Effects of foliar and root silicon application on mitigating water deficit stress in young Eucalyptus urophylla plants

Mateus Pires Barbosa
Paulo Araquém Ramos Cairo (pcairo@uol.com.br)
Universidade Estadual do Sudoeste da Bahia https://orcid.org/0000-0002-3619-7867
Raul Antonio Araújo do Bonfim
Leandro Dias da Silva
Milton Carriço Sá
Marcos Ferreira Almeida
Leonardo Santos de Oliveira
Sávio da Paz Brito
Fábio Pinto Gomes

Research Article

Keywords: Abiotic stress, water deficit, mineral nutrition, osmoregulation, antioxidant enzymes, woody plants

Posted Date: June 1st, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2993031/v1

Abstract

Background and Aims

Water restriction significantly affects the growth and survival of young plants following transplantation. Although silicon (Si) is not typically considered essential for plants, it can help mitigate abiotic stresses. We hypothesized that Si application in plants, depending on how it is applied, can improve the tolerance to water restriction. The objective was to investigate how applying Si to the leaves and roots of young *Eucalyptus urophylla* plants can mitigate water restriction effects.

Methods

A greenhouse experiment was conducted with one factor consisting of three Si application methods (via root substrate, foliar spray, and a combination of both) and a control group with no Si; the other factor involved well-watered and water-deficit irrigation regimes, based on 90% and 30% pot capacity, respectively.

Results

Silicon application improved the plant's tolerance to water restriction by maintaining stable cell turgor and increasing intrinsic water use efficiency. Silicon also helped to reduce stomatal conductance and water losses through transpiration, which prevented a decline in CO_2 assimilation and promoted osmoregulation. It also prevented a decrease in chlorophyll content and attenuated oxidative stress, by increasing superoxide dismutase and guaiacol peroxidase activity, which contributed to preventing an increase in lipid peroxidation and electrolyte leakage. The effectiveness of Si supply was higher when applied through the roots or a combination of roots and leaves compared to foliar spray alone.

Conclusion

These findings suggest that Si application can be a useful strategy for improving plant tolerance to water restriction, particularly when applied through the roots.

Introduction

In Brazil, the increasing demand for eucalyptus wood has led to the expansion of its cultivation, even in regions with scarce and irregular rainfall regimes. In many of these areas, irrigation is not a viable solution due to high costs or limited water resources. Soil water availability plays a crucial role in the success of new forest stands, especially after transplanting seedlings into the field, as water restriction during this period can significantly affect the initial growth and survival of young plants.

The effects of water deficit have been well documented (Li et al. 2020; Misra et al. 2020; Patmi et al. 2020); they mainly affect cell elongation and division, inhibiting plant growth at different stages. This growth reduction is partly due to a decrease in stomatal conductance to prevent cavitation and hydraulic

failure, leading to a sharp drop in transpiration and CO_2 assimilation (Johnson et al. 2022). Water restriction can also affect the contents of photosynthetic pigments, soluble and storage carbohydrates, proteins, and amino acids (Patmi et al. 2020), and negatively impact other photosynthetic traits, such as the quantum yield (F_v/F_0) and the maximum quantum efficiency of photosystem II photochemistry (Tripathi et al. 2015; Li et al. 2020). A decrease in CO_2 assimilation causes an accumulation of NADPH, hindering the transfer of electrons from the photosystem II reaction center (FSII) to $NADP^+$. As a result, chlorophylls reach an excited state and produce reactive oxygen species (ROS), such as superoxide, hydroxyl radicals, and hydrogen peroxide (H_2O_2), which can damage photosystems, thylakoid membranes, photosynthetic pigments, and enzymes (Salehi-Lisar and Bakhshayeshan-Agda 2016; Killi et al. 2020).

Silicon (Si), despite being a non-essential mineral for plant growth and metabolism, improves plants tolerance to water restriction, positively influencing their growth (Cao et al. 2020; Thorne et al. 2020; Vandegeer et al. 2020). However, the mechanisms by which Si reduces damage caused by water restriction remain unclear (Chen et al. 2011). Some authors have suggested that Si stimulates the antioxidant defense system (Ning et al. 2020), reducing photo-oxidative damage, maintaining the integrity of the chloroplast membrane, and improving water use efficiency, making plants more tolerant to water restriction (Cao et al. 2020). Silicon can also promote silicification, either of the cell wall in roots and stems, through self-polymerization to silica (Shakoor et al. 2014; Fleck et al. 2015; Khattab 2016) or the cuticle or stomatal pores on leaf surfaces (Khattab et al. 2016), thereby decreasing transpiration (Vandegeer et al. 2020). Furthermore, Si promotes an increase in the content of osmotically active solutes, favoring osmoregulation, which increases the volume and weight of the roots (Sonobe et al. 2010). Si is also advantageous because it is non-corrosive and non-polluting, making it a high-quality fertilizer for developing ecologically green agriculture.

Previous research has demonstred that Si is effective in reducing the negative impact of water stress on herbaceous crops, including wheat (Gong et al. 2005), potato (Crusciol et al. 2009), rice (Chen et al. 2011), and corn (Bianchini et al. 2019). However, little is known about the effects of Si on tree species, and its absorption benefits may vary depending on the application method – either by addition to the substrate for root absorption or by foliar spraying – due to diferences in uptake and ionic transport mechanisms among plant species (Farooq and Dietz 2015). In this sense, plant species can be classified as accumulators, intermediates, and non-accumulators (or excluders) based on their ability to accumulate Si in different tissues organs (Ma et al. 2016).

In this study, we hypothesized that the application of Si, depending on the method used, can positively influence stomatal control of transpiration and CO_2 assimilation, osmoregulation, and the antioxidant defense system, thereby mitigating the negative effects of water stress on plants. The main goal was to investigate the effects of different Si application methods (substrate addition and foliar spraying) on the biometric attributes, water status, gas exchange, chemical constituents, and antioxidant defense system of young *Eucalyptus urophylla* plants subjected to water restriction.

Materials and methods

Site description and experimental design

A greenhouse experiment was conducted at the State University of Southwestern Bahia, which is in Vitória da Conquista, Bahia State (BA), Brazil (14º53'08"S, 40º48'02"W, 881 m asl), from February to March 2022. The local climate is classified as *Cwb* type (dry-winter subtropical highland climate) according to the Köppen-Geiger's classification. The average annual rainfall is 733.9 mm, which concentrates from November to March. The average annual temperature is 20.2°C, with maximum and minimum values of 26.4°C and 16.1°C, respectively. During the experimental period, the average temperature and air relative humidity inside the greenhouse were 24 ± 2°C and 61.5% ± 5%, respectively.

A completely randomized design with a 4×2 factorial scheme was employed, with five replications and one plant per pot. One factor consisted of three Si application methods (via root substrate, foliar spray, and a combination of both) and a control group with no Si. The other factor was the irrigation regime, with two levels: well-watered (WW) and water-deficit (WD), based on 90% and 30% pot capacity, respectively.

