 Egypt. J. Soil Sci. **Vol. 64**, No. 3, pp: 845 - 854 (2024)

Egyptian Journal of Soil Science

http://ejss.journals.ekb.eg/

Glucose modulate Photosynthesis, Antioxidant System and Morpho-physiology in Tomato (*Solanum lycopersicum*) Plants under Cadmium Stress

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CADMIUM (Cd), a heavy metal that naturally exists in soil, is dangerous for plants, animals, and humans to be in contact with. The primary causes of Cd contamination in soil include manure, sewage sludge, aerial deposition, and excessive use of phosphate fertilizers. Cd toxicity significantly reduced the crop productivity and physio-biochemical attributes. Present research aim was to investigate the effect of glucose in *Solanum lycopersicum* under Cd stress. Seeds of *S. lycopersicum* soaked in 50 mM of glucose for 5hrs followed by sown in the maintain pots. Cd stress (0.8 mM) was applied directly through the soil at the stage of 15 days. Results of this study demonstrated that Cd stress reduced the length (shoot and root by 41 and 70%), weight (fresh [32 & 66%] and dry [28 & 29%]), SPAD value, photosynthesis and related characteristics, carbonic anhydrase (CA) and nitrate reductase (NR). Beside this, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content increased in the presence of Cd in *S. lycopersicum* plants. However, pre-treatment of glucose partially or fully counteracted the toxicity triggered by Cd in the aforesaid parameters. Moreover, antioxidant enzymes activity and proline content are further increased in the plants which previously received glucose through seeds. Our study reveals the beneficial effects of glucose for improved growth and will support new avenue on overcoming Cd toxicity and improving agricultural productivity.

Keywords: Shoot and root length, Proline content, Carbonic anhydrase activity, SPAD chlorophyll

1. Introduction

Cadmium (Cd) is a heavy metal (HM) that enters the environment through natural processes as well as increased human activities like mining, industrial processes, disposing of waste containing metals like batteries, sludge disposal, applying excessive amounts of fertilizer, and using products related to Cd (Yasin et al., 2024; Faizan et al., 2024a). There are serious concerns to human health and the food chain from this increased release of Cd into the ecosystem (Wang et al., 2023; Sana et al., 2024). A result of insufficient regulatory actions, plants is experiencing a worrisome increase in Cd bioaccumulation, which puts higher trophic organisms including humans at further risk (Retamal-Salgado et al., 2017). Even at low dosages, Cd has been shown to cause a variety of harmful health effects when consumed through food, such as cancer, heart disease, toxicity to the kidneys and liver, and reproductive system impairment (Abd Elnabi et al., 2023). Therefore, the creation of long-term remediation plans to deal with Cd-contaminated soils is urgently needed (Genchi et al., 2020). Cd and other necessary minerals can be easily absorbed by plants through their roots from the soil (Faizan et al., 2024b. Similar to other heavy metals, Cd damages the morphological, physiological, and biochemical processes of plants as well as their structural composition, which lowers agricultural productivity. Cadmium has a profound effect on the growth and general metabolism of plants due to their great sensitivity to it (Abd Elnabi et al., 2023). Cd is frequently not required for crops since it is not known to have any role in the growth and development of crop plants. Thus, even at low concentrations, Cd inhibits photosynthesis, modifies the ultrastructure of the chloroplast, enhances lipid peroxidation, and heightens the oxidative damage brought on by reactive oxygen species. Executing remediation expertise is typically linked to high budgets and major interruptions to ecologies (Fahad et al., 2020; Yousif et al., 2023).

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Received: 02/04/2024; Accepted: 17/04/2024
DOI: 10.21608/EJSS.2024.281121.1744

Sugars are metabolic substrates that influence a number of activities in plants at different stages of growth. Consequently, altering the metabolism of sugars can have a significant impact on the metabolism of plants. A soluble sugar that is present in all plants is glucose. Glucose is not just a common carbon source but also a signaling molecule that affects several metabolic developments in plants. Glucose controls a broad range of plant functions, from germination to senescence (Siddiqui et al., 2020). Plant organs, environmental factors, circadian rhythms, and developmental stage all have a significant influence on the exact regulation and storage, transport, and utilization of sugars in plant cells. Numerous environmental factors can affect different biochemical processes, often interfering with the balanced partitioning of sugars inside plant cells and their transfer from source to sink. These factors can vary in strength depending on climate change. Glucose promotes cell division, chlorophyll content and photosynthetic rate (Yusuf et al., 2021; Sami and Hayat 2019). Additionally, Glucose increases the activity of antioxidant enzymes and reduces the production of reactive oxygen species (ROS) in plants (Jiang et al. 2012; Huang et al. 2013).

