilized with 0.01% mercuric chloride followed by thorough washing with distilled water. Seeds were inoculated with the appropriate strain of *Rhizobium* sp. by soaking overnight in a thick slurry of *Rhizobium* culture mixed with activated charcoal and acacia gum. Trials were carried out in dome-shaped out-houses in the month of March with an average temperature of 30.0 ± 2°C and 34% humidity. Seedlings were raised in perforated polythene-lined earthen pots filled with approximately 5 kg of washed river sand. Calcium silicate (0.6 mM) was added to the soil before the sowing of seeds.[15] After germination, the seedlings were irrigated with distilled water only for the first 14 days, followed by Cd (0.3 and 0.5 mM $CdSO_4.7H_2O$) and Ascorbic acid (0.8 mM) treatments along with the nutrient medium. Trial consisted of 9 sets as Control, Cd(0.3mM), Cd0.3+CS0.6mM, Cd0.3+AsA0.8mM, Cd0.3+CS0.6+AsA0.8mM, Cd(0.5mM), Cd0.5+CS0.6mM, Cd0.5+AsA0.8mM, Cd0.5+CS0.6+AsA0.8mM. Treatments were repeated for fortnightly thrice. Control plants were grown with nutrient medium only. Observations were made at the reproductive stage of the crop, 45 DAS using fresh leaves samples on the same day.

[16,17]Standardized procedures were followed to measure parameters like chlorophyll and carotenoid content. Chlorophyll was extracted with 80% acetone repeatedly to ensure complete extraction and the absorbance was read at 480nm, 663nm, and 645nm against 80% acetone. Observations were made with Thermo-Scientific Evolution-201 UV-Visible Spectrophotometer. Electrolyte leakage (EL) was measured according to Lutts *et al.*[18] Relative leaf water contents (RLWC) were determined according to Chen *et al.*[19] Root-shoot length (cm) and fresh weight (g) were calculated at the sampling stage.

Statistical Analysis

All the values were in triplicate from a single sample and represented as mean ± SE (standard error). Data were statistically analyzed using randomized block design (RBD) one-way ANOVA in SPSS-16 by taking the probability level of 5%. A least significant difference (LSD) post-hoc test was used to compare the multiple comparisons of the mean.

Observations and Results

Chlorophyll Content

Cadmium (0.3 and 0.5mM) treatments reduced leaf chlorophyll content up to 19.08% and 30.84%. The pigment loss was only 5.88% ($Cd_{0.3}$+$CS_{0.6mM}$); 19.08% ($Cd_{0.5}$+$CS_{0.6mM}$), 2.18% ($Cd_{0.5}$+$AsA_{0.8mM}$) in CS and AsA supplemented Cd treatments in comparison to control plants. The percentage value of the contents was more than that of control in combination treatments +7.80% ($Cd_{0.3}$+$AsA_{0.8mM}$); +48.64% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$); and +20.18% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8\ mM}$). Observations revealed the effectiveness of the CS and AsA combination in neutralizing the deleterious effect of Cd (Fig.1).

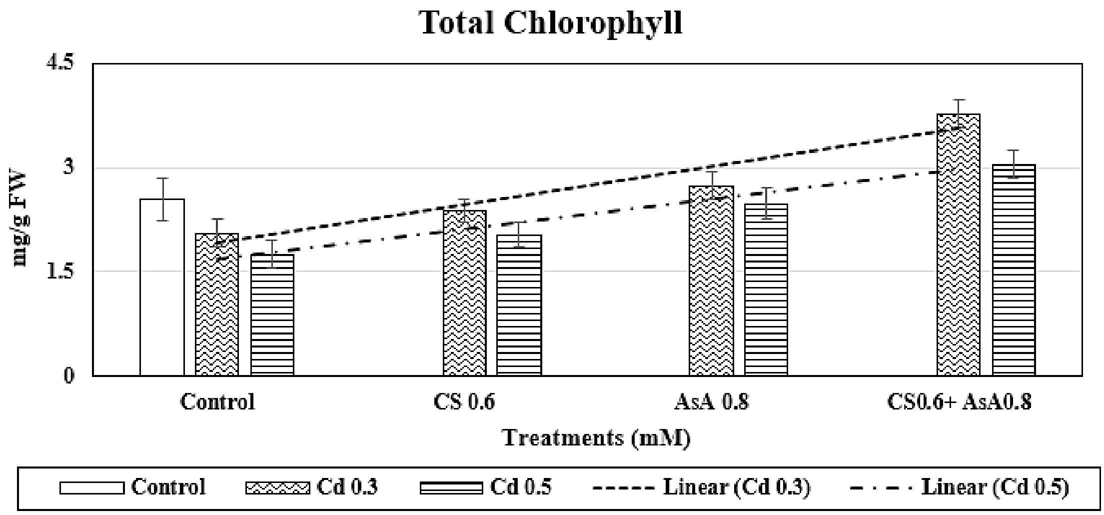

Fig. 1: Effect of Cd alone and in combination with CS and AsA on total chlorophyll in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=0.21).