Seedling management, Si pre-treatment, and transplantation

Eighty-day-old *Eucalyptus urophylla* clone AEC 144 seedlings were obtained from a nursery in Eunápolis City – BA, Brazil. These seedlings were grown in 54 cm^3 tubes filled with a substrate comprising Ceará coconut fiber (40%), carbonized rice husks (40%), and vermiculite (20%). The substrate was fertilized with 620 g of simple superphosphate and 625 g polyblend plus (N-P-K 10-15-20, with micronutrients) for each volume of 250 L.

At 110 days of age, the seedlings were transplanted into 20 L pots filled with sand passed through a 5 mm sieve. The sand was previously thoroughly washed using running water and sterilized using an HCl solution (0.4%), followed by another intensive wash to remove potential HCl residues. Sterilization aimed to eliminate residues of organic matter and minerals, including nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), and all potential sources of Si.

After transplantion, the first fertilization was performed, using a nutrient solution (Hoagland and Arnon 1952) with the NO_3^-/NH_4^+ ratio adjusted to 50/50, which is ideal for *Eucalyptus urophylla* according to Soares et al. (2021). The nutrient solution was initially supplied at 25% ionic strength, which was then increased to 50% 15 days after transplanting (DAT), to allow the seedlings adaptation to the new substrate. All plants were irrigated at 90% of pot capacity over 95 DAT. The substrate moisture was checked using the gravimetric method, according to Souza et al. (2020).

Silicon application was standardized at 50 mL of 2 mM Si plant^{-1} once a week from 80-day-old seedlings up to 95 DAT. Sifol Powder fertilizer was used as a Si source, an extremely pure nanosilica reactive source (92% SiO_2, 42.9% Si, 1-100 µm particle size, pH 6.0-7.5) with high dispersibility in water. For treatments using foliar spraying, 0.5 mL of Adesil adhesive spreader was added, and pot's surfaces were coated with a plastic film to prevent sprayed solution from running into the substrate. For treatments without Si (-Si), only water was sprayed. The two irrigation regimes were established at 95 DAT when irrigation in half of the pots was reduced to 30% of their capacity. Fifteen days after establishing the irrigation regimes (120 DAT), plant biometric attributes, plan water status, gas exchange, content of photosynthetic pigments and chemical constituents, and antioxidant enzyme activities were measured and evaluated.

Biometric attribute measurements

Plant height was measured using a graduated ruller, from the collar to the top of the stem. Stem diameter was measured using a digital caliper, 1 cm above the soil level. The total leaf area was estimated using the method outlined by Montelatto et al. (2020). The lengh of the main root was measured from the neck to the tip of the root. The total root volume was measured using a graduated cylinder. The dry masses of the root and shoot were determined after drying the plant material in an oven at 70 ± 5 ºC, until a constant mass was reached.

Determination of plant water status

Plant water status was evaluated at predawn (5:00 a.m.) on fully mature leaves situated in the middle of the shoot. Leaf water potential was determined with a pressure chamber (Model 1000, PMS) following the method of Scholander et al. (1965). Relative water content (RWC) was calculated using the equation: RWC (%) = [(FM – DM) / (TM – DM)] × 100; wherein: FM, DM, and TM represent the fresh, dry, and turgid masses, respectively (Weatherley 1950).

Leaf gas exchange measurements

Gas exchange measurements were conducted on mature leaves from the middle of the shoot; these leaves were fully expanded and physiologically active and collected between 8:00 a.m. and 10:00 a.m. Stomatal conductance (g_s), CO_2 assimilation (A), and transpiration (E) rates were determined using an Infrared Gas Analyzer (IRGA LI-6400, LI-COR™, Nebraska/USA). The leaves were exposed to an irradiance of 900 mmol photons m^{-2} s^{-1} (Silva et al. 1998), and the CO_2 concentration in the reference air supplied to the analyzer was 380 µmol mol^{-1}. The intrinsic water-use efficiency (iWUE) was calculated using the formula: iWUE = A/g_s.

Determination of the photosynthetic pigment contents

Leaf disc samples were used to extract photosynthetic pigments. The samples were immersed in 4 mL dimethyl sulfoxide (DMSO) saturated with $CaCO_3$ (Hiscox and Israelstam 1979) for 12 hours in the dark.

Chlorophylls *a* and *b* and carotenoids were quantified using a spectrophotometer, with readings taken at 665 nm, 649 nm, and 480 nm, respectively. The results were expressed as mg m^{-2} (Wellburn 1994).

Determination of chemical constituents and electrolyte leakage

Starch content in 125 mg physiologically mature and dried leaves, which were previously degreased in hexane, was determined by adding 5 mL of 0.5 M H_2SO_4 at 100°C for 1 hour. The solution was then brought to 250 mL by adding water. After cooling to 0°C, 1 mL of the solution was taken and mixed with 5 mL of 5 mM anthrone. The final solution was then heated to 100°C for 11 min, and cooled to room temperature, and measured using a spectrophotometer at 620 nm following Normative Instruction n° 20 (Brasil 1999).

Contents of soluble sugars, reducing sugars, proline, and thiobarbituric acid reactive substances (TBARS) were determined in 200 mg physiologically mature and dried leaves. For soluble and reducing sugars extraction, 15 mL of 0.1 M KH_2PO_4 buffer was used. After centrifugation was performed three times for 45 min at 2,500 *g*, the supernatant was collected. For soluble sugars determination (Yemm and Willis 1954), an aliquot of 1 mL of the supernatant was added to 2 mL anthrone solution under cooling and then heated in a water bath at 100°C for 3 min. After cooling to room temperature, readings were taken using a spectrophotometer at 620 nm, and the results were expressed as mg soluble sugars g^{-1} dry mass. For reducing sugars determination (Miller 1959), an aliquot of 0.8 mL of the supernatant was added to a reaction medium with 0.5 mL of dinitrosalicylic acid and 0.4 mL of water in a water bath at 100°C for 5 min. After cooling to room temperature, 3.5 mL of water was added to the reaction medium until reaching 5.0 mL. Readings were performed using a spectrophotometer at 540 nm, and the results were expressed as mmol reducing sugars g^{-1} dry mass.

For proline extraction, 6 mL of 3% sulfosalicylic acid (w/v) was used. After performing centrifugation for 10 min at 2,500 *g*, the supernatant was collected. For proline determination (Bates et al. 1973), an aliquot of 2 mL of the supernatant was added to 2 mL acidic ninhydrin solution (1.25 g ninhydrin, 30 mL glacial acetic acid, and 20 mL 6 M phosphoric acid) and 2 mL glacial acetic acid in a water bath at 100°C for 1 hour. After cooling using ice to stop the reaction, 4 mL toluene was added and the mixture was shaken for 20 seconds, for full extraction of proline. Readings were performed using a spectrophotometer at 520 nm, and the results were expressed as µmol proline g^{-1} dry mass.

