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Original Paper

Silicon-induced changes in morphophysiological and biochemical characteristics in *Enterolobium contortisiliquum* under cadmium stress

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Abstract

Cadmium (Cd) is a heavy metal that may bring about harmful pollution of water and soil. Phytoremediation involving elements beneficial for plant development is a strategy to alleviate this problem. Silicon (Si) has shown potential in neutralizing biotic and abiotic stresses in plants, especially those caused by heavy metals. Thus, the aim of this study was to evaluate whether Si could mitigate the effects of Cd toxicity on growth, photosynthetic activity, and oxidative stress in seedlings of *Enterolobium contortisiliquum*. The experiment consisted of a completely randomized design with four replications. In addition to a control treatment, the following amounts of Cd, Si, and combinations were added to the complete nutrient solution: 1.5 mM Si; 3.0 mM Si; 1.5 mM Si + 100 µM Cd; 3.0 mM Si + 100 µM Cd; 100 µM Cd. Each sampling unit consisted of a tray containing 16 plants. Silicon concentrations of 1.5 and 3.0 Si mM were adequate to mitigate the effects of cadmium toxicity on *Enterolobium contortisiliquum* seedlings. The results showed that Si promoted photosynthetic activity, increased total chlorophyll, and decreased shoot lipid peroxidation in the presence of Cd. Furthermore, the lack of significant differences in shoot and root dry weight among treatments and low peroxidation content in roots suggest that *E. contortisiliquum* is tolerant to cadmium.

Key words: beneficial elements, oxidative stress, photosynthesis, phytoremediation, timbaúva.

Resumo

O cádmio (Cd) é um metal pesado nocivo capaz de poluir a água e o solo. A fitorremediação ligada a elementos benéficos para o desenvolvimento vegetal é uma estratégia para amenizar esse problema. O silício (Si) tem mostrado potencial para neutralizar estresses bióticos e abióticos em plantas, principalmente aqueles causados por metais pesados. Assim, o objetivo deste estudo foi avaliar os efeitos do Si sobre a toxicidade do Cd no crescimento, na atividade fotossintética e no estresse oxidativo em mudas de *Enterolobium contortisiliquum*. O experimento constou de um delineamento inteiramente casualizado, com quatro repetições. Além de um tratamento controle, as seguintes combinações de Cd e Si foram adicionadas à solução nutritiva completa: 1,5 mM de Si; Si 3,0 mM; 1,5 mM de Si + 100 µM de Cd; 3,0 mM de Si + 100 µM de Cd; 100 µM Cd. Cada unidade amostral consistiu de uma bandeja contendo 16 plantas. Os resultados mostraram que o Si promoveu a atividade fotossintética, aumentou a clorofila total e diminuiu a peroxidação lipídica da parte aérea na presença de Cd. As concentrações de 1.5 e 3.0 Si mM de silício podem ser consideradas adequadas para amenizar os efeitos da toxicidade de cádmio em mudas de *Enterolobium contortisiliquum*. Além disso, a ausência de diferenças significativas no peso seco da parte aérea e da raiz entre os tratamentos e baixo teor de peroxidação nas raízes sugerem que *E. contortisiliquum* é tolerante ao cádmio.

Palavras-chave: elementos benéficos, estresse oxidativo, fotossíntese, fitorremediação, timbaúva.

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Introduction

Increasing environmental pollution from various sources such as industrialization, mining, and human activities, in addition to the changing climate, represents a high risk to the environment and human health (Kapoor *et al.* 2022). Among the numerous substances harmful to soil, water, plants, animals and humans are heavy metals which are persistent and highly toxic pollutants (Imtiaz *et al*. 2016; Alsherif *et al.* 2022). One of these elements is cadmium (Cd), which can reach the environment through natural sources, as well as artificial sources, such as the use of phosphate fertilizers, fossil fuels, and industrial wastewater (Behrouzi *et al.* 2018).

In plants, the most common symptoms of excess Cd are decreased root and shoot growth and reduced chlorophyll biosynthesis and photosynthetic rates (Woraharn *et al.* 2021). It also interferes with transpiration, stomatal conductance, and key enzyme activities in carbon assimilation (Naeem *et al.* 2018). Furthermore, excess Cd in plants may lead to an increase in hydrogen peroxide content, resulting in high levels of membrane lipid peroxidation that contribute to biomass reduction (Bamagoos *et al.* 2022). Cd also exacerbates ROS production and associated oxidative damage in plants through various mechanisms, such as inhibition of the electron transport chain and activation of lipoxygenase, resulting in lipid peroxidation. This lipid peroxidation from excessive ROS production leads to cell damage and ultimately halts plant growth (Souri *et al.* 2020).

Thus, plants grown in Cd-contaminated soils manifest metabolic problems, such as reduced growth and biomass production, oxidative stress, and pollutant accumulation. This represents a considerable risk of contamination via the food chain if such plants are intended for animal and human nutrition (Imtiaz *et al.* 2016; Shi *et al.* 2018). Cd, like other heavy metals, is not metabolized in the human body, so little of it is eliminated and it accumulates in the body, replacing essential salts and minerals. It ends up deposited in vascular tissues, muscles, bones, and joints (Behrouzi *et al.* 2018). Thus, elements such as lead (Pb), cadmium (Cd), and mercury (Hg) can be toxic to living beings, even at low concentrations.