Glucose is a naturally occurring photosynthetic product that is safe for the environment and functions as an osmo-protectant in plants. The scientific world is becoming increasingly interested in its exogenous application to plants in order to boost horticulture crops' growth and output. Consequently, seed soaking was employed as a novel mode of administration. This study aimed to investigate the effect of glucose on *S. lycopersicum* under Cd stress. According to the hypothesis, using glucose may be able to reduce the toxic effects of Cd stress on *S. lycopersicum*. This improvement is expected to show up as growth as well as improvements in physiological and biochemical characteristics.

1. Materials and Methods

2.1. Preparation of glucose solution

Glucose was dissolved in double distilled water (DDW) to create a 1 M stock solution. This was then diluted with DDW to achieve 50 mM.

2.2. Experimental layout and treatments

After soaking in 50 mM glucose for five hrs, *S. lycopersicum* seeds were planted in pots. Cd stress (0.8 mM) was applied directly through soil in the form of cadmium chloride at 15 days after sowing (DAS). The 20 pots were divided into four treatments were as follows:

- Set I: Control
- Set II: Cd (0.8 mM)
- Set III: Glucose (50 mM)
- Set IV: Cd (0.8 mM) + Glucose (50 mM)

The completely randomized design (CRD) was used for the planting of the *S. lycopersicum*, and sampling was done at 35 DAS to measured growth, physiological and biochemical attributes (Fig. 1).

2.3. Growth detection

S. lycopersicum plants were removed from the pots washed properly using DDW and measured length and weight (Fig. 1).

2.4. SPAD value and photosynthesis

SPAD value in the form of chlorophyll content was measure using SPAD chlorophyll meter (SPAD-502; Konica, Minolta Sensing, Inc., Sakai, Osaka, Japan). Photosynthesis and related attributes like net photosynthetic rate (P_N), stomatal conductance (gs), intercellular carbon dioxide concentration (Ci) and transpiration rate were recorded using infra-red gas analyzer (Li-COR 6200, Portable Photosynthesis System, Lincoln, NA, USA).

2.5. CA and NR activity

We calculated the CA activity in leaves by applying the method described by Dwivedi and Randhawa (1974). The method used to test nitrate reductase activity was Jaworski's (1971).

2.6. Antioxidant enzymes activity and proline content

The Aebi (1984) approach was used to measure the CAT activity. With minor adjustments, the Chance & Maehly (1955) process was used to quantity the POX activity. However, the Kono (1978) approach was used to measure SOD activity. Bates et al. (1973) method was applied to evaluate the proline content.

2.7. Estimation of H_2O_2 and MDA content

The amount of H_2O_2 in the leaves was measured using Patterson et al.'s (1984) method. Using the approach of Heath and Packer (1968), lipid peroxidation was related to MDA level.

2.8. Statistical analyses

To evaluate the existing data, SPSS 17.0 for Windows (SPSS, Chicago, IL, USA) was utilized. Using standard error computations and analyses of variance (ANOVA) at a significance level of $p < 0.05$, the least significant difference (LSD) between treatments was determined.

