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TREHALOSE AND GLUTATHIONE ROLE IN REDUCING CADMIUM TOXICITY IN MUNG BEAN (*VIGNA RADIATA* L.)

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SUMMARY

The experiment on mung bean (*Vigna radiata*) in 2023 commenced at the laboratory of the University of Kerbala, Kerbala, Iraq. It aimed to determine the role of trehalose and glutathione in reducing cadmium (Cd) toxicity. The mung bean cuttings served as an experimental material to evaluate the best method of applying trehalose and glutathione in reducing Cd toxicity. Root response, antioxidant defense, and peroxidation were the indicators used. With pre- and post-treatment of trehalose and glutathione and in combination with Cd, significant differences occurred for these treatments. By treating with Cd, trehalose and glutathione were able to remove Cd toxicity and showed an increase in rooting response. By using trehalose and glutathione together in mung bean, and using Cd during the first 24 hours, their effect was inhibitory. However, when treated together with the toxic Cd, the trehalose and glutathione removed the Cd toxicity in mung bean, improved the rooting response, and raised the levels of GSH, ASA, and CAT, and SOD activity. In addition, they decreased the level of MDA and the lipoxygenase activity compared with the control treatment. The study concluded that the combination of trehalose and glutathione showed a promising protection for mung bean plants from Cd toxicity.

Keywords: Mung bean (*Vigna radiata* L.), rooting response, antioxidant, glutathione, trehalose, cadmium toxicity.

Key findings: The combined effects of trehalose and glutathione before and after Cd application during the first 24 hours resulted in inhibitory action in mung bean (*Vigna radiata* L.). However, when applied in combination with the toxic Cd, the Cd toxicity vanished, with the rooting system enhanced along with the levels of GSH, ASA, CAT, and SOD.

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INTRODUCTION

In general, heavy metals are abiotic stresses that include highly toxic elements, with cadmium (Cd) considered as one of the most toxic heavy metals for living organisms having classified in the first group of carcinogen agents (Hussain *et al.*, 2021). Despite its easy uptake by crop plants, Cd has no nutritional value. In soil, Cd can either be in free or complex form of inorganic and organic molecules. In the cultivated soils, cadmium showed a negative impact on plant growth and reproduction, as well as on physiological and biochemical processes transpiring in crop plants (Haider *et al.*, 2021).

In comparison with other heavy metal ions, the Cd toxicity proved 2–20 times higher, and its highest concentration can stunt plant growth, cause root discoloration, and ultimately root death. Cadmium toxicity also leads to oxidative stress by generating ROS free radicals (Hasanuzzaman *et al.*, 2019). These radicals interact with fats and cause oxidation, resulting in damage to biological membranes and proteins. These processes result in enzyme inhibition, DNA, and RNA damage in plant cells after exposure to Cd, negatively affecting the proliferation and development of these cells (Žabka *et al.*, 2021). Plants develop different mechanisms to avoid Cd stress (El-Rasafi *et al.*, 2022).

In some crop plants, the disaccharide trehalose sugar works as a stress protectant and play a considerable role in Cd stress tolerance as an osmoprotectant. It also acts as a modulator of genes involved in detoxification and stress response. The trehalose also reduces Cd toxicity and its negative impact in rice and wheat, and minimize Cd accumulation in crop plants (Alzuaibr, 2023).

Glutathione is one of the enzymatic antioxidants composed of a sulfur compound with low molecular weight. It contributes to vital processes, such as participating in the enzymatic reaction that helps reduce the ROS accumulation and maintain the state of balance between the oxidation and reduction reactions (Jung *et al.*, 2019). In addition, GSH participates in the photochelatin synthesis that forms complexes with heavy metals in the

cytoplasm and then transferred to the vacuole to reduce its toxicity to plants.

Glutathione also participates in signal transmission in plant cells by controlling gene expression in various abiotic stress conditions, such as detoxification of heavy metals, where glutathione is crucial in reducing oxidative stress caused by Cd toxicity (El-Kafafi *et al.*, 2017). Therefore, mung bean (*Vigna radiata* L.) cuttings were selected samples to evaluate the best promising protocol on reducing the toxic effect of Cd in the cultivated fields using the combination of trehalose and glutathione.