For TBARS extraction, 2 mL 0.1% trichloroacetic acid (TCA) was used. After centrifugation for 6 min at 10,000 *g* at 4°C, an aliquot of the supernatant was collected and added to 1.5 mL 0.5% thiobarbituric acid (TBA) and 20% TCA in a water bath at 95°C for 30 min. After cooling to room temperature and centrifugation again for 6 min at 10,000 *g*, a reading was performed using a spectrophotometer at 532 nm (Heath and Paker 1968).

To determine electrolyte leakage, fresh leaves weighing 0.5 g were placed in 20 mL water at room temperature (25°C) and left for 24 hours. The initial electrical conductivity (L1) of the solution was measured using a conductivity meter (Tecnal-TEC 4MP). Afterward, the leaf samples were boiled at 100°C for 20 minutes and allowed to cool to room temperature. The final electrical conductivity (L2) was then measured, and the ratio of L1/L2 was expressed as a percentage to determine the level of electrolyte leakage (Valentovic et al. 2006).

Determination of antioxidant enzyme activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was determined by measuring the amount of enzyme required to cause 50% inhibition of the rate of nitro blue tetrazolium (NBT) reduction in the leaf tissue extract, according to Beauchamp and Fridovich (1971). In a reaction medium containing 50 mM NaH_2PO_4 buffer (pH 7.8), 0.1 mM EDTA, 13 mM L-methionine, and 75 µM NBT, a 100-µL aliquot of the enzymatic extract was added to initiate the reaction in the dark. The reaction was started by adding 2 µM riboflavin and exposing the reaction medium to 15 W fluorescent lamps for 15 min. SOD activity was read at 560 nm using a spectrophotometer and expressed as activity unit (AU) mg^{-1} protein.

Guaiacol peroxidase (GPX; EC 1.11.1.7) activity was determined by monitoring the kinetic evolution of the reaction in a reaction medium containing 50 mM KH_2PO_4 buffer (pH 7), 9 mM guaiacol, and 19 mM H_2O_2 according to Lin and Kao (1999). The kinetic evolution was measured for 1 min. GPX activity was calculated using the extinction coefficient (26.6 mM cm^{-1} at 470 nm). One unit of GPX was defined as the amount of enzyme required to form tetraguaiacol (1 mM) per minute at 470 nm.

Statistical analysis

The data from five separate replications were presented as mean ± standard deviation (SD). The Cochran test was used to evaluated data homogeneity, while the Lilliefors test was applied to verify the normal distribution of residuals. All data were analyzed using analysis of variance (ANOVA), and multiple comparisons of means were conducted using Tukey's test ($p < 0.05$). All statistical analysis were performed using the R software.

Results

Biometric attributes

The results showed a significant interaction between Si application and irrigation regime on stem diameter and root dry mass. The application of Si influenced total leaf area and shoot dry mass, irrespective of the irrigation regime, while the irrigation regime influenced root length and root volume irrespective of Si application. However, there was no significant effect of the factors on plant height. In plants under water deficit conditions, a smaller stem diameter was observed, especially in the absence of Si application. However, the effect of water restriction was mitigated by Si applications [(+) Si R] and [(+) Si L + R], which also allowed for a greater root dry mass accumulation under water deficit conditions.

Among the treatments with Si application, a smaller total leaf area was observed with the application [(+) Si R], while all methods of Si application allowed for a greater shoot dry mass. Lower root lenght and root volume values were observed under water deficit conditions; however, this effect was mitigated in root volume with the Si applications (Table 1).

Table 1
Plant height (H), stem diameter (D), total leaf area (LA), root length (RL), root volume (RV), root dry mass (RDM), and shoot dry mass (SDM) of young *Eucalyptus urophylla* plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in response to different Si application methods.

Biometric	**WW plants**				**WD plants**			
Attribute	(-) Si	(+) Si L	(+) Si R	(+) Si L + R	(-) Si	(+) Si L	(+) Si R	(+) Si L + R
H (cm)	5.7Aa	6.2 Aa	5.5 Aa	6.0 Aa	5.7 Aa	6.1 Aa	5.5 Aa	5.8 Aa
D (mm)	46.1 Aa	45.8 Aa	38.7 Bb	43.7 Aa	42.2 Ab	41.3 Ab	41.8 Aa	43.7 Aa
LA (cm^2)	779 ABa	814 Aa	724 Ba	828 Aa	750 ABa	814 Aa	693 Ba	869 Aa
RL (cm)	49.0 Aa	51.1 Aa	50.9 Aa	64.0 Aa	43.2 Ab	42.7 Ab	45.0 Ab	48.3 Ab
RV (mL)	54 Aa	52 Aa	46 Aa	48 Aa	42 Aa	48 Aa	46 Aa	44 Aa
RDM (g)	7.5 Ba	9.7 Aa	9.0 Aa	8.8 Aa	6.7 Ba	9.2 Aa	8.9 Aa	9.1 Aa
SDM (g)	6.5 Aa	6.3 Aba	6.5 ABb	5.1 Bb	6.4 Ba	6.9 Ba	8.8 Aa	7.5 ABa

Uppercase letters compare the control with Si application methods in each irrigation regime, while lowercase letters compare irrigation regimes in the control or in each Si application method ($n = 5$) using Tukey's test ($p < 0.05$).

Plant water status

The irrigation regime had a significant impact on leaf water potential, which was reduced in plants subjected to water deficit; however, the application of Si did nor affect leaf water potential. On the other hand, there was a significant interaction between the two factors for relative water content. Control plants (–S) subjected to water deficit showed a decrease in relative water content. In contrast, in treatments involving Si application, the negative effect of water restriction was attenuated with [(+) Si L], while relative water content remained stable with [(+) Si R] and [(+) Si L + R] (Fig. 1).

Leaf gas exchange

There was a notable interaction of factors on stomatal conductance, CO_2 assimilation, and intrinsic water-use efficiency, while transpiration was influenced by each fator separately without any interaction. In plants subjected to water deficit, a reduction was observed in stomatal conductance, CO_2 assimilation, and transpiration was observed. However, the negative effect on CO_2 assimilation was ameliorated by Si application, especially with [(+) Si R], and to a lesser extent with [(+) Si L] and [(+) Si L + R]. In contrast, the reduction in transpiration was more pronounced with [(+) Si R] application. Water restriction did not have a significant effect on intrinsic water-use efficiency, but Si application increased it, primarly with [(+) Si R], and to a lesser extent with [(+) Si L] and [(+) Si L + R] (Fig. 2).

Photosynthetic pigments

A significant interaction of factors was observed in chlorophyll content. In plants subjected to water deficit, a decline in chlorophyll content was observed in control plants, but the application of [(+) Si L] helped mitigate this reduction. Furthermore, the use of [(+) Si R] and [(+) Si L + R] prevented the decrease in chlorophyll content altogether. The irrigation regime did not affect the carotenoid content, but it was noted that the application of Si increased carotenoid content compared to the control, regardless of the irrigation regime (Fig. 3).