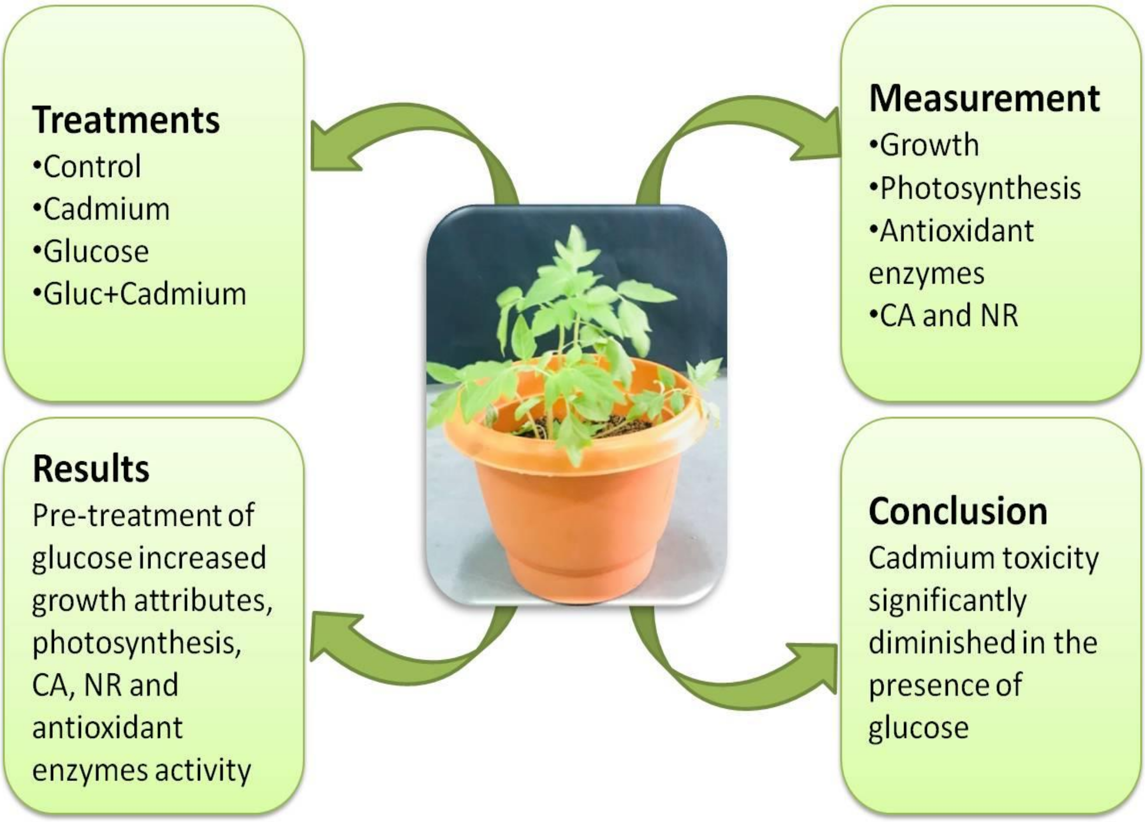

Fig. 1. Diagrammatic representation of current investigation, include treatment, measurement, results and conclusion.

3. Results

3.1. Glucose-mediated growth indices of *S. lycopersicum* under Cd stress

In the present investigation, glucose-mediated response of growth attributes of *S. lycopersicum* under Cd stress recorded (Fig. 2 and 3). It is found that, Cd stress diminished all the growth attributes with significant loss. However, glucose application completely/partially reverts the toxicity of Cd. Plants grown with glucose treatment significantly increased growth by 12% (SL), 09% (RL), 14% (SFW), 10% (RFW), 06% (SDW), and 09% (RDW) of *S. lycopersicum* under Cd stress compared to water treated plants (Fig. 2 and 3).

3.2. Glucose-mediated response on SPAD value and photosynthesis

SPAD value represents the chlorophyll content in the leaves. It is one of the key indicators for estimating photosynthesis, and an increase in chlorophyll value will have an impact on photosynthesis. The level of SPAD chl (Fig. 4) and photosynthesis (Fig. 5) were shown to rise throughout the entire soaking duration when glucose was present. Cd stress significantly reduced the chlorophyll content and photosynthesis. This lessens effect of Cd overcome by the glucose and increased all the attributes in the presence/absence of Cd stress in *S. lycopersicum* (Fig. 4 and 5).