MATERIALS AND METHODS

Genetic material and procedure

The presented experiment proceeded in 2023 at the laboratory of the University of Kerbala, Kerbala, Iraq. It aimed to determine the role of trehalose and glutathione in reducing cadmium (Cd) toxicity in mung bean (*V. radiata* L.).

Seed sterilization

Mung bean seeds sterilization followed the method as described by Coumans *et al.* (2009) in the succeeding steps: 1) Seeds soaked in sterile distilled water for 5 min, then another soaking for 5 min in a sterilization solution (4% bleach, 10% absolute ethanol in sterile distilled water, 2) The sterile mung bean seeds rinsed eight times in sterile distilled water, and 3) The sterile seeds allowed to germinate on YMA (yeast mannitol agar plates) at 25 °C for 2–3 days in the dark.

Seeds planting

The germinated non-contaminated mung bean seeds were choice samples planted uniformly in parallel rows in sawdust-filled perforated plastic tubs under sterile conditions. Then, larger non-perforated plastic tubs contained the tubs with mung bean seeds for incubation under standard conditions of light, temperature, and humidity in the growth chamber. Adding water continued for 10 days as needed. The selected seedlings will serve in

preparation of the cuttings and carry out the experiment. Preparing the cuttings was according to the method described by Thomas and Archana (2023), which contains a terminal bud and a pair of fully expanded leaves with epicotyl and hypocotyl, 3 cm long, after removing the root system.

The mung bean cuttings, contained in three vials (4 cuttings per vial), received solutions of Cd, trehalose, and glutathione, with the hypocotyls immersed in a solution of 15 ml of the previously mentioned solutions for 24 hours. Then, the transfer of cuttings to the rooting medium followed (vials containing 15 ml of boric acid at a concentration of 5 µg/ml for five days). Afterward, calculating the average number of exposed roots ensued. The leaf content from the mung bean cuttings incurred estimations for MDA, ASA, and GSH, as well as the activity of the lipoxygenase enzyme SOD and CAT following described methods below:

The SOD enzyme activity determined according to Beauchamp and Fridovich (1971);

The CAT enzyme activity determined according to Chandlee and Scandalios (1984);

The lipoxygenase enzyme activity determined according to a method described by Wang *et al.* (2008); and

The MDA content estimated by the method of Jana and Choudhuri (1982).

RESULTS AND DISCUSSION

The presented study investigated the role of trehalose sugar and GSH antioxidants in enhancing mung bean (*V. radiata* L.) plants' tolerance to Cd toxicity using the nutrient solution technique. Adopting Hawkland's solution at half strength, feed the mung bean seedlings for the rooting response of the cuttings, and enzymatic and non-enzymatic antioxidants. In mung bean cuttings, reducing the number of developed roots (50%) determined the concentration of Cd, in addition to the appearance of morphological symptoms associated with toxicity.

Determination of Cd toxicity

The effect of different concentrations of Cd in the form of CdCl₄ on the rooting response of mung bean cuttings appears in Table 1. Mung bean cuttings grew in the light for 10 days, until the cuttings of the control sample (treated with distilled water only) reached 8.7 roots cutting⁻¹. The cuttings' treatment with different concentrations of Cd (25–300 µg mL) underwent detecting in the treated roots. Results revealed 8.3, 7.4, 6.3, 5.5, 4.2, and 1.8 roots per cutting, while the concentrations at 400 and 500 showed a lethal effect (the rooting response was zero). The lowest concentration (200 µg ml) was considerably a toxic concentration (reducing the growth rate for the number of roots per cutting reached ≈ 50% and becoming selected for use in subsequent experiments). The mung bean cuttings with low concentration were inhibiting, with the number of roots reduced from a concentration of 200 (Table 1), whereas the number of roots declined to less than half (4.2), with a decrease of 51.72% compared with the control treatment. These results agreed with past findings, which indicated a decrease in the rooting response of mung bean cuttings by exposing to Cd toxicity (Ali-Hussein, 2018).

Determination of trehalose concentration

The effect of trehalose with different concentrations on the rooting response of mung bean cuttings reached estimation (Table 2). After 24 hours, results indicated the control sample with 8.5 roots cutting⁻¹. The calculation of cuttings treated with different concentrations of trehalose and trehalose response in rooting of the cutting progressed, starting from low to high concentration (from 8.8 to 14.8 roots cutting⁻¹). The trehalose improved the mung bean plant's ability to absorb essential nutrients better, such as zinc and iron, and reduced the absorption of cadmium. Generally, when the plant receives sufficient amounts of essential nutrients, it becomes less willing to absorb harmful

Table 1. Effect of cadmium on the rooting response of mung bean cuttings.