Carotenoids

The contents of accessory pigment carotenoids were reduced up to 17.72% and 28.09% with Cd (0.3 and 0.5mM) application, respectively. Loss of accessory pigment noticed with CS or AsA supplements was only 7.94% ($Cd_{0.3}$+$CS_{0.6mM}$); 21.57% ($Cd_{0.5}$+$CS_{0.6mM}$), 2.27% ($Cd_{0.5}$+$AsA_{0.8mM}$) in comparison to control. The combination treatments were found to be promotive such as +9.48% ($Cd_{0.3}$+$AsA_{0.8mM}$); +52.66% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$); and 15.96% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8mM}$). Thus, the effectiveness of combination treatments of CS and AsA was more when used with low cadmium (0.3mM) concentrations (Fig. 2).

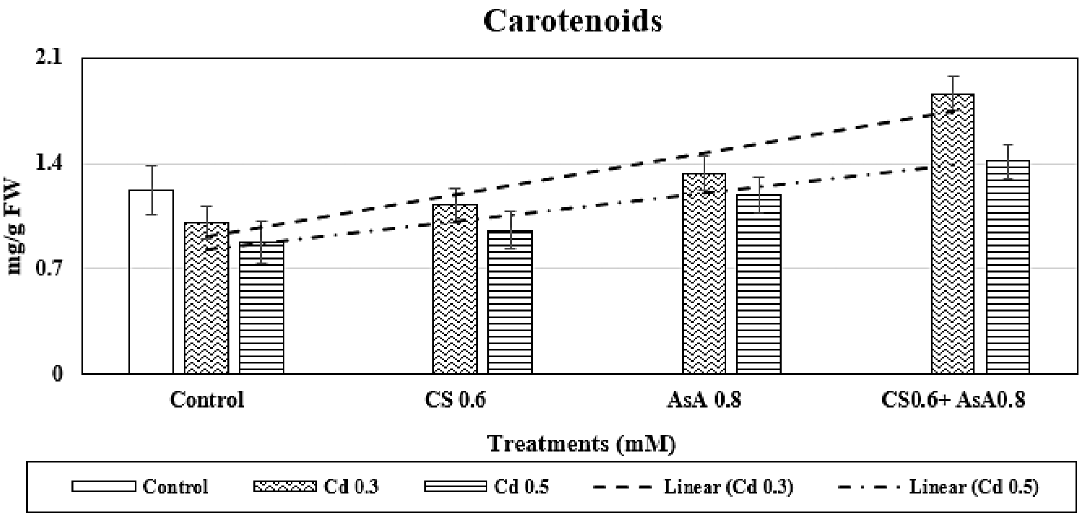

Fig. 2: Effect of Cd alone and in combination with CS and AsA on carotenoids in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=0.12).

Relative Leaf Water Content (RLWC)

RLWC of the leaves dropped with Cd treatments up to 9.31% ($Cd_{0.3\ mM}$) and 11.74% ($Cd_{0.6\ mM}$). Such losses in water content with the application of CS or AsA were only up to 6.07% ($Cd_{0.3}$+$CS_{0.6\ mM}$); 8.09% ($Cd_{0.5}$+$CS_{0.6mM}$); and 4.86% ($Cd_{0.3}$+$AsA_{0.8mM}$);

7.29% ($Cd_{0.5}$+$AsA_{0.8mM}$). The results with combination treatments of CS and AsA with Cd were encouraging, minimizing losses of water content to 1.21% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 4.05% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8mM}$). Thus, RLWC could nearly be restored to control levels using a combination of CS and AsA with low concentrations of Cd (Fig.3).