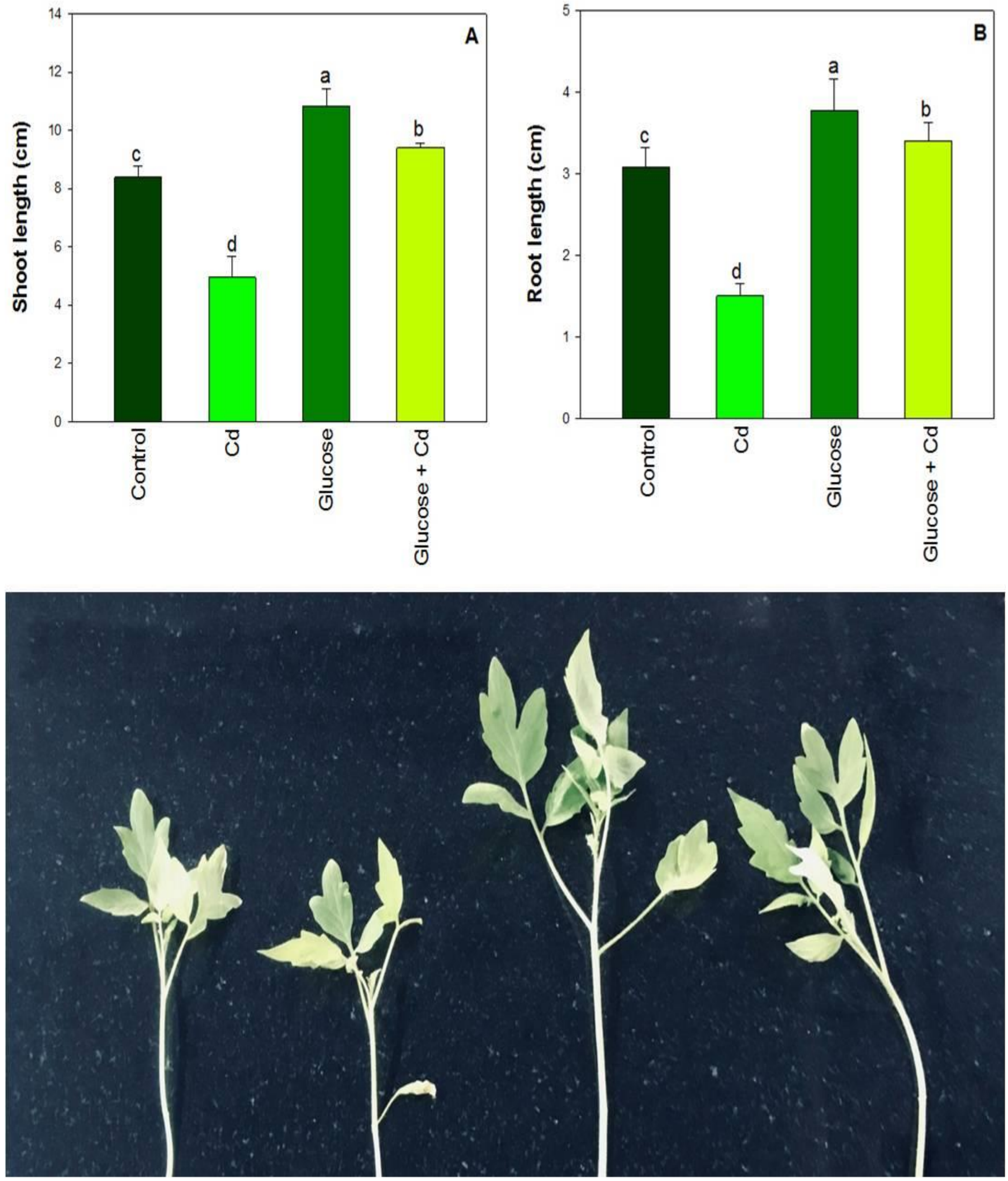

Fig. 2. Glucose-mediated response on shoot length (A) and root length (B) of *S. lycopersicum* under Cd stress.

3.3. CA and NR activity influenced by the presence of glucose

The contents of CA and NR in comparison to control are significantly reduced when plants emerge in Cd-treated soil (Fig. 6). Furthermore, the application of seed-glucose treatments significantly enhanced the activity of CA and NR. Highest content of CA and NR was noted in the plants, which seeds prior received glucose over control (Fig. 6).