A good strategy to mitigate this problem is using beneficial elements that help plants develop well and not absorb such pollutants (in the case of plants used for food), or that help plants assimilate elements, thus remediating soils. The application of a chemical regulator or mediator is considered a viable and economical approach to safer food production (Hussain *et al.* 2020). Silicon (Si), the second most abundant element in the earth's crust, has the potential to play this role, as it has been shown to be vital to plant growth and to neutralization of biotic and abiotic stresses, reducing metal toxicity by ion adsorption or by improving the antioxidant system (Shi *et al.* 2018). Thus, Si decreases biotic and abiotic stresses such as pathogen infection, salt stress, and water stress; and it simultaneously increases plant tolerance to otherwise toxic heavy metals, including aluminum (Al), manganese (Mn), zinc (Zn), chromium (Cr), and cadmium (Cd) (Jesus *et al.* 2017). The action of Si in reducing the negative effects of Cd has been described in the literature, including improvement of antioxidant responses in *Arachis hypogaea*, maintenance of membrane integrity in *Echium amoenum*, increased biomass in *Zea mays*, increased growth in *Cucumis sativus* and *Solanum lycopersicum*, improved photosynthetic performance in *Cucumis sativus*, and reduced Cd content in *Triticum turgidum* (Pereira *et al*. 2017).

Phytoremediation using tree species which are not used for human and animal food is a promising alternative to improve soils polluted with heavy metals (Capuana 2020). Using leguminous species for this process is an interesting option as they provide extensive canopy coverage, serve as a windbreak, and are able to associate with and enhance the heterotrophic microbial community, contributing to nutrient uptake and increasing plant tolerance to environmental stresses (De Melo *et al.* 2014). Among the tree species belonging to the Leguminosae family is *Enterolobium contortisiliquum* (Vell.) Morong, commonly known as *timbaúva* or *tamboril* (LPWG 2017). It is a fast-growing species with wide use, in addition to being tolerant to heavy metals (De Melo *et al.* 2014), which makes it promising for reforestation programs and recovery of degraded areas (Lorenzi 2016).

Our hypothesis is that Si reduces Cd toxicity in *E. contortisiliquum* without causing damage to plant development and biomass production. Therefore, the aim of this study was to evaluate whether silicon reduces the effects of cadmium toxicity on morphological, photosynthetic, and oxidative stress parameters in *E. contortisiliquum*.

Materials and Methods

Study site and experiment

The experiment was carried out in the Plant Physiology and Nutrition Laboratory and in the greenhouse of the Biology Department of the Federal University of Santa Maria, located in the city of Santa Maria, state of Rio Grande do Sul (RS), Brazil. The greenhouse had a controlled temperature of approximately 25 ºC and average air humidity of 60%.

The seeds of *Enterolobium contortisiliquum* were supplied by the Center for Research in Forests of the Department of Agricultural Diagnosis and Research (DDPA/RS). The seeds underwent acid scarification in 98% sulfuric acid for 40 min. to overcome dormancy (Da Silva *et al.* 2014) and were then placed in Petri dishes with Germitest® paper (sterilized seed germination material) moistened with 2.5 times its weight in deionized water and placed in a germination room (25±1 °C and constant photoperiod) until radicle emission. In the greenhouse, the seeds were placed in trays containing commercial substrate and were irrigated daily; and every five days, the nutrient solution of Hoagland & Arnon (1950), with pH 5.5 ± 0.1, was added (50 ml per seedling). The composition of the nutrient solution was (in µM) 6090.5 N, 974.3 Mg, 4986.76 Cl, 2679.2 K, 2436.2 Ca, 359.9 S, 243.592 P, 0.47 Cu, 2.00 Mn, 1.99 Zn, 0.17 Ni, 24.97 B, 0.52 Mo, and 47.99 Fe ($FeSO_4$/Na-EDTA). Upon reaching a height greater than 10 cm, homogeneous plants were separated and placed in a hydroponic system.

In a hydroponic system composed of 24 plastic trays with 16 liters of Hoagland's nutrient solution each, under constant aeration, the seedlings were fixed on polystyrene plates with the aid of a sponge. All the trays received a plate with 10 plants suspended in nutrient solution. At seven-day intervals, the solutions were changed, and the pH adjusted to 4.5 ± 0.1. During the first seven days, the plants were left to acclimate in the complete nutrient solution alone. After that period, six treatments with Si (Na_2SiO_4) and Cd ($CdCl_2.H_2O$) were distributed in the system as follows: 0 mM Si + 0 µM Cd (control), 1.5 mM Si + 0 µM Cd, 3.0 mM Si + 0 µM Cd, 1.5 mM Si + 100 µM Cd, 3.0 mM Si + 100 µM Cd, and 0 mM Si + 100 µM Cd. The concentrations and the pH of the solution were determined based on preliminary tests and by reviewing scientific literature. The experiment was carried out in a completely randomized design, with four replications of 10 plants, for a total of 24 experimental units.

Sixty days after sowing, the plants were kept in the hydroponic system for an additional 22 days, seven for acclimatization and 15 for exposure to the treatments. The period of acclimatization and treatment exposure were also based on preliminary tests and on the scientific literature.

Morphophysiological parameters

After the acclimatization period, before and after exposure to the treatments, the taproot length of four plants from each experimental unit was measured with a millimeter ruler. From these data, the increase in taproot length was determined as the difference in the values measured before and after application of the treatments.

To determine root surface area, root volume, total root length, and average root diameter, four seedlings were removed from the experiment and the shoots and roots were separated. Root images were digitized with a 11000XL Epson scanner and analyzed in WinRHIZO Pro software.