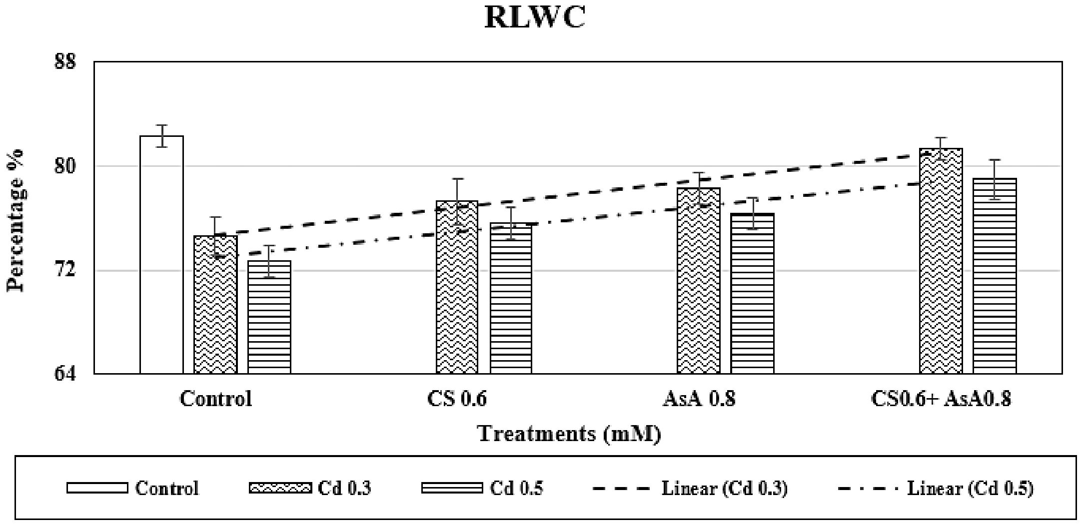

Fig. 3: Effect of Cd alone and in combination with CS and AsA on RLWC in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=1.28).

Electrolyte Leakage

Electrolyte leakage from membranes increased substantially with the application of Cd and was found to be up 19.02% ($Cd_{0.3\,mM}$) and 27.60% ($Cd_{0.5\,mM}$). Both supplement (CS and AsA) applications in cadmium treatment were able to check the rise in EL and leakage it was up 15.80% ($Cd_{0.3}$+$CS_{0.6\,mM}$), 21.27% ($Cd_{0.5}$+$CS_{0.6\,mM}$); and 16.36% ($Cd_{0.3}$+$AsA_{0.8\,mM}$), and 17.57% ($Cd_{0.5}$+$AsA_{0.8mM}$). This EL in leakage combination treatments was minimum and recorded as up 4.35% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 15.18% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8mM}$) to that of control (Fig.4).

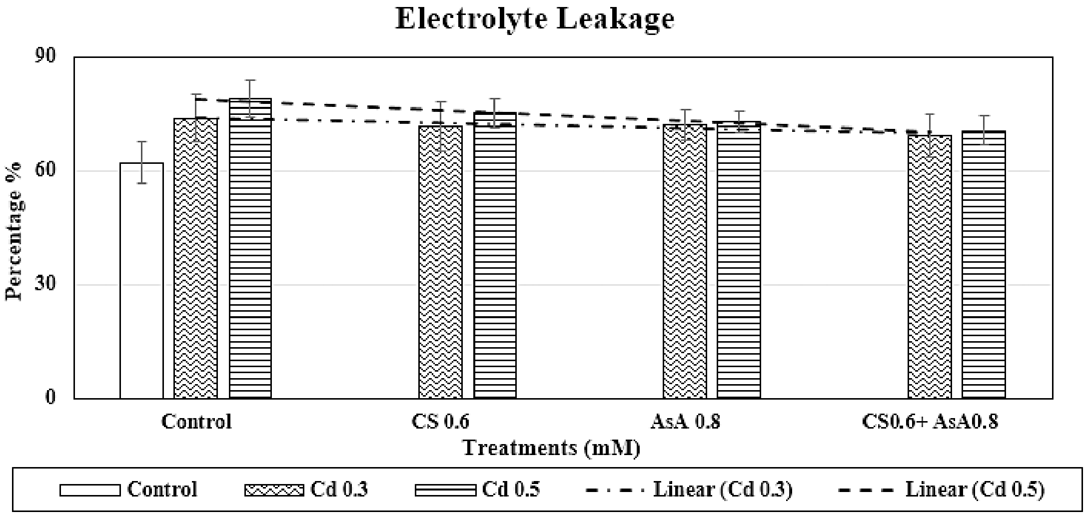

Fig. 4: Effect of Cd alone and in combination with CS and AsA on electrolyte leakage in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=6.19).

Root and Shoot Length

The overall length of mung bean plants was reduced with Cd treatments. It was found that the percentage decline in the length of roots was 38.44% in $Cd_{0.3mM}$ and 49.34% in $Cd_{0.5mM}$ to that of control. The same reduction in root length in CS or AsA supplemented Cd treatments was comparatively lesser, 14.32% ($Cd_{0.3}$+$CS_{0.6mM}$); 32.16% ($Cd_{0.5}$+$CS_{0.6mM}$) and 12.44% ($Cd_{0.3}$+$AsA_{0.8mM}$); 27.75% ($Cd_{0.5}$+$AsA_{0.8mM}$). Their combination treatments with Cd minimized the losses to 5.84% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 19.27% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8\ mM}$) (Fig.5).