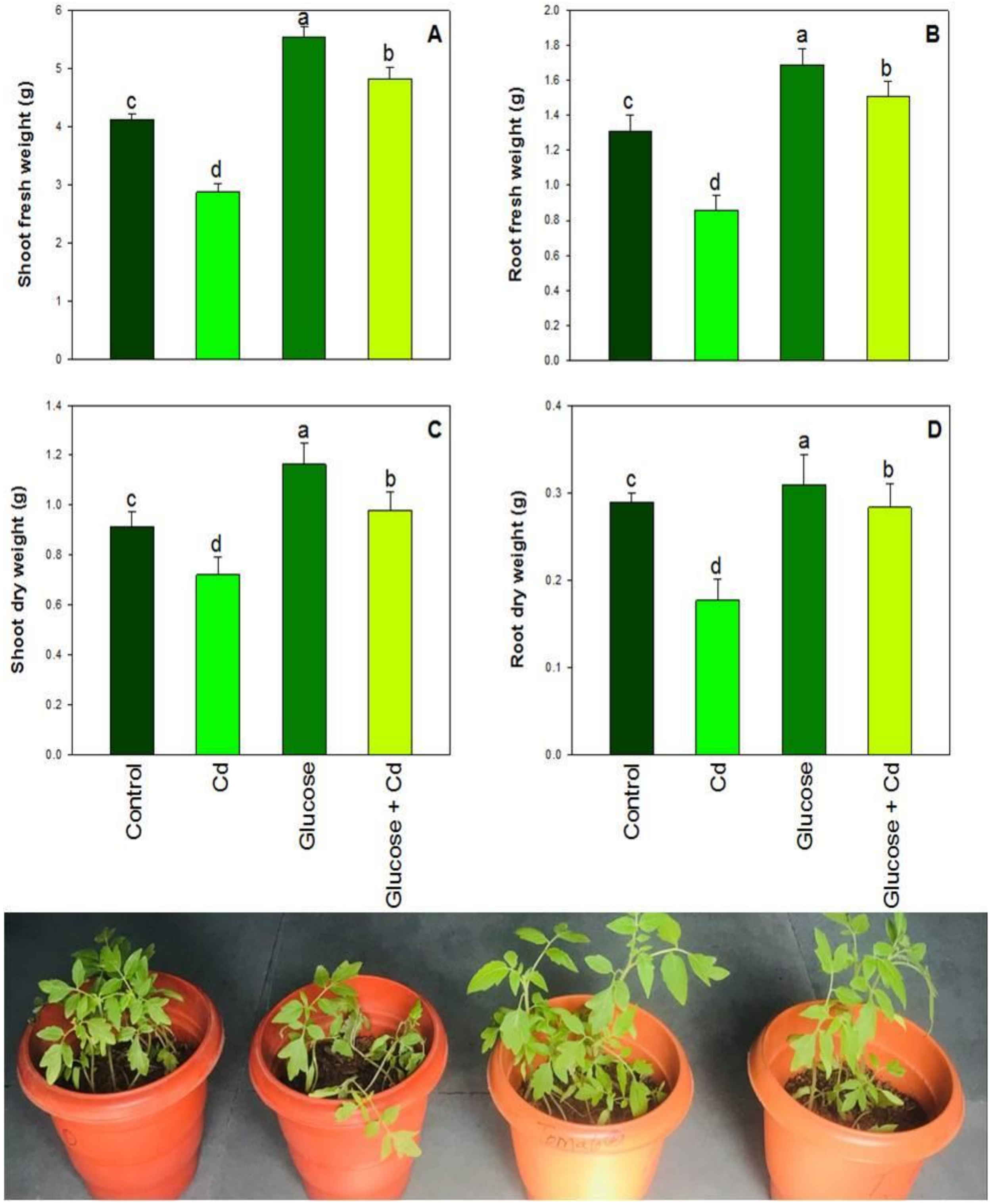

Fig. 3. Glucose-mediated response on shoot fresh weight (A), root fresh weight (B), shoot dry weight (C), and root dry weight (D) of *S. lycopersicum* under Cd stress.

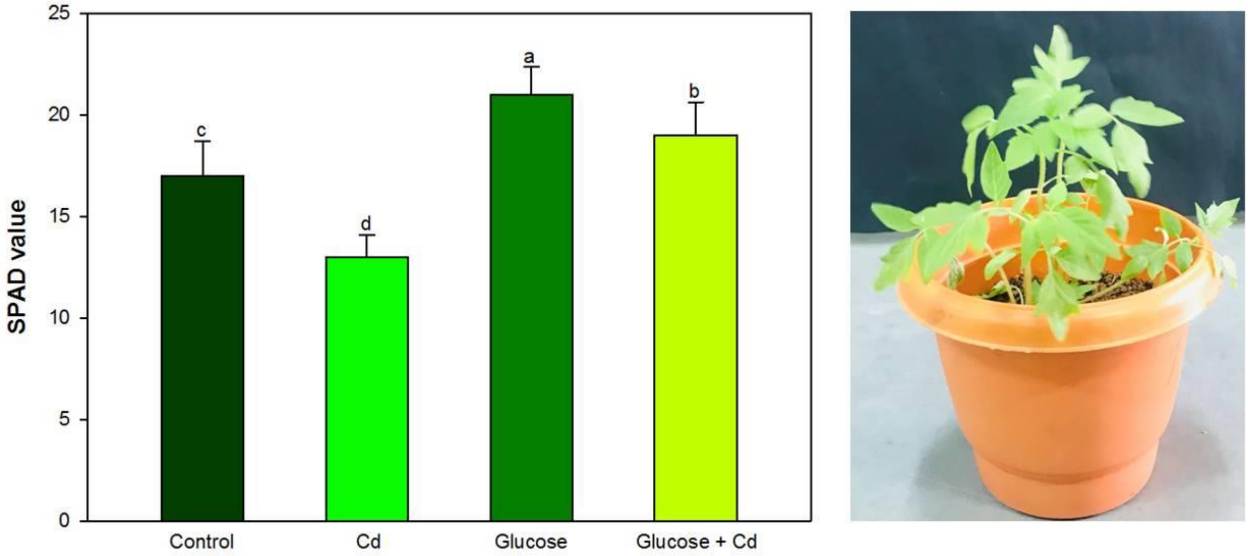

Fig. 4. Glucose-mediated response of SPAD values (chlorophyll content) on *S. lycopersicum* under Cd stress.