To determine dry weight, the plants were separated into shoots and roots, placed in Kraft paper bags, and then immediately placed in a forced air circulation laboratory oven at 65 °C until reaching constant weight. Values were measured on a precision balance to determine shoot and root dry weight.

Photosynthetic parameters

The photosynthetic parameters of one plant from each experimental unit were analyzed on the 7th and 15th day of exposure to the treatments. This process was carried out in the morning, between 8:00 and 11:00, with a portable infrared CO_2 meter (LI-COR, model LI-6400XT). The following parameters were assessed: net CO_2 assimilation rate / photosynthetic rate (A), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), transpiration rate (E), water use efficiency (WUE), and Rubisco carboxylation efficiency (A/Ci) at an ambient CO_2 concentration of 400 µmol mol^{-1} at 20–25 °C, 50 ± 5% relative humidity, and photon flux density of 1500 µmol m^{-2} s^{-1}.

Biochemical parameters

The seedlings were collected after 15 days of exposure to the treatments and separated into shoots and roots, washed with distilled water, and then immediately frozen in liquid nitrogen for sample conservation. This material was kept in an ultrafreezer (-80 °C) until samples were prepared for analysis. This process consisted of macerating samples with liquid nitrogen until a fine powder was obtained, then weighing the amount of specific material for each analysis on a precision digital scale.

Pigment concentrations (chlorophyll *a* and *b* and carotenoids) were determined using leaf samples, according to the Hiscox & Israelstam (1979) method, and the results were defined by the equation of Lichtenthaler (1987). Total chlorophyll is the sum of chlorophyll *a* + chlorophyll *b*. Membrane lipid peroxidation was determined by the method presented by El-Moshaty *et al.* (1993), and the degree of lipid peroxidation was given as nmol malondialdehyde (MDA) mg^{-1} protein. Hydrogen peroxide content was estimated according to Loreto & Velikova (2001), and the concentration expressed in µmol g^{-1} fresh weight.

Superoxide dismutase (SOD) activity was defined according to Giannopolitis & Ries (1977), and a unit of SOD is considered the amount of enzyme that inhibits the photoreduction of nitroblue tetrazolium (NBT) by 50% (Beauchamp & Fridovich 1971). Guaiacol peroxidase (POD) activity was estimated according to Zeraik *et al.* (2008), and results were given in enzyme unit per mg of protein (U mg^{-1} protein).

Statistical analysis

The experimental data were checked for normality of errors by the Shapiro-Wilk test and for homogeneity of variances by the Bartlett test (Storck *et al.* 2016). If the assumptions were met, analysis of variance (ANOVA) was carried out, and significant difference of the means was analyzed by Tukey's test at 5% probability of error, using the SISVAR software (Ferreira 2014).

Results

Effects of Si and Cd on growth parameters

The highest values for increase in taproot length of *E. contortisiliquum* seedlings were found in the control, which did not differ statistically from 3.0 mM Si + 0 µM Cd (Fig. 1a). Lower values for increase in taproot and total root length were found in the presence of Cd (1.5 mM Si + 100 µM Cd; 3.0 mM Si + 100 µM Cd, and 100 µM Cd) (Fig. 1a,b), while total root length did not differ from the control with the presence of Si alone (Fig. 1b). Lower values of root surface area were observed in the presence of Cd alone and for 3.0 Si mM + 100 µM Cd, but there was no difference between the control and 1.5 Si + 100 µM Cd for this parameter (Fig. 1c).

Furthermore, the presence of Si alone provided the highest values for shoot dry weight, but only diverged from 3.0 mM Si + 100 µM Cd. (Fig. 1d). No significant difference was observed for root dry weight (Fig. 1e), average root diameter, and root volume, regardless of the Si and Cd combinations applied (data not shown).

Effects of Si and Cd on photosynthetic parameters

Photosynthetic parameters were analyzed after 7 and 15 days of exposure to treatments with different concentrations of Si and Cd (Fig. 2). On the seventh day, the concentrations of Si (1.5 mM and 3.0 mM) showed higher values of photosynthetic rate (A). The combinations with Si and Cd (1.5 mM Si + 100 µM Cd and 3.0 mM Si + 100 µM Cd) did not differ from the control, while 100 µM Cd showed the lowest value (Fig. 2a). On the fifteenth day, 100 µM Cd still showed the lowest value, and the other treatments did not show significant differences among themselves for this parameter (Fig. 2a). Si attenuated Cd toxicity on photosynthetic rate, unlike some of the growth parameters, both at 7 and 15 days of exposure. In the first assessment of stomatal conductance (Gs) (day 7), 1.5 mM Si and 3.0 mM Si showed higher rates, which were significantly higher than those found in the other treatments; while lower values in the second assessment were found in treatments with Si and Cd (1.5 mM Si + 100 µM Cd and 3.0 mM Si + 100 µM Cd) (Fig. 2c).