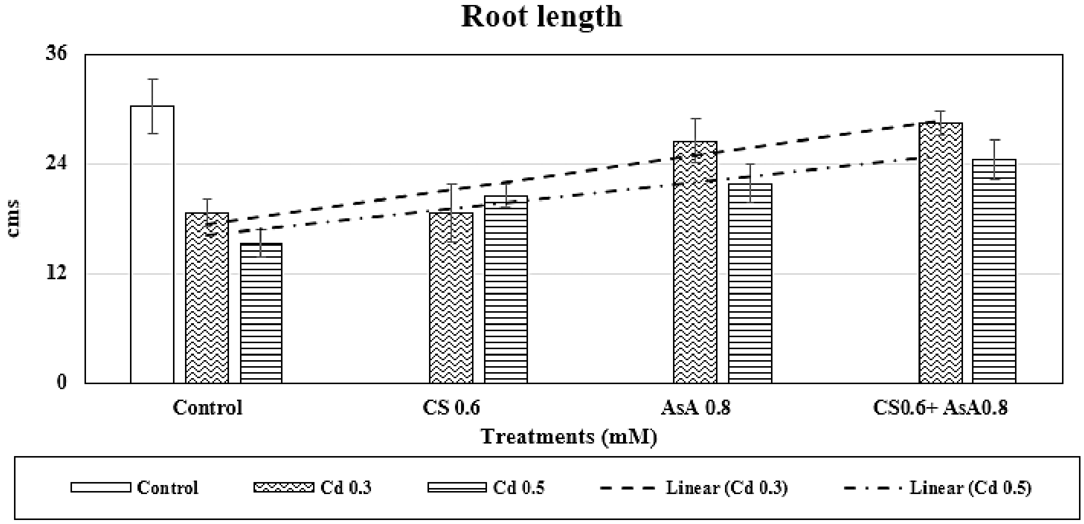

Fig. 5: Effect of Cd alone and in combination with CS and AsA on root length in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=2.38).

The length of shoots also declined up to 40.56% ($Cd_{0.3mM}$) and 45.59% ($Cd_{0.5mM}$) in comparison to the control. The addition of CS or AsA to Cd lowered the drop in height to 36.77% (Cd0.3+CS0.6mM); 39.71% ($Cd_{0.5}$+$CS_{0.6mM}$) and 28.15% ($Cd_{0.3}$+$AsA_{0.8mM}$); 36.97% ($Cd_{0.5}$+$AsA_{0.8mM}$). The combination of CS and AsA to Cd was able to minimize the drop in height to 4.64% in ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 27.96% in ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8\ mM}$) (Fig.6).

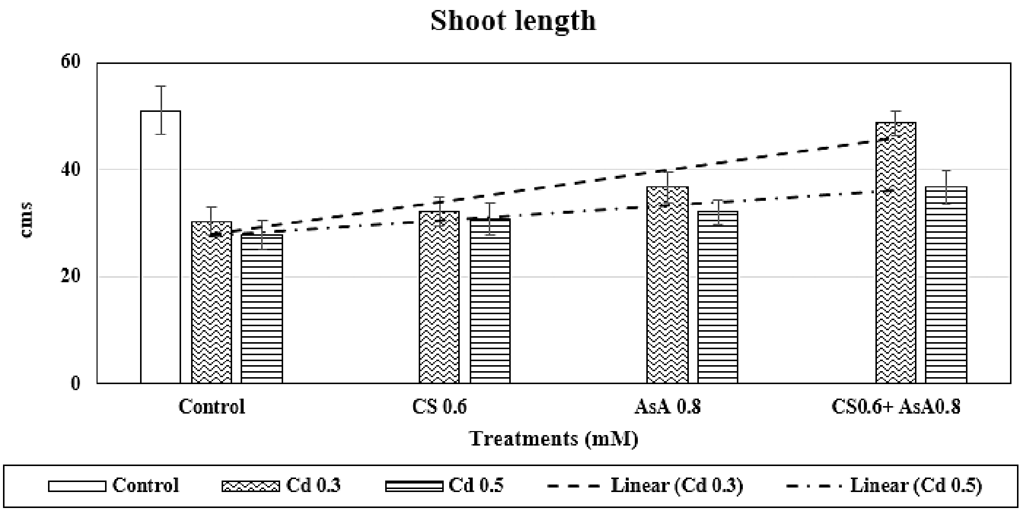

Fig. 6: Effect of Cd alone and in combination with CS and AsA on shoot length in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=2.49).