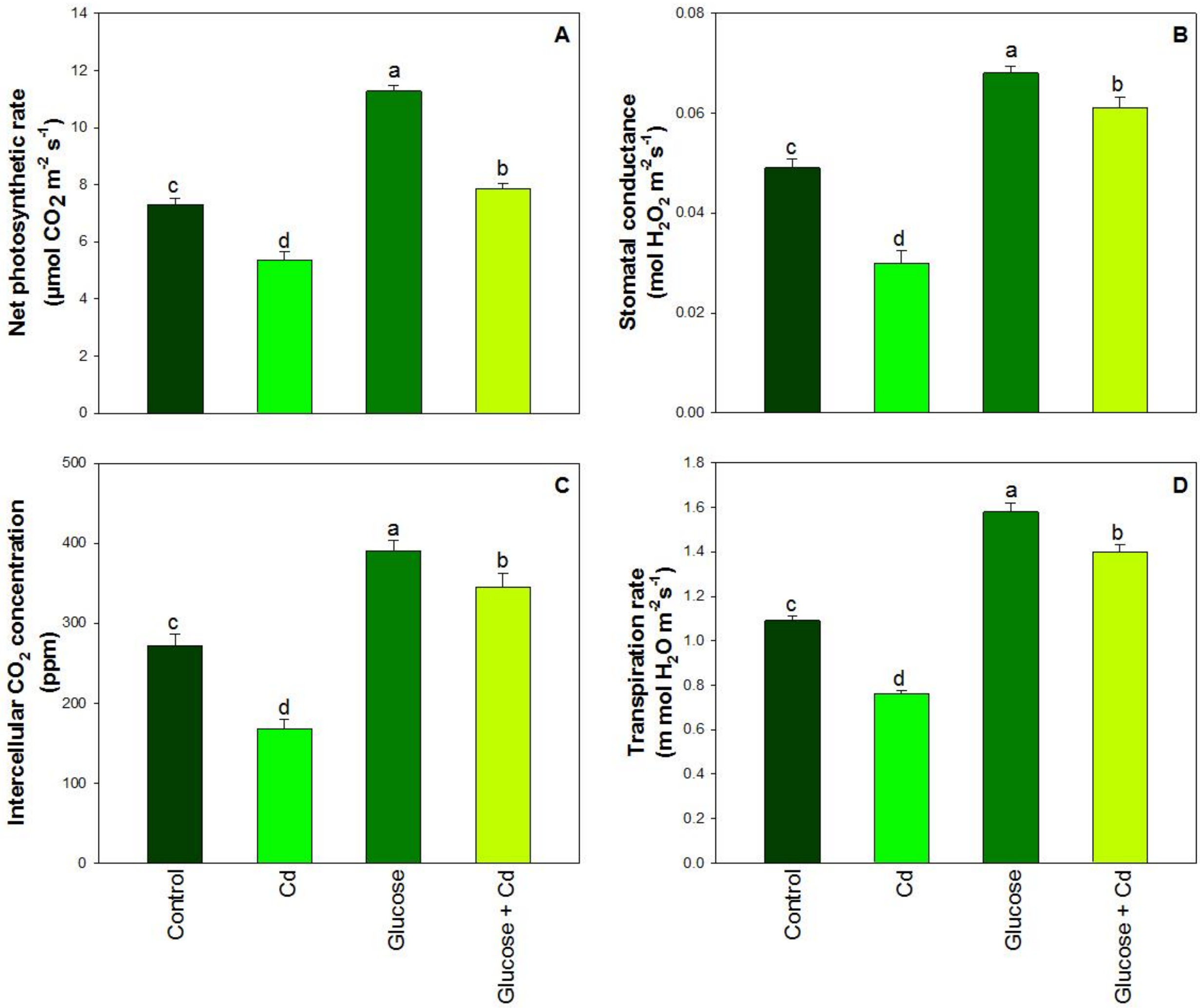

Fig. 5. Glucose-mediated response of net photosynthetic rate (A), stomatal conductance (B), intercellular CO_2 concentration (C), and transpiration rate (D) of *S. lycopersicum* under Cd stress.

3.4. Glucose-mediated antioxidant enzymes activities and proline content

Over control plants, the actions of CAT, POX, and SOD in the leaves of glucose pre-treated plants increased knowingly (Fig. 7A-C). Compared to control, Cd stress significantly increases the activities of CAT, POX, and SOD by 58%, 65%, 68% respectively (Fig. 7A-C). Moreover, in comparison to Cd stressed plants; the glucose pre-treatment significantly increased all these enzymes activity (Fig. 7A-C).