For intercellular CO_2 concentration (Ci), both in the first and in the second assessments, no significant differences were observed between the treatments and the control (data not shown). For transpiration rate (E), at day 7, the treatments with Si and Cd (1.5 mM Si + 100 µM Cd and 3.0 mM Si + 100 µM Cd) and 100 µM Cd alone did not differ from the control, while 1.5 mM Si and 3.0 mM Si showed the highest values (Fig. 2c). At 15 days, 3.0 mM Si showed the highest transpiration rate and 3.0 mM Si + 100 µM Cd the lowest; the other treatments did not differ from one another (Fig. 2c). In the first assessment of water use efficiency (WUE), significant differences were not found between the treatments and the control, whereas

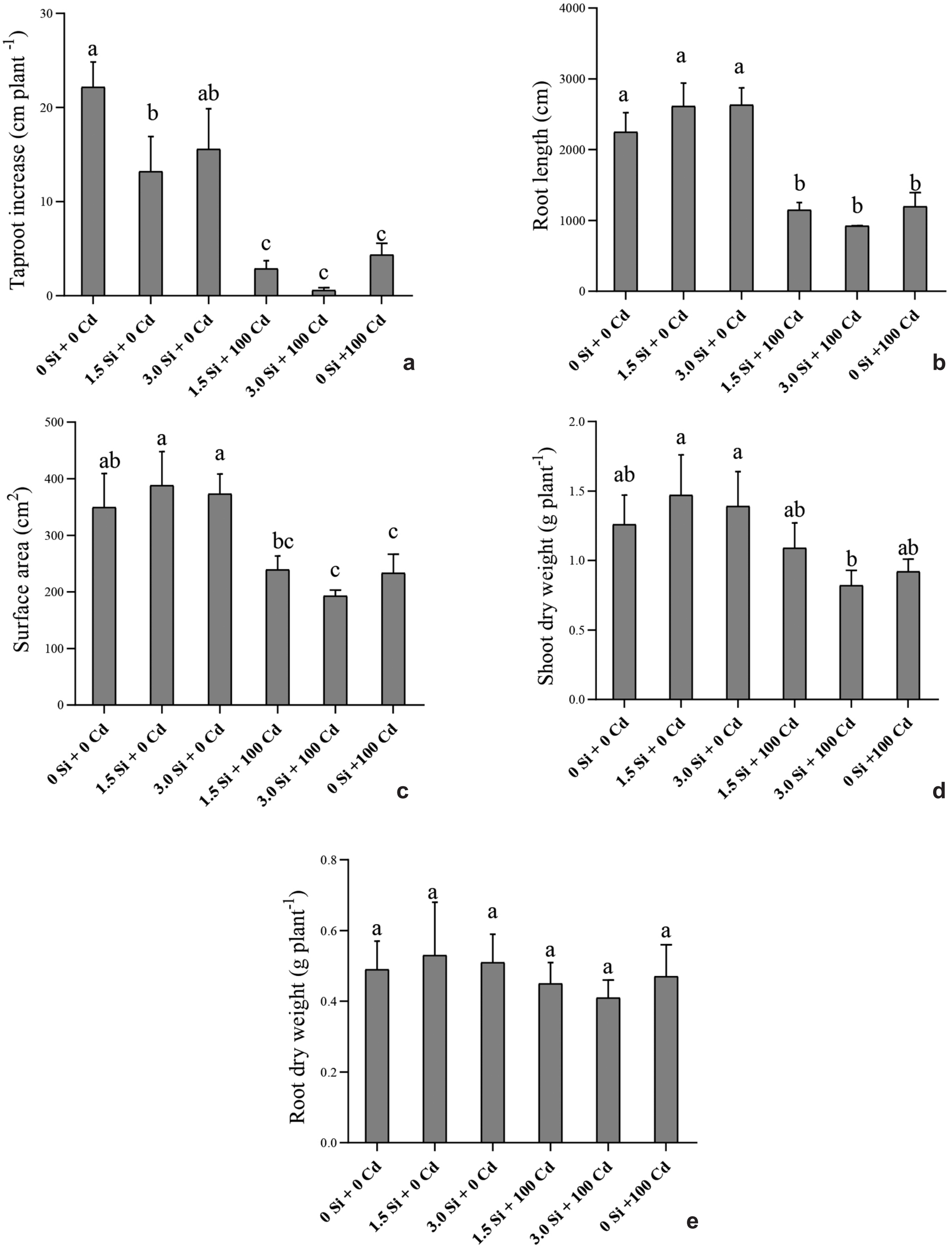

Figure 1 – a-e. *Enterolobium contortisiliquum* plants exposed to Cd and Si in a hydroponic system. Mean values recorded – a. for increase in taproot length; b. in root length; c. in root surface area; d. in shoot dry weight; e. in root dry weight. Different letters between treatments represent statistically significant difference by Tukey's test. Bars represent the mean ± standard deviation.

in the second assessment, we found a higher WUE value for 3.0 mM Si + 100 µM Cd than for 100 µM Cd (Fig. 2d). However, there was no significant difference in WUE between the treatments (with Cd and Si) and the control (Fig. 2d).

Effects of Si and Cd on biochemical parameters

Lower total chlorophyll content resulted from the Cd treatments compared to the control (Fig. 3a). However, higher values for carotenoid content were found in the control, differing significantly from the other treatments (Fig. 3b).

There was no significant difference for SOD activity in shoots, regardless of the treatment (Fig. 3c). However, higher values for SOD activity in roots were found for 1.5 mM Si + 0 Cd and for 100 µM Cd alone (Fig. 3d). For POD activity in shoots, the presence of Si (3.0 mM) led to the highest value, but it differed only from the control and from 1.5 mM Si alone (Fig. 3e). The highest value for POD activity in roots was observed for