Fresh and Dry Weight of Plants

In Cd alone treatments (0.3 and 0.5 mM), the fresh weight of the plants declined up to 54.57% and 59.86% respectively. It was comparatively lesser in CS or AsA supplemented Cd treatments 31.13% ($Cd_{0.3}$+$CS_{0.6mM}$); 53.48% ($Cd_{0.5}$+$CS_{0.6mM}$) and 20.23% ($Cd_{0.3}$+$AsA_{0.8mM}$); 51.28% ($Cd_{0.5}$+$AsA_{0.8mM}$). Combination treatments of CS and AsA with Cd checked the fresh weight losses to 13.87% in ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 35.51% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8mM}$) to that of control (Fig. 7).

The dry weight of plants was reduced by up to 16.06% and 19.36% in Cd treatments (0.3 and 0.5 mM) in comparison to the control. CS and AsA supplemented Cd treatments had lesser dry weight loss, 13.27% ($Cd_{0.3}$+$CS_{0.6mM}$); 15.18% ($Cd_{0.5}$+$CS_{0.6mM}$) and 11.77% ($Cd_{0.3}$+$AsA_{0.8mM}$); 17.69% ($Cd_{0.5}$+$AsA_{0.8mM}$). CS and AsA combination treatment with Cd was effective in minimizing dry weight loss to 4.56% ($Cd_{0.3}$+$CS_{0.6}$+$AsA_{0.8mM}$) and 11.69% ($Cd_{0.5}$+$CS_{0.6}$+$AsA_{0.8mM}$) to that of control (Fig. 8).

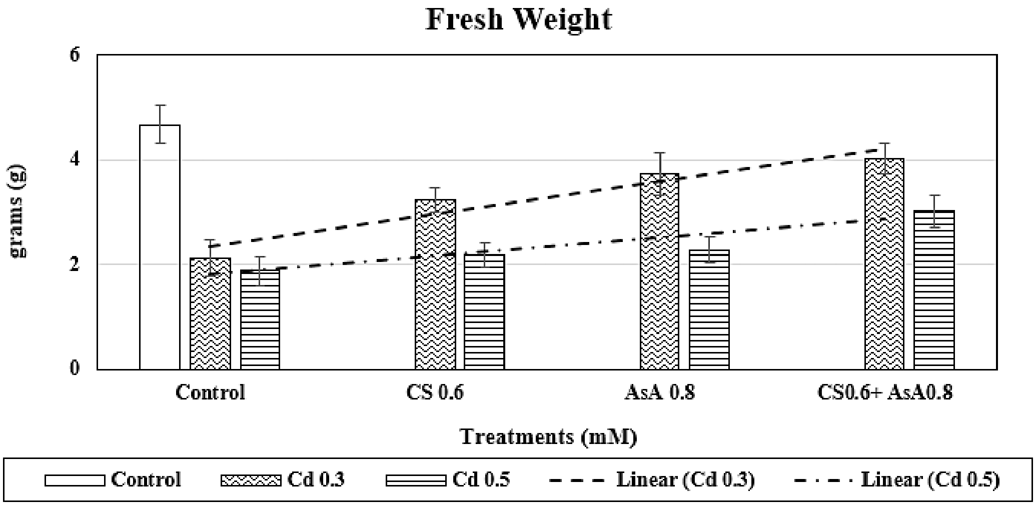

Fig. 7: Effect of Cd alone and in combination with CS and AsA on fresh weight in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=0.47).