Chemical constituents

A significant interaction of factors was noted in contents of starch, soluble sugars, reducing sugars, and proline. In plants subjected to water deficit, there was a reduction in starch content observed in both control and Si-treated plants. The application of [(+) Si R] or [(+) Si L + R] resulted in a more significant increase in starch content, while [(+) Si L] resulted in a lesser increase. The content of both soluble and reducing sugars increased in control and Si-treated plants, but the levels were higher with Si application than in the control. The proline content increase only with the application of Si, particularly using [(+) Si R] (Fig. 4).

Oxidative stress-related characteristics

A significant interaction of factors was observed in TBARS content, electrolyte leakage, SOD, and GPX activities. In plants subjected to water deficit, an increase in TBARS content was noted. However, this increase was attenuated by the application of [(+) Si L] and [(+) Si L + R], and reversed by [(+) Si R] application. The activities of SOD and GPX were not significantly influenced by water restriction, except when using [(+) Si R] application, which resulted in a significant increase in the activity of both enzymes (Fig. 5).

Discussion

Environmental stresses can significantly impact plant growth in both natural and agricultural ecosystems. The majority of the Earth's surface (96,5%) is subject to environmental limitations, as noted

by Cramer et al. (2011). Since plants are sessile organisms, they are constantly exposed to abiotic stresses, such as water restriction, which can affect their development and metabolism. Unlike anual plants, woody plants generally lack the phenological plasticity to escape drought by interrupting their vegetative life cycle. Adult trees typically possess a higher level of tolerance to water restriction than annual plants (Pallardy 2008). Therefore, in eucalyptus, the symptoms of water deficit are more noticeable in young plants, after transplanting seedlings, than in adult plants.

Stem diameter and root dry mass were the only biometric attributes significantly affected by the interaction of the water regime and Si application method. These results suggest that a 15-day period of water restriction may not have been long enough to cause widespread damage to most biometric attributes. This finding supports the understanding that the plant's phenological response to water restriction is depend on the duration of the stress (Pugnaire et al. 1999) and varies across different plant species (Kovács et al. 2022). Despite this, the application of Si in plants subjected to water deficit resulted in higher shoot and root dry masses, particularly with [(+) Si R] and [(+) Si L + R] applications.

Although the impact of water restriction on biometric attributes was moderate, significant changes were observed in other growth-related traits (e.g. relative water content, gas exchange, photosynthetic pigments, chemical constituents, and oxidative stress). In plants subjected to water deficit, there was a reduction in relative water content observed in control plants (-S), but Si application proved to be beneficial in terms of enhancing plant tolerance to water restriction. Applying Si using [(+) Si R] or [(+) Si L + R] prevented the decrease in relative water content, while the use of [(+) Si L] attenuated this decrease (Fig. 1B). This effect of Si application on relative water content can be partially attributed to a stomatal control of water loss by transpiration, as it is closely related to reductions in stomatal conductance and transpiration. According to Pimentel (2004), stomatal control is a foliar mechanism that tends to favor a plant exposed to short-term water restriction, as observed in this study. Stomatal closure is often the first line of defense against desiccation, even before a decrease in leaf RWC occurs (Lang et al. 2018). In contrast, Johnson et al. (2022) argued that a stomatal closure primarily aims to prevent cavitation and a catastrophic hydraulic failure that could result in significant drops in transpiration and CO_2 assimilation.

A significant decrease in stomatal conductance was observed in our study, irrespective of the Si application method (Fig. 2A). However, the use of Si, particularly [(+) Si R], resulted in lower stomatal conductance rates, with [(+) Si L] and [(+) Si L + R] having a lesser effect. Such a reduction in stomatal conductance may be partly attributed to a Si deposition in the cell walls of guard cells, which can lead to structural changes that indirectly affect stomatal deformation and opening capacity (Vandegeer et al. 2020). Additionally, Si application can cause a decrease in guard cell turgor and stomatal pore regulation, contributing to a reduction in stomatal conductance (Gao et al. 2006; Luyckx et al. 2017).

The lower stomatal conductance rates contributed to a decrease in transpiration, which was more significant with the Si application, particularly with [(+) Si R], and to a lesser extent with [(+) Si L] and [(+) Si L + R] (Fig. 2B). Water loss during transpiration is known to reach about 90%, which can be either stomatal or cuticular (Domingues et al. 2017). The effect of Si application can be partly attributed to a

thickening of the cuticle layer, which results from H_4SiO_4 polymerization in phytoliths, an amorphous, hydrated silica ($SiO_2 \times nH_2O$) (Keutmann et al. 2015). Phytoliths are deposited on cell and intercellular walls or below the cuticle as amorphous silica (Mandlik et al. 2020; Singh et al. 2020), forming a silica double layer (Thorne et al. 2020), which reduces leaf water loss (Bukhari et al. 2021).

In this study, the Si-induced reduction in stomatal conductance caused a decrease in leaf water loss that was more significant than the CO_2 influx into the chloroplasts, resulting in a greater reduction in transpiration than CO_2 assimilation. The lower rates of stomatal conductance also led to a reduction in CO_2 assimilation, but the Si application mitigated this reduction, mainly with [(+) Si R], maintaining higher rates of CO_2 assimilation than the control treatment (–S) (Fig. 2C). Similar results were observed in *Pistacia vera* L. (Habibi and Hajiboland, 2013) and rice (Kuhla et al. 2021) with Si application under water restriction. In previous studies on strawberries under water restriction, more than 90% of the Si absorbed by the roots was translocated to the plant shoot when Si was supplied via the substrate (Ma and Takahashi 2002).

In plants that accumulate large amounts of Si, supply must be made mainly through the roots using a nutrient solution (Ma and Takahashi 1990) or via soil fertilization (Camargo et al. 2019). After absorption by roots, Si is transported to the plant shoot, using specialized transporters in cell membranes (Mitani et al. 2009). In sugarcane, a tipically accumulating species, Si supply via substrate provided more effective physiological responses to mitigate water stress than supply via spraying because Si was better absorbed by the roots than by the leaves (Teixeira et al. 2020).

According to Ma and Yamaji (2015) and Queiroz et al. (2018), eucalyptus is a non-Si accumulating species; for this reason, we chose to use the commercial fertilizer Sifol Powder as a source of Si in the presente study. Sifol Powder is an extremely pure reactive source of nanossílica that is more effective when applied via the roots than by foliar spray (Suriyaprabha et al. 2014). Although the mechanism of nanosilica root absorption is not well understood, it occurs at a higher rate than that of other silicates (Schaller et al. 2013; Asgari et al. 2018), mainly following the apoplastic route as Si transporters are less sensitive to nanosilica (Nazaralian et al. 2017). In the present study, Si application using [(+) Si L] and [(+) Si L + R] also proved effective in mitigating, to a lesser extent, the decrease in CO_2 assimilation in plants subjected to water deficit. Although the mechanism of foliar Si uptake is unclear, leaves can absorb Si, but the effect of Si on plants subjected to short-term water restriction may depend less on the amount of Si accumulated due to a the restriction in its foliar absorption compared to root absorption (Teixeira et al. 2021). Some researchers have suggested that leaves can directly also absorb silicic acid and/or stimulate plants to absorb more nutrients, including Si, from the soil (Zhu et al. 2019).