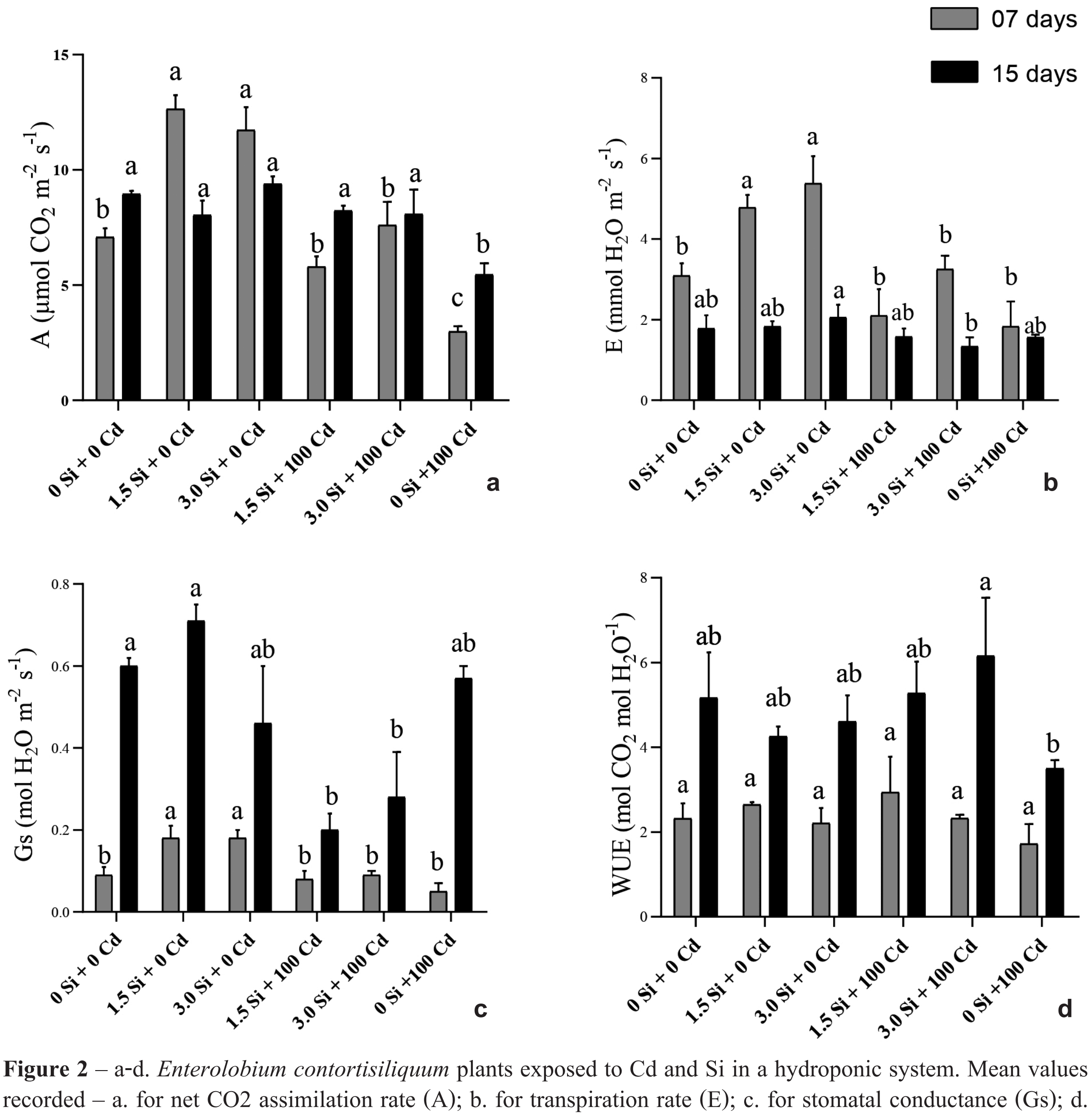

Figure 2 – a-d. *Enterolobium contortisiliquum* plants exposed to Cd and Si in a hydroponic system. Mean values recorded – a. for net CO2 assimilation rate (A); b. for transpiration rate (E); c. for stomatal conductance (Gs); d. for water use efficiency (WUE). Different letters between treatments represent statistically significant difference by Tukey's test. Bars represent the mean ± standard deviation.