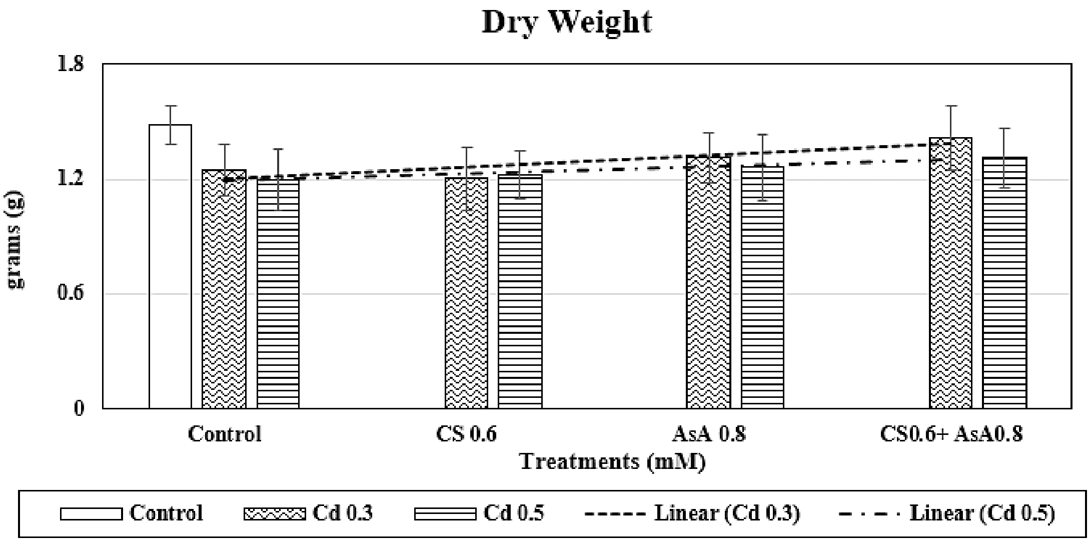

Fig. 8: Effect of Cd alone and in combination with CS and AsA on dry weight in mungbean plants. Each value represents the mean ± SE of three replicates. ($LSD_{0.05}$=0.10).

Discussion

Heavy metal exposure hampers the growth and productivity of a plant. The present investigation indicated that reduced leaf pigments like chlorophyll and carotenoids in Cd-stressed plants could be restored using supplements like CS and/or AsA.[20,21] An improved content of chlorophyll and carotenoids have been linked to the lowering of Cd-induced toxicity with supplements like CS and AsA.[22,23] Different findings correlated CS elevating pigment levels and[24] AsA enhancing chlorophyll and membrane stability index during stress.[25] As a redox buffer, AsA neutralizes the superoxide radicals and other singlet oxygen species, thus, preventing chlorophyll degradation and increasing its content.[26] The decline in photosynthetic pigments like chlorophyll and carotenoids is accompanied by enhanced leakage of ions in Cd-contaminated chickpea genotypes. As noticed in our present study, enhanced electrolyte leakage due to the action of heavy metal could be suppressed largely by using a combination treatment of CS and AsA in Cd-stressed mung bean.[27] Production and storage of ROS in heavy metal stress destroys membrane lipids distorting the structure of lipid-protein membrane which increase membrane permeability.[28] AsA help in combating stress by decreasing electrolyte leakage and maintaining membrane integrity. Negatively impacted RLWC recovered using CS and AsA supplements in Cd treatments.[29] Long term exposure to Cd causes water imbalance leading to decreased RLWC and transpiration.[30] Cd reduces turgor pressure, relative water content, and water potential of plant cells.[31] Excess Cd levels have been reported to alter the osmotic balance and water content. As reported by Kaya *et al.,*[32] RLWC and chlorophyll levels lowered with Cd toxicity were accompanied by enhanced electrolyte leakage in Capsicum annuum.[33] Introduction of CS enhanced water content owing to deposition of silica in the cell wall that reduces transpiration rate to mitigate plant stress.[34] AsA have also been reported to improve water content in various plant species.

[35]Higher Cd levels lead to a drop in fresh-dry weight, root-shoot length, and total pod-seeds in different legumes. The impact of heavy metal stress on the growth profile of mung bean plants like root-shoot length, and fresh and dry weight in the present study was based upon the strength of Cd treatment.[36] Lesser growth and yield of Cd stressed plants was related to the suppression of growth rate of cells because of irreversible inhibition of proton pump responsible for the process.[37] A direct interference of Cd with some hydrolytic enzymes was also suggested to play a pivotal role in restricting food supply to root and shoot. Further, both supplements used in combination proved useful in alleviating metal toxicity.[38] Addition of CS improved root-shoot length, biomass, and related yield attributes in Cd-stressed *Vigna radiata*.[39] Silicates (Si) help in cell enlargement by enhancing the tissue extensibility.[40] AsA is also a co-factor for growth hormones like auxin which is essential for cell expansion and hydroxyproline-rich glycoproteins in cell division. Hence, it can be said that the interaction of CS and AsA with heavy metal Cd improved the morpho-physiological characteristics of mung bean by enhancing RLWC, restoring photosynthetic pigments, and suppressing electrolyte leakage to maintain the integrity of membranes.

Conclusion

CS and AsA improve morpho-physiological characteristics by restoring the content of chlorophyll and carotenoids, RLWC, and suppressing EL to mitigate Cd-induced heavy metal toxicity in mung bean.

Acknowledgment

The financial support of the University Grants Commission, New Delhi in conducting present investigations is gratefully acknowledged.

Funding

University Grants Commission, New Delhi.

Conflict of Interest

There is no conflict of interest.