In this study, Si application may have caused a cuticle thickening due to silica deposits, leading to changes in the physical state and mechanical properties of cell walls. This solid barrier may have contributed to reducing water losses (Gao et al. 2005; Bukhari et al. 2020; Wang et al. 2021) and improving intrinsic water-use efficiency, which was higher with [(+) Si R] (Fig. 2D). Similar results were found with Si application in other species (Ming et al. 2012; Asmar et al. 2013; Habibi 2014). Silicon

deposition on xylem vessel walls has also been observed in many plants, with Si nanoparticles intertwining with organic macromolecules such as cellulose, pectin, glycoprotein, and lignin, to form amorphous colloidal complexes with large surface areas of absorption (Parry and Winslow 1977). These nanoparticles could influence the transport rate of water and solutes in vascular bundles, improving intrinsic water-use efficiency (Gao et al. 2005). Therefore, Si application mitigated the decrease in CO_2 assimilation in plants subjected to water deficit, despite reductions in stomatal conductance and transpiration, contributing to an increase in intrinsic water-use efficiency and improving plant tolerance to water restriction (Ma et al. 2004).

In contrast, the effect of Si application in preventing or mitigating relative water content reduction occurred simultaneously with a decrease in leaf water potential (Fig. 1A). Similar observations on Si application under water restriction were also reported in *Sorghum bicolor* (Sonobe et al. 2010), olive trees (Karimi et al. 2018), and plums (Hassan et al. 2021). Thiese results suggest that the Si-induced regulation of relative water content associated with leaf water potential decrease might be caused by osmoregulation, resulting from the hydrolysis of starch into sugars and proteins into amino acids. In this study, the Si application intensified starch hydrolysis (Fig. 4A), along with an increase in contents of soluble sugars (Fig. 4B) and reducing sugars (Fig. 4C), promoting ionic homeostasis that favors water absorption and improving the antioxidante system (Zhang et al. 2018). Si-induced changes in soluble sugars content, associated with water restriction, were also observed in *Sorghum bicolor* (Sonobe et al. 2010) and *Glycyrrhiza uralensis* (Zhang et al. 2018). These changes can promote protoplasmic tolerance and osmotic adjustment, where osmolyte accumulation reduces the osmotic potential and increases water influx, thus maintaining turgor pressure (Farroq et al. 2019). The osmotic adjustment may also result from a Si-induced increase in proline content, mainly using [(+) Si R] (Fig. 5D). Similar results using Si application under water restriction were found in orange (El Sayed et al. 2014) and mango (Elsheery et al. 2020). Proline, in addition to acting as an osmoprotectant, plays a crucial role in the defense mechanism of plants, promoting anatomical changes, protein stability, and elimination of free radicals that can damage the photosynthetic apparatus (Ghafoor et al. 2019).

In plants subjected to water deficit, chlorophyll content decreased in control plants (-S), but Si had a beneficial effect on plant tolerance to this water restriction effect. Si application using [(+) Si R] or [(+) Si L + R] prevented the decrease in chlorophyll content while using [(+) Si L], this decrease was attenuated (Fig. 3A). Si application can keep chlorophyll content stable under water restriction by protecting chloroplast structure (preventing the degradation of grana and stroma lamellae) and inducing photosynthetic pigment biosynthesis to protect chloroplast enzymes (Cao et al. 2015). Similar results have been reported in other studies using Si application under water restriction (Muneer et al. 2014; Cao et al. 2015; Hajiboland et al. 2017; Sienkiewicz-Cholewa et al. 2018; Hassan et al. 2022). Carotenoid content was not influenced by the irrigation regime, but was higher with Si application compared to the control treatment (-Si) (Fig. 3B). Carotenoids, apart from being pigments that help capture light for photosynthesis, also serve as photoprotectors by rapidly quenching the excited states of chlorophylls (Taiz et al. 2017) and thus preventing the formation of damaging singlet oxygen ($^1O_2^*$), which may

damage many cellular components, especially lipids (Uarrota et al. 2018). Although Si application was unlikely to be related to carotenoid photoprotection in the present study, its beneficial effect on maintaining stable chlorophyll content in plants subjected to water deficit was likely due to its influence on increasing antioxidant enzyme activity and mitigating oxidative stress.

Control plants (-Si) under water deficit showed an increase in TBARS, indicating a rise in membrane lipid peroxidation and electrolyte leakage. This happens due to water deficiency stress, which causes an increase in levels of cell membrane lipid oxidative stress indicators, such as malondialdehyde and H_2O_2, increasing electrolyte leakage (Ogbaga et al. 2020). The Si application using [(+) SI L + R] prevented an increase in TBARS, whereas using [(+) Si L] or [(+) Si R] mitigated this increase (Fig. 5A). Overall, unlike control plants (-Si), Si supply preserved membrane and electrolyte leakage (Fig. 5B). SOD and GPX activities were not influenced by the water regime, but Si supply using [(+) Si R] resulted in a significant increase in plants subjected to water deficit (117.5% and 184.5%, respectively) (Figs. 5C and 5D). This helps prevent lipid peroxidation and electrolyte leakage, thus mitigating oxidative stress. Previous reports have supported this effect of Si in increasing antioxidant enzyme activity in other species (Gong et al. 2005; Gunes et al. 2007; Shi et al. 2014). Although the mechanisms by which Si influences the activation of antioxidant enzymes are not yet clear, studies have suggested that it regulates genes (e.g., TaSOD and TaCAT) that synthesize and activate those enzymes (Ma et al. 2016; Mostofa et al. 2021).

Conclusion

In summary, young *Eucalyptus urophylla* plants have improved tolerance to water restriction with Si application, as this element helps to maintain stable cell turgor and improves intrinsic water-use efficiency. Silicon reduces stomatal conductance and transpiration, preventing a decline mitigating a decline in CO_2 assimilation and promoting osmoregulation. Moreover, Si prevents a decrease in chlorophyll content and attenuates oxidative stress by inducing an increase in SOD and GPX activities, thus preventing lipid peroxidation and electrolyte leakage.

Silicon application through the roots, or combined with leaf spray, is more effective in mitigating the effects of water restriction than using only foliar spray. As eucalyptus is a non-Si accumulating species, nanossílica becomes an efficient source of Si, with benefits optimized when applied mainly through the roots.

Declarations

Acknowledgments The authors wish to express their sincere thanks to the following Brazilian research support institutions for providing salaries and research grants: Universidade Estadual do Sudoeste da Bahia (UESB), Universidade Estadual de Santa Cruz (UESC), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB).