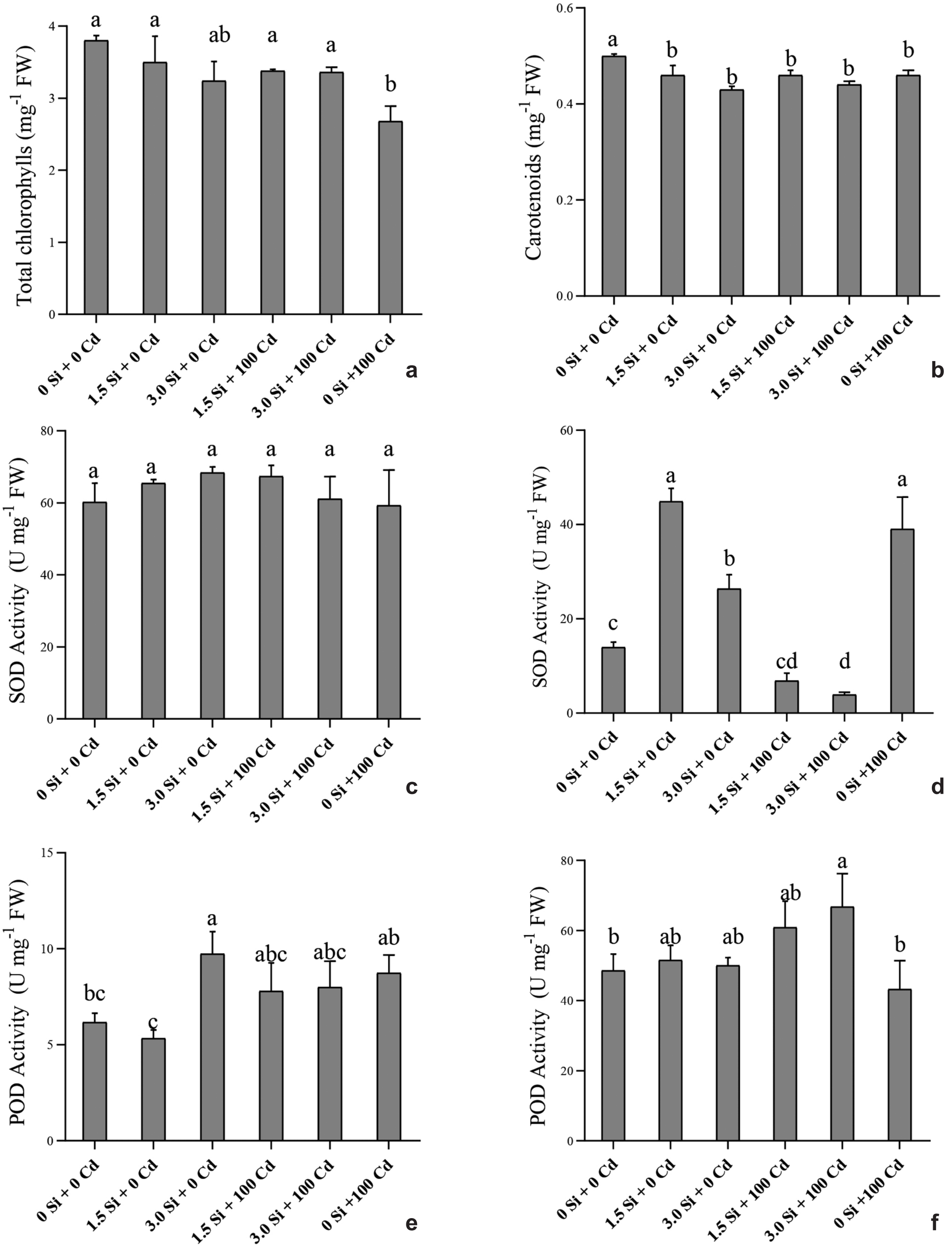

Figure 3 – a-f. *Enterolobium contortisiliquum* plants exposed to Cd and Si in a hydroponic system. Mean of – a. total chlorophyll; b. carotenoids; c. superoxide dismutase (SOD) activity in shoots; d. superoxide dismutase (SOD) activity in roots; e. guaiacol peroxidase (POD) activity in shoots; f. guaiacol peroxidase (POD) activity in roots. Different letters between treatments represent statistically significant difference by Tukey's test. Bars represent the mean ± standard deviation.

3.0 mM Si + 100 µM Cd, differing only from the control and from Cd alone (Fig. 3f).

The lowest values of hydrogen peroxide (H_2O_2) content in shoots and roots were found in the control (Fig. 4a,b). Furthermore, we found the lowest value for MDA contents in shoots and the highest in roots for 1.5 mM Si + 100 µM Cd (Fig. 4c,d). However, there was no difference for MDA levels in shoots between the control and 3.0 mM Si + 100 µM Cd (Fig. 4c).

Discussion

Lower values for increase in taproot length, total root length, and root surface area were observed in the presence of Cd (Fig. 1a-c). This is because Cd toxicity negatively affects the mitotic division of root meristematic cells and causes abnormal changes in the cortical cell layers and apical region of the epidermis (Subaši *et al.* 2022). These changes decrease water and nutrient uptake and result in reduction of root length (Haider *et al.* 2021), and subsequently, they may decrease plant biomass in the long term. However, the addition of Si did not mitigate the negative effects induced by Cd for these parameters (Fig. 1a-c). That may have occurred because Si failed to expand the plastic and elastic extension of the cell wall in the root elongation zone, and consequently the root system did not grow (Lux *et al.* 2020).