Cd conc. ($\mu\text{mol L}^{-1}$)	Root number (roots cutting ⁻¹)
d.w (0.0)	8.7
25	8.3
50	7.4
100	6.3
150	5.5
200	4.2
300	1.8
400	0
500	0
LSD _{0.05}	0.43

Table 2. Effect of trehalose sugar on rooting response of mung bean cuttings.

Trehalose conc. ($\mu\text{mol L}^{-1}$)	Root number (roots cutting ⁻¹)
d.w (0.0)	8.5
1	8.8
2	8.4
5	9.1
10	11
25	12.5
50	14.8
LSD _{0.05}	2.97

Table 3. Effect of GSH on rooting response of mung bean cuttings.

GSH conc. ($\mu\text{mol L}^{-1}$)	Root number (roots cutting ⁻¹)
d.w (0.0)	8.5
10 ⁻⁹	11.3
10 ⁻⁷	15.7
10 ⁻⁵	18.9
10 ⁻³	17.0
LSD _{0.05}	1.98

elements, such as cadmium. Therefore, optimal concentrations of trehalose can improve the plant growth and development and reduce its response to toxic elements, particularly cadmium (Rehman *et al.*, 2022).

Determine of glutathione concentration

The effect of different glutathione (GSH) concentrations on the rooting response of mung bean cuttings emerged (Table 3). Outcomes showed that the mung bean cuttings with the control treatment providing 8.5 roots cutting⁻¹. However, the highest concentration of GSH (10⁻⁵) revealed 18.9 roots cutting⁻¹ and considerably a significant increase in rooting number, estimated at 122.35%. Therefore, the

study considered that the optimal GSH concentration could remove the Cd toxicity in subsequent experiment. An exceeding glutathione concentration (10⁻⁵) may correlate to several factors, and when using in crop plants, the glutathione level must maintain a balance to achieve optimal benefits. The possible reasons for this superiority may refer to an increase in the defensive ability of plants, connected to an escalation in the production of glutathione in crop plants. It further strengthens its defense system against the harmful effects of cadmium. The other reason may be a positive role of glutathione in stimulating antioxidants that inhibit cadmium toxicity by improving its removal and disposal from plant tissues (Ali-Hussein, 2018).

Table 4. Effect of trehalose sugar in removing cadmium toxicity.

Treatments for 24 h	Sub-treatment (5 µg/ml days)	Root number (roots cutting ⁻¹)
d.w (0.0)	6	9.2
toxic Cd	6	5.5
Optimum trehalose	6	15.7
Trehalose 24 h → Toxic Cd 24 h	5	9.4
Toxic Cd 24 h → Trehalose 24 h	5	8.5
Toxic cd + Trehalose	6	17.11
LSD _{0.05}	4.09	

Table 5. Effect of GSH in removing cadmium toxicity.

Treatments for 24 h	Sub-treatment (5 µg/ml days)	Root number (roots cutting ⁻¹)
d.w (0.0)	6	8.8
d.w (0.0)	6	4.7
Toxic Cd	6	17.3
Optimum GSH	5	10.7
Trehalose 24 h → Toxic Cd 24 h	5	9.8
Toxic Cd 24 h → Trehalose 24 h	6	16.0
LSD _{0.05}	3.76	

Trehalose and Cd interaction

The effects of the mutual reaction of Cd and trehalose when preparing Cd before, after, and in combination with trehalose resulted (Table 4). The control treatment revealed 9.2 roots cutting⁻¹ in mung bean. However, treating Cd and trehalose separately during the first 24 hours also had a stimulating effect (15.7 roots cutting⁻¹) for the trehalose sugar and an inhibitor (5.5 roots cutting⁻¹), in relation with Cd with a significant difference compared with the control treatment. The Cd processed before trehalose during the second 24 hours and after the Cd, effects were nonsignificant in both cases regarding the processing time. Meanwhile, equipping the mung bean cutting with trehalose and Cd at the same time stimulated the rooting response (17.11 roots cutting⁻¹) to almost double compared with the control treatment (9.2 roots cutting⁻¹).