M.P.B.: conceptualization, plant cultivation and data collection, laboratory chemical analysis, data analysis and interpretation, writing – review & editing; P.A.R.C.: conceptualization, data analyses and interpretation, writing – review & editing; R.A.A.B.: plant cultivation and data collection, laboratory chemical analysis; L.D.S.: plant cultivation and data collection, laboratory chemical analysis, data analysis and interpretation; M.C..S.: plant cultivation and data collection, laboratory chemical analysis; M.F.A.: plant cultivation and data collection, laboratory chemical analysis; L.S.O.: plant cultivation and data collection, laboratory chemical analysis; S.P.B.: plant cultivation and data collection, laboratory chemical analysis.

Data availability Data will be made available on request.

Code availability Not applicable.

Ethics approval Not applicable.

Consent to participate The authors declare consent to participate.

Consent for publication The authors declare consent for publication.

Conflict of interest The authors declare no competing interests.

References

1. Asgari F, Majd A, Jonoubi P, Najafi F (2018) Effects of silicon nanoparticles on molecular, chemical, structural and ultrastructural characteristics of oat (*Avena sativa* L). Plant Physiol Biochem 127:152–160. https://doi.org/10.1016/j.plaphy.2018.03.021
2. Asmar SA, Castro EM, Pasqual M, Pereira FJ, Soares JDR (2013) Changes in leaf anatomy and photosynthesis of micropropagated banana plantlets under different silicon sources. Sci Hortic 161:328–332. https://doi.org/10.1016/j.scienta.2013.07.021
3. Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060
4. Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem 44:276–287. https://doi.org/10.1016/0003-2697(71)90370-8
5. Bianchini HC, Marques DJ (2019) Tolerância ao estresse hídrico em cultivares de milho adubadas com silício: absorção e eficiência no uso da água. Biosci J 35:527–539. http://dx.doi.org/10.14393/BJ-v35n2a2019-42347
6. Brasil (1999) Instrução Normativa nº 20, de 21 de jul. de 1999. Oficializa os métodos analíticos físicoquímicos, para controle de produtos cárneos e seus ingredientes – sal e salmoura. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária. Diário Oficial da União. Brasília, DF, 27 jul. 1999

7. Bukhari MA, Ahmad Z, Ashraf MY, Afzal M, Nawaz F, Nafees M, Jatoi WN, Malghani NA, Shah AN, Manan A (2021) Silicon mitigates drought stress in wheat (*Triticum aestivum* L.) through improving photosynthetic pigments, biochemical and yield characters. Silicon 13:4757–4772. https://doi.org/10.1007/s12633-020-00797-4
8. Camargo MS, Bezerra BKL, Holland LA, Oliveira AL, Vitti AC, Silva MA (2019) Silicon fertilization improves physiological responses in sugarcane cultivars grown under water deficit. J Soil Sci Plant Nutr 19:81–91. https://doi.org/10.1007/s42729-019-0012-1
9. Cao BL, Ma Q, Xu K (2020) Silicon restrains drought-induced ROS accumulation by promoting energy dissipation in leaves of tomato. Protoplasma 257:537–547. https://doi.org/10.1007/s00709-019-01449-0
10. Cao BL, Ma Q, Zhao Q, Wang L, Xu K (2015) Effects of silicon on absorbed light allocation, antioxidant enzymes and ultrastructure of chloroplasts in tomato leaves under simulated drought stress. Sci Hortic 194:53–62. https://doi.org/10.1016/j.scienta.2015.07.037
11. Chen W, Yao X, Cai K, Chen J (2011) Silicon alleviates drought stress of rice plants by improving plant water status, photosynthesis and mineral nutrition absorption. Biol Trace Elem Res 142:67–76. https://doi.org/10.1007/s12011-010-8742-x
12. Cramer GR, Urano K, Delrot S, Pezzotti M, Shinozaki K (2011) Effects of abiotic stress on plants: a systems biology perspective. BMC Plant Biol 11:163. https://doi.org/10.1186/1471-2229-11-163
13. Crusciol CAC, Pulz AL, Lemos LB, Soratto RP, Lima GPP (2009) Effects of silicon and drought stress on tuber yield and leaf biochemical characteristics in potato. Crop Sci 49:949–959. https://doi.org/10.2135/cropsci2008.04.0233
14. Domínguez E, Heredia-Guerrero JA, Heredia A (2017) The plant cuticle: old challenges, new perspectives. J Exp Bot 68:5251–5255. https://doi.org/10.1093/jxb/erx389
15. El Sayed OM, El-Gammal OHM, Salama ASM (2014) Effect of proline and tryptophan amino acids on yield and fruit quality of Manfalouty pomegranate variety. Sci Hortic 169:1–5. https://doi.org/10.1016/j.scienta.2014.01.023
16. Elsheery NI, Helaly MN, El-Hoseiny HM, Alam-Eldein SM (2020) Silicon improves seed germination and alleviates oxidative stress of bud seedlings in tomato under water deficit stress. Plant Physiol Biochem 10:558. https://doi.org/10.3390/agronomy10040558
17. Farooq MA, Dietz K (2015) Silicon as versatile player in plant and human biology: overlooked and poorly understood. Front Plant Sci 6:994–1008. https://doi.org/10.3389/fpls.2015.00994
18. Farooq M, Hussain M, Ul-Allah S, Siddique KHM (2019) Physiological and agronomic approaches for improving water-use efficiency in crop plants. Agric Water Manag 219:95–108. https://doi.org/10.1016/j.agwat.2019.04.010
19. Fleck AT, Schulze S, Hinrichs M, Specht A, Waßmann F, Schreiber L, Schenk MK (2015) Silicon promotes exodermal casparian band formation in si-accumulating and si-excluding species by forming phenol complexes. PLoS ONE 10:1–10. https://doi.org/10.1371/journal.pone.0138555