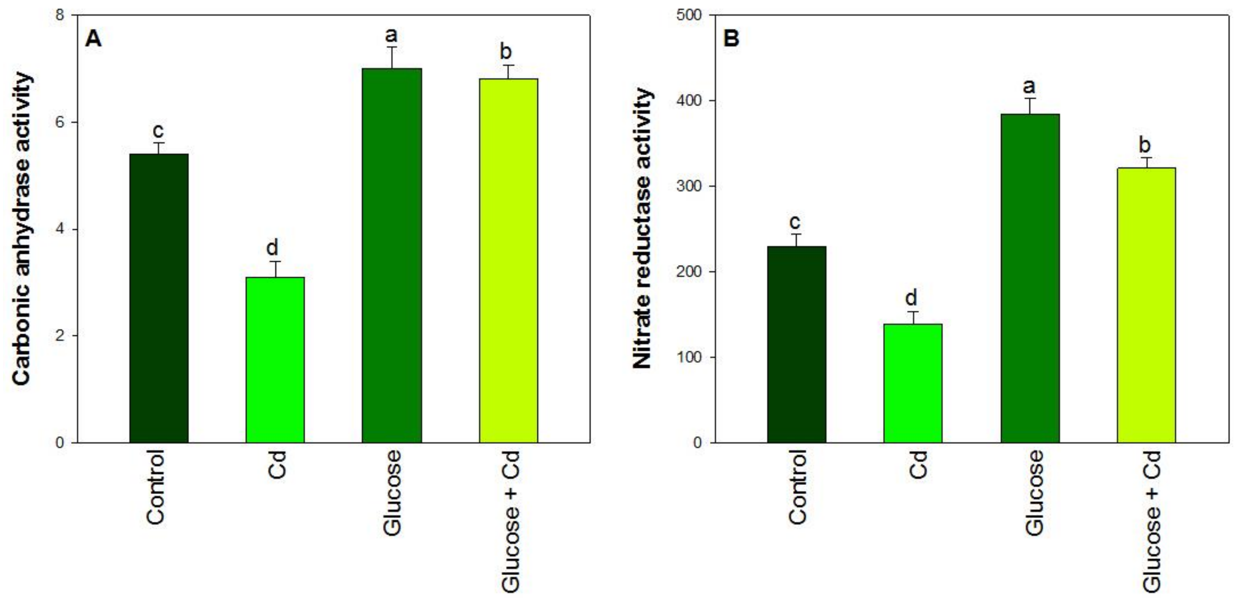

Fig. 6. Glucose-mediated response of carbonic anhydrase (A) and nitrate reductase (B) activity of *S. lycopersicum* under Cd stress.

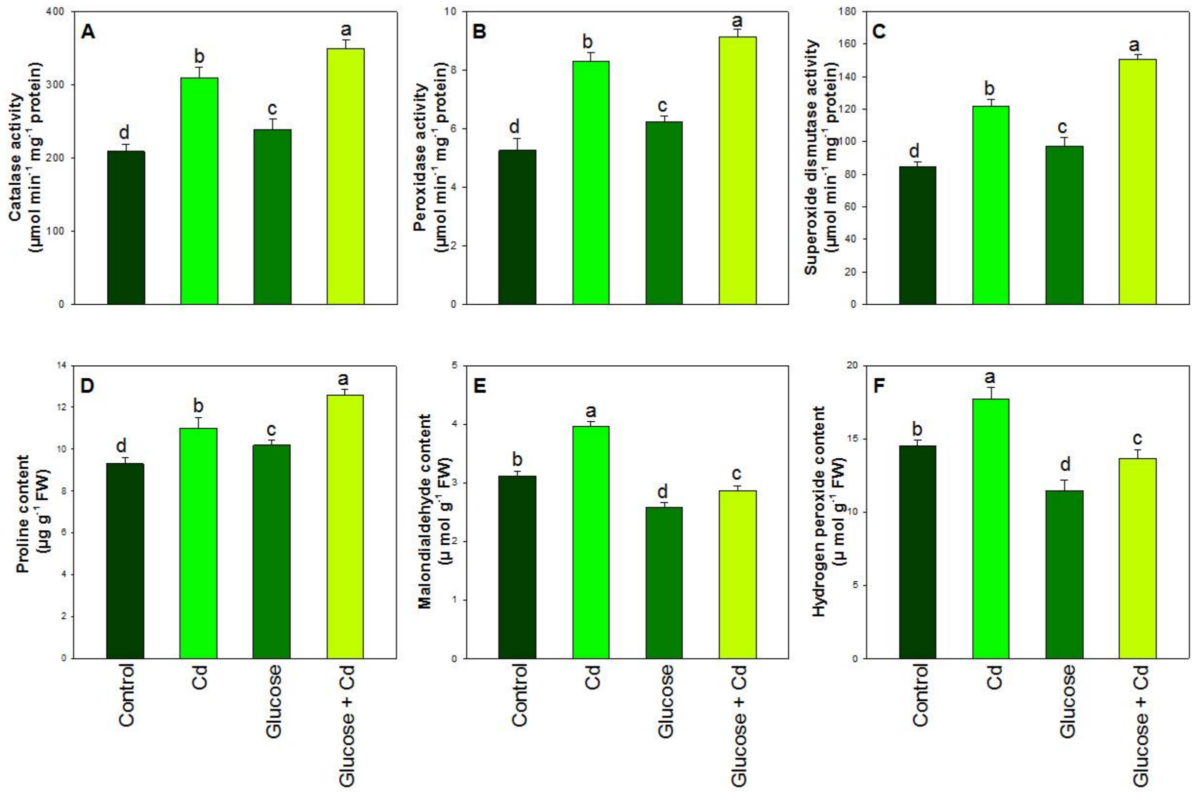

Fig. 7. Glucose-mediated response of catalase (A), peroxidase (B), superoxide dismutase (C), proline (D), malondialdehyde (E), and hydrogen peroxidase (F) content of *S. lycopersicum* under Cd stress.

3.5. Glucose-mediated H_2O_2 and MDA content under Cd stress

In comparison to the control, the MDA and H_2O_2 content in the leaves dramatically decreased following the glucose pretreatment. Figure 7E and F indicates that Cd-stressed plants showed a large increase in lipid peroxidation (27%) and H_2O_2 (22%) concentration, the glucose-pretreated plants showed a significant decrease in both parameters (Fig. 7E and F).