References

1. Qin S.Y., Liu H.E., Nie Z.J., Rengel Z., Gao W., Li C., Zhao P. Toxicity of cadmium and its competition with mineral nutrients for uptake by plants: A review. *Pedosphere,* 2020; 30(2): 168-180.
2. Jaishankar M., Tseten T., Anbalagan N., Mathew B.B., Beeregowda K.N. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.*, 2014; 7(2): 60-72.
3. Rizwan M., Ali S., Rehman M.Z., Rinklebe J., Tsang D.C., Bashir A., Maqbool A., Tack F.M., Ok Y.S. Cadmium phytoremediation potential of *Brassica crop* species: a review. *Sci. Total Environ.,* 2018; 631: 1175-1191.
4. Stoyanova Z., Doncheva S. The effect of zinc supply and succinate treatment on plant growth and mineral uptake in pea plant. *Braz. J. Plant Physiol.*, 2002;14:111-116.
5. Tian S., Xie R., Wang H., Hu Y., Ge J., Liao X., Gao X., Brown P., Lin X., Lu L. Calcium deficiency triggers phloem remobilization of cadmium in a hyperaccumulating species. *Plant Physiol.,* 2016; 172(4): 2300-2313.
6. Kapur D., Singh K.J. Zinc alleviates cadmium induced heavy metal stress by stimulating antioxidative defense in soybean [*Glycine max* (L.) Merr.] crop. *J. Appl. Nat. Sci.,* 2019; 11(2): 338- 345.
7. Paciolla C., Fortunato S., Dipierro N., Paradiso A., Leonardis S.D., Mastropasqua L., Pinto M.C.D. Vitamin C in Plants: From Functions to Biofortification. *Antioxidants,* 2019; 8: 519.
8. Smirnoff N. Ascorbic acid: metabolism and functions of a multi-facetted molecule. *Curr. Opin. Plant Biol.*, 2000; 3: 229-235.
9. Soundharya N., Srinivasan S., Sivakumar T., Kamalkumaran P.R. Effect of foliar application of nutrients and silicon on yield and quality traits of tomato (*Lycopersicon esculentum* L.). *Int. J. Pure Appl. Biosci.,* 2019; 7(2): 526-531.
10. Marschner H. Mineral nutrition of higher plants. 2012;3rd ed. Academic Press, London.
11. Zhao X.L., Masaihiko S. Amelioration of cadmium polluted paddy soils by porous hydrated calcium silicate. *Water Air Soil Pollut.,* 2007; 183: 309-315.
12. MittlerR., Vanderauwera S., Gollery M., Van Breusegem F. Reactive oxygen gene network of plants. 2019; 8: 490-498.
13. Epstein E. The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci. U.S.A.*, 1994; 91: 11-17.
14. Rizwan M., Ali S., Ibrahim M., Farid M., Adrees M., Bharwana S.A., Rehman M.Z., Qayyum M.F., Abbas F. Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. *Environ. Sci. Pollut. Res.,* 2015; 22: 15416-15431.
15. Minchin F.R., Pate J.S. Effects of water, aeration and salt regime on nitrogen fixation in a nodulated legume: definition of an optimum root environment. *J. Exp. Bot.,* 1975; 26: 60-80.
16. Arnon D.I. Copper enzyme in isolated chloroplast: polyphenol oxidase in *Beta vulgaris. Plant Physiol.,* 1949; 24: 1-15.
17. Kirk J.T.O., Allen R.L. Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. *Cell Biochem. Biophys.*, 1965; 27: 523-530.
18. Lutts S., Kinet J.M., Bouharmont J. NaCl-induced senescence in leaves of rice (Oryza sativus L.) cultivars differing in salinity resistance. *Ann. Bot.*, 1996; 78: 389-398.
19. Chen J., Shiyab S., Han F.X., Monts D.J., Waggoner A.W., Su Z.Y. Bioaccumulation and physiological effects of mercury in *Pteris vittata* and *Nephrolepis exaltata. Ecotoxicology,* 2009; 18: 110-121.
20. Fernandes P.B., Bitencourt L.P., Theodoro G.F., Curcio U.A., Theodoro W.A., de Arruda C.O.C.B. Influence of calcium silicate on soil fertility and corn morphology. *J. Agric. Stud.,* 2020; 8(1): 51-63.
21. Kumar U., Kaviraj M., Rout S., Chakraborty K., Swain P., Nayak P.K., Nayak A.K. Combined application of ascorbic acid and endophytic N-fixing *Azotobacter chroococcum* Avi2 modulates photosynthetic

efficacy, antioxidants and growth-promotion in rice under moisture deficit stress. Microbio. Res., 2021; 250: 126808.