20. Gao X, Zou C, Wang L, Zhang F (2006) Silicon decreases transpiration rate and conductance from stomata of maize plants. J Plant Nutr 29:1637–1647. https://doi.org/10.1080/01904160600851494
21. Gao X, Zou C, Wang L, Zhang F (2005) Silicon improves water use efficiency in maize plants. J Plant Nutr 27:1457–1470. https://doi.org/10.1081/PLN-200025865
22. Ghafoor R, Akram NA, Rashid M, Ashraf M, Iqbal M, Lixin Z (2019) Exogenously applied proline induced changes in key anatomical features and physio-biochemical attributes in water stressed oat (*Avena sativa* L.) plants. Physiol Mol Biol Plants 25:1121–1135. https://doi.org/10.1007/s12298-019-00683-3
23. Gong H, Zhu X, Chen K, Wang S, Zhang C (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. Plant Sci 169:313–321. https://doi.org/10.1016/j.plantsci.2005.02.023
24. Gunes A, Inal A, Bagci EG, Pilbeam DJ (2007) Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. Plant Soil 290:103–114. https://doi.org/10.1007/s11104-006-9137-9
25. Habibi G, Hajiboland R (2013) Alleviation of drought stress by silicon supplementation in pistachio (*Pistacia vera* L.) plants. Folia Hortic 25:25–29. https://doi.org/10.2478/fhort-2013-0003
26. Habibi G (2014) Silicon supplementation improves drought tolerance in canola plants. Russ J Plant Physiol 61:784–791. https://doi.org/10.1134/S1021443714060077
27. Hajiboland R, Cheraghvareh L, Poschenrieder C (2017) Improvement of drought tolerance in tobacco (*Nicotiana rustica* L.) plants by silicon. J Plant Nutr 40:1661–1676. http://doi:10.1080/01904167.2017.1310887
28. Hassan IF, Gaballah MS, El-Hoseiny HM, El-Sharnouby ME, Alam-Eldein SM (2021) Deficit irrigation to enhance fruit quality of the 'African Rose' plum under the egyptian semi-arid conditions. Agron 11:1405. https://doi.org/10.3390/agronomy11071405
29. Heath R, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1
30. Hiscox JD, Israelstam GF (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot 57:1332–1334. https://doi.org/10.1139/b79-163
31. Hoagland DR, Arnon DI (1952) The water culture method for growing plans without soil. University of California / College of Agriculture, Berkley
32. Johnson DM, Katul G, Domec J-C (2022) Catastrophic hydraulic failure and tipping points in plants. Plant Cell Environ 45:2231–2266. https://doi.org/10.1111/pce.14327
33. Karimi S, Rahemi M, Rostami AA, Sedaghat S (2018) Drought effects on growth, water content and osmoprotectants in four olive cultivars with different drought tolerance. Int J Fruit Sci 18:254–267. https://doi.org/10.1080/15538362.2018.1438328
34. Keutmann I, Melzer B, Seidel R, Thomann R, Speck T (2015) Review: The functions of phytoliths in land plants. In: Hamm C (ed) Evolution of lightweight structures: analyses and technical applications. Springer, Dordrecht

35. Khattab H (2016) Roles of silicon in improving drought tolerance in plants. In: Tripathi DK, Singh VP, Ahmad P, Chauhan DK, Prasad SM (eds) Silicon in plants: advances and future prospects. CRC Press, Boca Raton
36. Killia D, Raschi A, Bussotti F (2020) Lipid peroxidation and chlorophyll fluorescence of photosystem II performance during drought and heat stress is associated with the antioxidant capacities of C3 sunflower and C4 maize varieties. Int J Mol Sci 21:4846. https://doi.org/10.3390/ijms21144846
37. Kovács S, Kutasy E, Csajbók J (2022) The multiple role of silicon nutrition in alleviating environmental stresses in sustainable crop production. Plants 11:9. https://doi.org/10.3390/plants11091223
38. Kuhla J, Pausch J, Schaller J (2021) Effect on soil water availability, rather than silicon uptake by plants, explains the beneficial effect of silicon on rice during drought. Plant Cell Environ 44:3336–3346. https://doi.org/10.1111/pce.14155
39. Lang Y, Wang M, Xia J, Zhao Q (2018) Effects of soil drought stress on photosynthetic gas exchange traits and chlorophyll fluorescence in *Forsythia suspensa*. J For Res 29:45–53. https://doi.org/10.1007/s11676-017-0420-9
40. Li W, Yajun W, Yubao Z, Ruoyu W, Zhongkui X (2020) Impacts of drought stress on the morphology, physiology, and sugar content of Lanzhou lily (*Lilium davidii* var. Unicolor). Acta Physiol Plant 42:127. http://dx.doi.org/10.1007/s11738-020-03115-y
41. Lin CC, Kao CH (1999) NaCl induced changes in ionically bound peroxidase activity in roots of rice seedlings. Plant Soil 216:147–153. https://doi.org/10.1023/A:1004714506156
42. Luyckx M, Hausman JF, Lutts S, Guerriero G (2017) Silicon and plants: current knowledge and technological perspectives. Front Plant Sci 8:411. https://doi.org/10.3389/fpls.2017.00411
43. Ma CC, Gao YB, Guo HY, Wang JL (2004) Photosynthesis, transpiration, and water use efficiency of *Caragana microphylla*, *C. intermedia*, and *C. korshinskii*. Photosynthetica 42: 65–70. https://doi.org/10.1023/B:PHOT.0000040571.63254.c2
44. Ma D, Sun D, Wang C, Qin H, Ding H, Li Y, Guo T (2016) Silicon application alleviates drought stress in wheat through transcriptional regulation of multiple antioxidant defense pathways. J Plant Growth Regul 35:1–10. https://doi.org/10.1007/s00344-015-9500-2
45. Ma JF, Takahashi E (1990) Effect of silicon on the growth and phosphorus uptake of rice. Plant Soil 126:115–119. https://doi.org/10.1007/BF00041376
46. Ma JF, Takahashi E (2002) Soil, fertilizer, and plant silicon research in Japan. Elsevier, Amsterdam
47. Ma JF, Yamaji N (2015) A cooperative system of silicon transport in plants. Trends Plant Sci 20:435–442. https://doi.org/10.1016/j.tplants.2015.04.007
48. Mandlik M, Thakral V, Raturi G, Shinde S, Nikolić M, Tripathi DK, Sonah H, Deshmukh R (2020) Significance of silicon uptake, transport, and deposition in plants. J Exp Bot 79:6703–6718. https://doi.org/10.1093/jxb/eraa301
49. Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428. https://doi.org/10.1021/ac60147a030