The trehalose sugar notably increased the rooting response, by preparing sugar as pre-, post-, and simultaneous with Cd at the same time (Table 4). It was also evident that sugar remarkably stimulates compared with the control. The Cd has a significant inhibitory influence compared with the control. Sugar does not significantly affect the removal of Cd toxicity when prepared as pre- and post-Cd

during the first and second 24 hours. However, it can remove Cd toxicity while inducing an increase in the rooting response by treating together simultaneously. The estimated increase was at 85.97% compared with the control and was nearly twice as much. Trehalose enhances the stress tolerance in plants by increasing antioxidant effectiveness, balancing cellular redox, and boosting photosynthesis (Sarkar and Sadhukhan, 2022).

GSH and Cd interaction

The effects of the interaction between GSH and Cd, and when treating with glutathione before, after, and in combination with Cd at once in the mung bean cuttings' rooting underwent assessment (Table 5). The cuttings of the control sample revealed 8.8 roots cutting⁻¹. When processing Cd and GSH separately during the first 24 hours, it had an inhibitory effect (4.7 roots cutting⁻¹) in relation to Cd and a stimulating outcome in relation to GSH and glutathione, both of which were significant. The processing of GSH (during the first 24 hours) before Cd (during the second 24 hours) or vice versa was significantly effective in the case of processing after Cd (10.7 roots cutting⁻¹) compared to cuttings prepared with Cd only (4.7 roots cutting⁻¹) and with stimulation after

Cd (9.8 roots cutting⁻¹). However, when processing GSH and Cd together simultaneously during the first 24 hours, it caused the removal of Cd toxicity through the number of roots (16.0 roots cutting⁻¹), which was almost equal to the number of roots in the cuttings processed with GSH alone (17.3 roots cutting⁻¹). It confirms the dominance of GSH over the toxic effect of Cd.

The interaction of glutathione with cadmium in mung bean plants can be a natural defense mechanism against cadmium toxicity. The interaction between glutathione and cadmium occurs in plants that lead to the formation of compounds that are insoluble in water, which reduces the movement of cadmium in tissues and its absorption. Glutathione improves the effectiveness of the plant defense system against cadmium effects and reduces its toxic effect on plants. When exposing plants to cadmium contamination, glutathione can react with it to form stable metal compounds, which reduces the toxic effects of cadmium on plants and enhances cadmium instability, weakening its ability to cause greater damage to crop plants. Thus, the interaction of glutathione with cadmium contributes to lessening the cadmium absorption by plants and improving its defense system efficiency against toxins' effects (Dorion *et al.*, 2021).

GSH, trehalose, and Cd interaction

The mutual effect among GSH, trehalose, and Cd, by preparing GSH and trehalose before, after, and together with Cd at the same time in mung bean cuttings' rooting were indicative (Table 6). The mung bean cutting's control treatment (with distilled water only) revealed 7.7 roots cutting⁻¹. However, the trehalose, GSH, and Cd alone during the first 24 hours had a stimulatory effect on trehalose (13 roots cutting⁻¹) and on GSH (15.6 roots cutting⁻¹). Both were significantly comparable to the control and inhibitor (3.8 roots cutting⁻¹) for Cd. Equipping the trehalose and the GSH during the first 24 hours before the Cd (during the second 24 hours), and after the Cd, indicated their inhibitory effects from the first (12.0 roots cutting⁻¹) and the second case (10 roots cutting⁻¹). However, supplying together with Cd at the same time removed the Cd toxicity, with the response to rooting raised slightly more than the control treatment (16.6 roots cutting⁻¹). Thus, it removed the toxic effects of Cd significantly. Trehalose and GSH individually had a stimulatory effect (13 and 15.6 roots cutting⁻¹, respectively), compared with the control (7.7 roots cutting⁻¹). Furthermore, Cd individually had an inhibitory effect (3.8 roots cutting⁻¹) compared with the control. When treating trehalose and GSH together during the first and second 24 hours only, their effect was inhibitory. However, preparing together and with Cd removed the Cd toxicity, and the rooting response rose to a higher level (16.6 roots cutting⁻¹) than the control treatment (Hassan *et al.*, 2023).

Table 6. Effect of trehalose and glutathione (GSH) combined in removing cadmium toxicity.