22. Al-Mayahi A.M.W. Effect of silicon (Si) application on *Phoenix dactylifera* L. growth under drought stress induced by polyethylene glycol (PEG) *in vitro. Am. J. Plant Sci.*, 2016; 7:1711-1728.
23. Wasti S., Manaa A., Mimouni H., Nsairi A., Ibtissem M., Gharbi E., Gautier H., Ahmed H.B. Exogenous application of calcium silicate improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars. *J. Plant Nutr.,* 2017; 40(5): 673-684.
24. Alves R.C., Oliveira K.R., Lucio J.C.B., Silva J.D.S., Carrega W.C., Queiroz S.F., Gratao P.L. Exogenous foliar ascorbic acid applications enhance salt-stress tolerance in peanut plants throughout an increase in the activity of major antioxidant enzymes. *S. Afr. J. Bot.,* 2022; 150: 759-767.
25. Noctor G., Foyer C.H. Ascorbate and glutathione: keeping active oxygen under control. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* Versailles, 1998; 49(1): 249-279.
26. Wani A.S., Tahir I. Screening of different chickpea varieties for their sensitiveness and tolerance to cadmium and/or salt stress. *J. Adv. Plant Sci.,* 2019; 2: 104.
27. Anjum N.A., Ahmad I., Mahmood I., Pacheco M., Duarte A.C., Pereira E., Umar S., Ahmad A., Khan N.A., Iqbal M., Prasad M.N.V. Modulation of glutathione and its related enzymes in plants' responses to toxic metals and metalloids- A review. *Environ. Exp. Bot.*, 2012; 75: 307-324.
28. Elkelish A., Qari S.H., Mazrou Y.S.A., Abdelaal K.A.A., Hafez Y.M., Abu-Elsaoud A.M., Batiha G.E., El-Esawi M.A., El-Nahhas N. Exogenous ascorbic acid induced chilling tolerance in tomato plants through modulating metabolism, osmolytes, antioxidants, and transcriptional regulation of catalase and heat shock proteins. *Plants,* 2020; 9:431.
29. Barceló J., Poschenrieder C. Plant water relations as affected by heavy metal stress: A review. *J. Plant Nutr.,* 1990; 13: 1-37.
30. Barceló J., Poschenrieder C., Andreu I., Gunsé B. Cadmium-induced decrease of water stress resistance in bush bean plants (Phaseolus vulgaris L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. *J. Plant Physiol.,* 1986; 125: 17-25.
31. El-Esawi M.A., Elkelish A., Soliman M., Elansary H.O., Zaid A., Wani S.H. Serratia marcescens BM1 enhances cadmium stress tolerance and phytoremediation potential of soybean through modulation of osmolytes, leaf gas exchange, antioxidant machinery, and stress-responsive genes expression. *Antioxidants*, 2020; 9:43.
32. Kaya C., Akram N.A., Ashraf M., Alyemeni M.N., Ahmad P. Exogenously supplied silicon (Si) improves cadmium tolerance in pepper (*Capsicum annuum* L.) by upregulating the synthesis of nitric oxide and hydrogen sulfide. *J. Biotech.*, 2020; 316: 35-45.
33. Marques D.J., Ferreira M.M., Lobato A.K.S., de Freitas W.A., Carvalho J.A., Ferreira E.D., Broetto F. Potential of calcium silicate to mitigate water deficiency in maize. *Bragantia*, 2016; 75(3):275-285.
34. Zhang K., Wang G., Bao M., Wang L., Xie X. Exogenous application of ascorbic acid mitigates cadmium toxicity and uptake in maize (*Zea mays* L.). *Environ. Sci. Pollut. Res.,* 2019; 26: 19261-19271.
35. Ghani A. Effect of cadmium toxicity on the growth and yield components of mungbean [*Vigna radiata* (L.) Wilczek]. *World Appl. Sci.* J., 8 (Special Issue of Biotechnology and Genetic Engineering): 2010; 26-29.
36. Aidid S.B., Okamoto H. Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc. *Biometals*, 1993; 6: 245-249.
37. Mondal N.K., Das C., Roy S., Datta J.K., Banerjee A. Effect of varying cadmium stress on chickpea (Cicer arietinum L.) seedlings: an ultrastructural study. *Ann. Environm. Sci.,* 2013; 7: 59-70.
38. Ahmed N., Ahsen S., Ali M.A., Hussain M.B., Hussain S.B., Rasheed M.K., Butt B., Irshad I., Danish S. Rhizobacteria and silicon synergy modulates the growth, nutrition and yield of mungbean under saline soil. *Pak. J.*

Bot., 2020; 52(1): 9-15.

39. Ouzounidou G., Giannakoula A., Ilias I., Zamanidis P. Alleviation of drought and salinity stresses on growth, physiology, biochemistry and quality of two *Cucumis sativus* L. cultivars by Si application. *Braz. J. Biol*., 2016; 39(2): 531-539.

40. Carpita N.C., Gibeaut M.D. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.,* 1993; 3: 1-30.