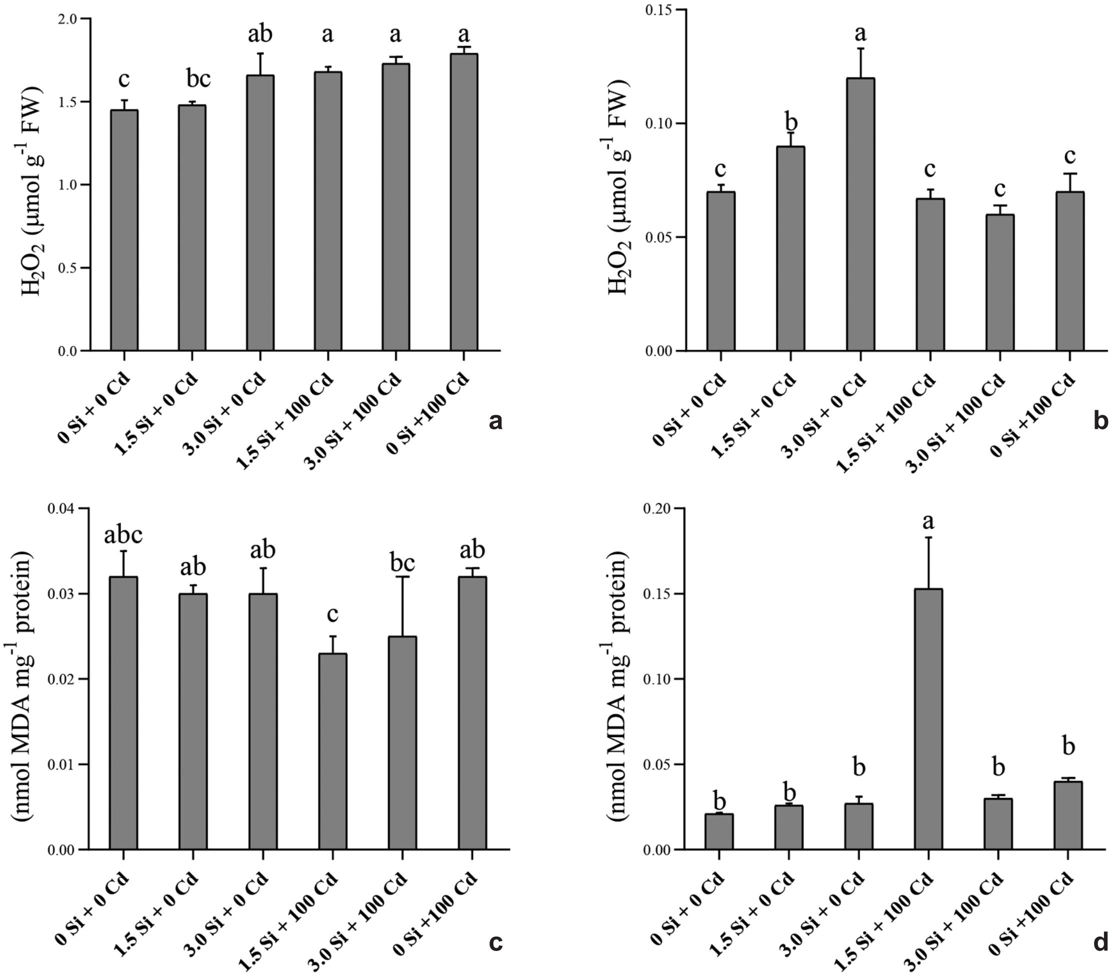

Figure 4 – a-d. *Enterolobium contortisiliquum* plants exposed to Cd and Si in a hydroponic system. Mean values recorded for – a. hydrogen peroxide (H_2O_2) concentration in shoots; b. hydrogen peroxide (H_2O_2) concentration in root; c. membrane lipid peroxidation in shoots; d. membrane lipid peroxidation in roots. Different letters between treatments represent statistically significant difference by Tukey's test. Bars represent the mean ± standard deviation.

The reduction brought about by Cd in the morphological parameters of the root system did not result in reduced biomass production, as there was no significant difference for shoot and root dry weight of *E. contortisiliquum* seedlings in comparison to the control treatment (Fig. 1d,e). This was probably because Cd-tolerant species are able to maintain biomass production under stress, due to their strategies of Cd compartmentalization in the cell vacuole and Cd complexation/chelation to decrease Cd bioavailability (Subaši *et al.* 2022).

Regarding the photosynthetic parameters, the lowest values for net CO_2 assimilation rate (A) were found in the presence of Cd alone, both at 7 and 15 days of exposure (Fig. 2a). The addition of Si led to maintenance of photosynthetic rate, even in the presence of Cd (Fig. 2a). This response may be associated with beneficial effects caused by Si addition, which contributed by improving leaf architecture, allowing better light absorption and resulting in a higher photosynthetic rate (Lee *et al.* 2010). Even with this positive effect of Si on photosynthetic activity, we did not observe an increase in biomass production of *E. contortisiliquum* (Figs. 2a; 1d,e). This lack of increase may also be because the exposure time to the treatments was not enough for the positive effect of Si on photosynthetic activity to bring about greater biomass production.

The lowest value of transpiration rate (E) was for 3.0 mM Si + 100 μM Cd at 15 days (Fig. 2b). That probably occurred because plants that have more CO_2 in the intercellular spaces of the leaf close their stomata more often, decreasing stomatal conductance (Gs), resulting in increased water use efficiency (WUE) (Engineer *et al.* 2016). This reduction in stomatal conductance and, subsequently, in transpiration rate can also be considered the main inhibition mechanism of Cd translocation from roots to shoots. Si-induced inhibition of stomatal conductance with no effect on photosynthetic rate also appears to be associated with Si-triggered improvement in photosynthetic pigments and Calvin cycle enzymes (Naeem *et al.* 2018).

Nwugo & Huerta (2011) evaluated the effect of silicon on *Oryza sativa* L. under cadmium stress and found an increase in transpiration and stomatal conductance, while intercellular CO_2 concentration declined in the presence of Si + Cd. According to these authors, this reduction in Ci may be related to the enhanced performance of the RUBISCO enzyme during CO_2 fixation, an effect that is possibly due to the addition of Si in plants exposed to toxicity. In studies with *Cucumis sativus*, Feng *et al.* (2010) found that 1.5 mM Si mitigated the negative effects caused by Cd toxicity on gas exchange, significantly increased transpiration rate and stomatal conductance, and considerably reduced Ci after 10 and 15 days exposure to treatments. According to these authors, Si brought about increases in WUE and A/Ci in plants exposed to Cd + Si. The higher WUE was explained by the benefits from Si on photosynthesis, while the increase in A/Ci was due to reduction in Ci.