Treatments	Sub treatment (5 µg/ml days)	Root number (roots cutting ⁻¹)
d.w (0.0)	6	7.7
Trehalose	6	13
GSH	6	15.6
GSH + Tre	6	17.5
Toxic Cd	5	3.8
Toxic cd 24 h→ GSH+Tre 24 h	5	12.0
GSH+Tre 24 h + Toxic Cd 24 h	5	10
GSH+Tre 24 h + Toxic Cd 24 h	6	16.6
LSD _{0.05}	5.05	

Table 7. Effect of glutathione (GSH) in removing cadmium Cd toxicity in terms of fat oxidation.

Treatments for 24 h	MDA	Lipoxygenase
Control	25.12	0.277
Cd Toxic	88.5	0.411
Tre + GSH + Toxic Cd	31.34	0.201
LSD _{0.05}	5.34	0.012

Table 8. Effect of Tre and Glutathione GSH in removing Cd toxicity in terms of antioxidants in mung bean leaves.

Treatments for 24 h	ASA	GSH	SOD	CAT
Control	6.2	277.3	3.07	14.26
Cd Toxic	10.01	180.5	3.47	16.66
Tre + GSH + Toxic Cd	7.04	379.8	3.25	14.98
LSD _{0.05}	2.98	56.87	0.072	0.31

Trehalose and GSH role in removing Cd toxicity in lipid oxidation

The mung bean cuttings with a toxic concentration of Cd were noticeable (Table 7). The final product of the fat oxidation process rose based on MDA by more than 252.3% than the control treatment. Likewise, the activity of the lipoxygenase enzyme was superior by more than 48.37%. Equipping the cuttings with a synthesis of trehalose and GSH together with the toxic concentration of the Cd managed Cd toxicity. The considerable differences between MDA and Lox disappeared with the control and were almost equal to the effectiveness of Lox. Therefore, the results suggested that trehalose (Trihalomethanes) and glutathione (GSH) could contribute positively to cadmium detoxification in plants via lipid oxidation processes. As an antioxidant, trehalose reacts with harmful molecules, while glutathione is an influential compound in cytolytic processes, protecting cells from harmful effects of heavy metals (Roslim *et al.*, 2015; Wang *et al.*, 2021; Naeem and Mamoon-Ur-Rashid, 2024).

Trehalose and GSH role in Cd detoxification through antioxidants

By taking together the GSH and trehalose to remove the Cd toxicity via enzymatic and non-enzymatic antioxidants appears in Table 8. The

Cd toxicity caused an increase in the concentration of ASA (01.10) and a decrease in the concentration of GSH. By exposing along and simultaneously with Cd toxicity, an increase in ASA content emerged, which were close to the control. Likewise, an increase in GSH concerning the toxic concentration treatments and the control (110.41 and 36.96, respectively) occurred. Similarly, the toxic concentration caused an upsurge in the activity of antioxidant enzymes, i.e., SOD (3.47) and CAT (16.66), compared with the control treatment. The treatment combination caused a decrease in the activity of enzymes versus the toxic concentration and raised it to higher levels than the control treatment.

When preparing the cuttings with the combination of trehalose and GSH at optimal concentrations with the Cd toxic concentration, it prevented the oxidation of fats indicative of MDA. Thus, it maintained the level of 33.1, which was not different from the control. This may refer to preserving the structure and integrity of the cell membrane and scavenging free radicals. It could also be due to an increase in the concentration of enzymatic antioxidants, and exposure to Cd toxicity stimulates the production of free radicals (ROS), and the treatments with trehalose sugar occurs by strengthening the antioxidant system (Wang *et al.*, 2020; Jinhua *et al.*, 2022).

CONCLUSIONS

The Cd toxicity reduced the rooting response of mung bean (*Vigna radiata* L.) cuttings and activated the antioxidant defense mechanisms of SOD, CAT, GSH, and ASA. Treatment with trehalose and GSH improved the rooting response of the cuttings, reduced antioxidant levels to normal, and prevented membrane damage. However, the inability of GSH and Tre-sugar to remove Cd toxicity when prepared as pre- and post-Cd was because Cd inhibits the action of GSH. However, when preparing glutathione at the optimal concentration along with Cd during the first 24 hours, their antagonistic effect on each other will balance the equation and eliminate Cd toxicity.

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