4. Discussion

Soluble sugars are necessary primary metabolites in plants and many other organisms. They are vital supervisory particles that regulate the appearance of genes, Plant development, metabolism, physiology, and the cell cycle (Gibson, 2005). Increases in root and shoot length, fresh and dry mass of both the root and the shoot, and the leaf area index all demonstrated the growth performance of plants when glucose was added. This might result from glucose complex effects on essential functions including developmental processes (Yaseen et al., 2013). This aligns with the findings of other researchers, who have demonstrated that the application of GLC enhances root growth and development (Singh et al., 2014), shoot induction, and proliferation (Gauchan 2012; Huang et al., 2013). A small number of further observations (Huang et al. 2013) show similar outcomes with a notable rise in the fresh and dry weight of plants. Moreover, Glc controls the expression of several genes involved in a variety of cellular processes, including growth, development, transcription, metabolism, transport, and signaling (Sheen, 2014; Eveland and Jackson, 2012).

Photosynthetic attributes show higher P_N, which is likely the result of improved non-stomatal activities, such as more effective CO_2 substrate usage brought on by improved photosynthetic enzyme performance and increased PSII efficiency (Bhat et al., 2023; Faizan et al., 2022; Faraz et al., 2023; Liet et al., 2022; Zhang et al., 2022). Because stomatal conductance controls CO_2 and water exchanges, it is also closely related to P_N (Habib, 2021). This is lined by the study of Hu et al. (2009), which found that exogenous glucose causes a subsequent rise in *Triticum aestivum* seedlings. Several other studies also noticed increased photosynthesis in glucose-treated plants, which is consistent with our findings (Jiang et al. 2012).

More interestingly, we found that NR activity is enhanced by pre-treatment of glucose (Fig. 6B). Actually, it is assumed that NR is efficiently induced by NO_3^- in the metabolic pool. Glucose, in instance, has many of the following factors have a direct and indirect impact on NR activity: (1) main foundation of influence reduction for NR activity; (2) supply carbon essential for NR synthesis. The same findings were also documented by Aslam and Oaks (1975), who found that adding glucose causes active NO_3^- to accumulate in corn roots. Additionally, in aerobic environments, glucose can serve as a stand-in for light requirements (El-Ramady et al., 2021). These findings suggested that the availability of NO_3^- within leaf cells may be controlled by light or glucose. Both light and glucose have the potential to impact the amount of NO_3^- recovered in the metabolic NO_3^- pool. This investigation revealed that the CA activity was increased by the pre-treatment of glucose (Fig. 6A). Results of Novichkova et al. (2006), which show that Glc pretreatment increases CA activity in Raphanus sativus leaves, are consistent with this. The proline content of *S. lycopersicum* leaves was shown to increase in the current study when glucose was present (Fig. 7D). Here, the oxidative pentose phosphate pathway and respiration use glucose as an energy source to make NADPH and ATP, respectively. These two compounds are sources of proline biosynthesis from α-ketoglutarate and glutamate.

The present study found that the occurrence of Glc also raised the amount of antioxidant enzymes (Fig. 7). The results of this investigation are consistent with those of Huang et al. (2013), who found that glucose in cucumber seedlings increases the activity of antioxidant enzymes during drought stress. Additionally, when *T. aestivum* was subjected to salt stress, exogenous glucose administration increased its antioxidant capacity (Hu et al., 2012).

In the current investigation, exogenous glucose reduces the levels of MDA and H_2O_2 in *B. juncea* roots and leaves. It's interesting to note that sugars have two distinct roles in plants: (1) they are linked to the respiration process, which produces ROS, and (2) they are linked to the oxidative pentose-phosphate pathways, which produce NADPH and are linked to the pathway associated with ROS foraging (Cuoee et al. 2006). However, plants have the capability to forage ROS thanks to sugars (fructose and glucose) that play roles in membrane contact (Keunen et al. 2013). As was previously said, sugar-mediated elevation of antioxidant content lowers the ROS level. This is lined by the previous study of Huang et al. (2013), which showed that glucose application decreased MDA and H_2O_2 levels in *Cucumis sativus*.

5. Conclusions

Based on the current observation, it can be concluded that Cd toxicity seriously reduced the morphology and physio-biochemical attributes of *S. lycopersicum*. However, pre-treatment of glucose on *S. lycopersicum* plant improves its growth, enzymes activity and gaseous exchange parameters. To comprehend the molecular mechanisms that give plants tolerance to Cd stress, more research is required in this field. For crops to successfully establish in heavy metal-affected areas, an integrated strategy that combines physiological and biochemical events with molecular approaches is still required.

Author Contributions

Conceptualization, P.A.; investigation, P.A., M.F.; resources, M.F., writing-original draft preparation, P.A.; M.F.; writing-review and editing, P.A., M.F., visualization, P.A. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

There are no conflicts to declare.

Formatting of funding sources

The authors extend their appreciation to Prince Sattam bin Abdulaziz University for funding this research work through the project number (PSAU/2024/01/27831).

Acknowledgments

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