The presence of Si in the growth medium reduced the effects of Cd toxicity on total chlorophyll content. In treatments in which Si was present with Cd, the total chlorophyll levels were higher than in those with Cd alone (Fig. 3a). This can be explained by Si increasing the rigidity of epidermal cells and lignification. Consequently, a mechanical barrier is formed, decreasing the entry of Cd, resulting in less damage to the pigments (Pereira *et al.* 2018). Thus, Si mitigated the toxic effects of Cd on total chlorophyll, and possibly induced the formation of thylakoids, improving the efficiency of photosynthesis (Vaculík *et al.* 2015). Thylakoid formation may be a consequence of decreased soluble Cd and increased amounts of Cd bound to the leaf cell walls of plants treated with Si (Imtiaz *et al.* 2016). However, the highest values for carotenoid content were found in the control, and Si could not reverse the negative effect caused by Cd on that parameter (Fig. 3b).

The stress caused by Cd generally disturbs redox homeostasis and stimulates excessive generation of reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot -}$), and singlet oxygen ($^1O_2$) (Rahman *et al.* 2021). Plants have several antioxidant enzymes to eliminate ROS, which can cause oxidative damage. Among these enzymes, superoxide dismutase (SOD) is the first involved in the detoxification process, as it converts superoxide radicals into hydrogen peroxide (H_2O_2) at a very rapid rate (Zhang *et al.* 2020). Another antioxidant enzyme is guaiacol peroxidase (POD), which fights free radicals primarily in the cell wall (Alsherif *et al.* 2022).

There was no significant difference among treatments for SOD activity in shoots (Fig. 3c), while Cd stress resulted in increased SOD activity in roots (Fig. 3d). This may indicate that the roots most likely accumulated higher concentrations of Cd, causing greater stress. However, the addition of Si associated with Cd led to a decrease in root SOD activity (Fig. 3d), indicating reduction in the stress caused by Cd. This rapid decrease in SOD activity can be explained by the effect of Si in preventing contact between the enzyme and its phenolic substrate, or even by the removal of free monophenols as a consequence of the formation of Si-phenol complexes (Maksimovic *et al.* 2012).

A higher value for POD activity in roots was found in the treatment with the presence of Si (3.0 mM Si + 100 μM Cd) (Fig. 3f) than in the control. This suggests that the ability to detoxify ROS is being upregulated (Shi *et al.* 2018). Thus, we can conclude that the presence of Si was beneficial in reducing the effects of Cd toxicity, as there was an increase in POD activity. This suggests that Si can increase the activity of antioxidant enzymes and protect cells and tissues against oxidative damage caused by Cd stress (Pereira *et al.* 2018).

Higher values of H_2O_2 content in shoots were found in the 100 μM Cd alone, 1.5 mM Si + 100 μM Cd, and 3.0 mM Si + 100 μM Cd treatments than in the control, suggesting that Si did not prevent the formation of H_2O_2 in shoots (Fig. 4a). This increase in H_2O_2 concentration may be related to SOD and POD activity, as neither was higher in plants exposed to these treatments than in the control (Fig. 3c,e).

MDA content may indicate that exposure to Cd causes oxidative damage. Silicon has the potential to alleviate these effects by reducing MDA levels and increasing fatty acid unsaturation (Pereira *et al.* 2018). There was no significant difference between the treatments and the control for shoot MDA content. However, Si (1.5 Si + 100 Cd) reversed the harmful effect of Cd, with lower lipid peroxidation levels than in plants treated with Cd alone. This shows that Si collaborated in reducing MDA levels in this stress situation. In this context, Si may be acting more significantly in relieving Cd phytotoxicity as it promotes antioxidant activity (Dorneles *et al.* 2016). Similar results were reported by Shi *et al.* (2018), who found Si supplementation only reduced MDA levels in the presence of Cd and showed no significant difference in comparison to the control when using Si alone.

However, the highest MDA levels in roots of *E. contortisiliquum* were found for the 1.5 mM Si + 100 μM Cd treatment (Fig. 4d). This may have happened because cadmium stress resulted in damage to the cell membrane structure, and Si failed to reverse the phytotoxic effect of Cd up to the point of preventing damage to membrane lipids, as evidenced by increased levels of MDA.

The concentration of 100 μM Cd used in this experiment is equivalent to 11.24 mg L^{-1}, an amount much higher than what the National Environmental Council recommends through Resolution No. 420/2009 (Brasil 2009), which establishes values that indicate risk and contamination of soils. A soil that contains 1.3 mg Kg^{-1} Cd already offers risks of contamination, while 3 mg Kg^{-1} Cd in soil is classified as contaminated (Akbar *et al.* 2006; Brasil 2009).

In general, silicon decreased oxidative stress in *Enterolobium contortisiliquum* seedlings grown under cadmium stress. Silicon interfered with the regulation and/or functioning of POD and SOD enzymes, at times increasing or otherwise reducing activity according to the needs of homeostasis within plant cells. Consequently, this promoted greater plant vigor, causing an increase in total chlorophyll content and improvement in the photosynthetic performance of the seedlings exposed to Si. That resulted in greater biomass production, which highlights the contribution of Si within a situation of exposure to Cd, ensuring greater plant adaptability to the contaminated environment.

Silicon concentrations of 1.5 and 3.0 Si mM were adequate to mitigate the effects of cadmium toxicity on *Enterolobium contortisiliquum* seedlings. Furthermore, silicon was beneficial for photosynthetic activity and total chlorophyll content and provided relief from oxidative stress through a decrease in lipid peroxidation considering MDA levels in shoots.

The lack of significant differences in shoot and root dry weight in the Cd treatment compared to the control, as well as low contents of H_2O_2 and lipid peroxidation in roots, suggests that *E. contortisiliquum* is tolerant to Cd.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that all data are available within the manuscript.

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