50. Ming DF, Pei ZF, Naeem MS, Gong HJ, Zhou WJ (2012) Silicon alleviates PEG-induced water-deficit stress in upland rice seedlings by enhancing osmotic adjustment. J Agron Crop Sci 198:14–26. https://doi.org/10.1111/j.1439-037X.2011.00486.x
51. Misra V, Solomon S, Mall AK, Prajapati CP, Hashem A, Abd Allah EF, Ansari MI (2020) Morphological assessment of water stressed sugarcane: A comparison of waterlogged and drought affected crop. Saudi J Biol Sci 27:1228–1236. http://doi.org/10.1016/j.sjbs.2020.02.007
52. Mitani N, Yamaji N, Ma JF (2009) Identification of maize silicon influx transporters. Plant Cell Physiol 50:5–12. https://doi.org/10.1093/pcp/pcn110
53. Mostofa MG, Rahman MM, Ansary MMU, Keya SS, Abdelrahman M, Miah MG, Tran L-SP (2021) Silicon in mitigation of abiotic stress-induced oxidative damage in plants. Crit Rev Biotechnol 41:918–934. https://doi.org/10.1080/07388551.2021.1892582
54. Nazaralian S, Majd A, Irian S, Najafi F, Ghahremaninejad F, Landberg T, Greger M (2017) Comparison of silicon nanoparticles and silicate treatments in fenugreek. Plant Physiol Biochem 115:25–33. https://doi.org/10.1016/j.plaphy.2017.03.009
55. Ning D, Qin A, Liu Z, Duan A, Xiao J, Zhang J, Liu Z, Zhao B, Liu Z (2020) Silicon-mediated physiological and agronomic responses of maize to drought stress imposed at the vegetative and reproductive stages. Agron 10:1136–1155. https://doi.org/10.3390/agronomy10081136
56. Ogbagaa CC, Athar H, Bano H, Chater CCC, Jellason NP (2020) Clarity on frequently asked questions about drought measurements in plant physiology. Sci Afr 8:e00405. https://doi.org/10.1016/j.sciaf.2020.e00405
57. Osakabe Y, Osakabe K, Shinozaki K, Tran LP (2014) Response of plants to water stress. Front Plant Sci 5:86–93. https://doi.org/10.3389/fpls.2014.00086
58. Pallardy SG (2008) Physiology of woody plants, 3rd edn. Elsevier, New York
59. Parry DW, Winslow A (1977) Electron-probe microanalysis of silicon accumulation in the leaves and tendrils of *Pisum sativum* (L.) following root severance. Ann Bot 41:275–278. https://doi.org/www.jstor.org/stable/42770148
60. Patmi YS, Pitoyo A, Solichatun S (2020) Effect of drought stress on morphological, anatomical, and physiological characteristics of Cempo Ireng cultivar mutant rice (*Oryza sativa* L.) strain 51 irradiated by gamma-ray. J Phys: Conference Series 1436: 12–19. http://dx.doi.org/10.1088/1742-6596/1436/1/012015
61. Pimentel C (2004) A relação da planta com a água. Editora Universidade Federal Rural do Rio de Janeiro, Rio de Janeiro
62. Pugnaire FI, Serrano L, Pardos J (1999) Constraints by water stress on plant growth. In: Pessarakli M (ed) Handbook of plant and crop stress, 2nd edn. Marcel Dekker, Inc., New York
63. Queiroz DL, Camargo JMM, Dedecek RA, Oliveira EB, Zanol KMR, Melido RCN (2018) Absorção e translocação de silício em mudas de *Eucalyptus camaldulensis*. Cienc Florest 28:632–640. https://doi.org/10.5902/1980509832053

64. Salehi-Lisar SY, Bakhshayeshan-Agdam H (2016) Drought stress in plants: causes, consequences, and and tolerance. In: Hossain MA, Wani SH, Bhattacharjee S, Burritt DJ, Tran LSP (eds) Drought tress tolerance in plants. Springer, Switzerland
65. Schaller J, Brackhage C, Paasch S, Brunner E, Bäucker E, Dudel EG (2013) Silica uptake from nanoparticles and silica condensation state in different tissues of *Phragmites australis*. Sci Total Environ 442:6–9. https://doi.org/10.1016/j.scitotenv.2012.10.016
66. Scholander PF, Bradsterret ED, Hemmingsen EA, Hammel HT (1965) Sap pressure in vascular plants. Science 148:339–346. https://doi.org/10.1126/science.148.3668.339
67. Shakoor SA (2014) Silicon biomineralisation in plants: a tool to adapt global climate change. J Res Biol Sci 1:1–3
68. Shi Y, Zhang Y, Yao H, Wu J, Sun H, Gong H (2014) Silicon improves seed germination and alleviates oxidative stress of bud seedlings in tomato under water deficit stress. Plant Physiol Biochem 78:27–36. https://doi.org/10.1016/j.plaphy.2014.02.009
69. Sienkiewicz-Cholewa U, Sumisławska J, Sacała E, Dziągwa-Becker M, Kieloch R (2018) Influence of silicon on spring wheat seedlings under salt stress. Acta Physiol Plant 40:1–8. https://doi.org/10.1007/s11738-018-2630-y
70. Silva W, Sediyama T, Silva AA, Ferreira FA (1998) Condutância estomática de *Eucalyptus citriodora* e *E. grandis*, em resposta a diferentes níveis de água no solo e de convivência com *Brachiaria brizantha* Stapf. Bragantia 57:339–347. https://doi.org/10.1590/S0006-87051998000200015
71. Singh A, Kumara A, Hartley S, Singh IK (2020) Silicon: its ameliorative effect on plant defense against herbivory. J Exp Bot 71:6730–6743. https://doi.org/10.1093/jxb/eraa300
72. Soares PPS, Cairo PAR, Silva LD, Matsumoto SN, Bonfim RAA, Barbosa MP, Sá MC, Almeida MF, Mesquita NLS (2022) Mitigating water stress by increasing NO_3^-: NH_4^+ ratio in young *Eucalyptus urophylla* plants. Trees 36:81–91. https://doi.org/10.1007/s00468-021-02183-y
73. Sonobe K, Hattori T, An P, Tsuji W, Eneji AE, Kobayashi S, Kawamura Y, Tanaka K, Inanaga S (2010) Effect of silicon application on sorghum root responses to water stress. J Plant Nutr 34:71–82. https://doi.org/10.1080/01904167.2011.531360
74. Souza CC, Oliveira FA, Silva IF, Amorim Neto MS (2020) Avaliação de métodos de determinação de água disponível e manejo da irrigação em terra roxa sob cultivo de algodoeiro herbáceo. Rev Bras Eng Agric Ambient 4:338–342. https://doi.org/10.1590/S1415-43662000000300006
75. Suriyaprabha R, Karunakaran G, Kavitha K, Yuvakkumar R, Rajendran V, Kannan N (2014) Application of silica nanoparticles in maize to enhance fungal resistance. IET Nanobiotechnol 8:133–137. https://doi.org/10.1049/iet-nbt.2013.0004
76. Taiz L, Zeiger E, Møller IM, Murphy A (2017) Fisiologia e desenvolvimento vegetal, 6th edn. Artmed, Porto Alegre
77. Teixeira GCM, Prado RM, Rocha AMS (2021) Low absorption of silicon via foliar in comparison to root application has an immediate antioxidant effect in mitigating water deficit damage in sugarcane. J Agron Crop Sci 208:805–814. https://doi.org/10.1111/jac.12511

78. Teixeira GCM, Prado RM, Rocha AMS, Santos LCN, Santos MMS, Gratão PL, Fernandes C (2020) Silicon in presprouted sugarcane seedlings mitigate the effects of water deficit after transplanting. J Soil Sci Plant Nutr 1:1–11. https://doi.org/10.1007/s42729-019-00170-4
79. Thorne SJ, Hartley SE, Maathuis FJM (2020) Is silicon a panacea for alleviating drought and salt stress in crops? Front Plant Sci 11:1221–1237. https://doi.org/10.3389/fpls.2020.01221
80. Tripathi DK, Singh VP, Prasad SM, Chauhan DK, Dubey NK, Rai AK (2015) Silicon-mediated alleviation of Cr (VI) toxicity in wheat seedlings as evidenced by chlorophyll florescence, laser, induced breakdown spectroscopy and anatomical changes. Ecotoxicol Environ Saf 113:133–144. https://doi.org/10.1016/j.ecoenv.2014.09.029
81. Uarrota VG, Stefen DLV, Leolato LS, Gindri DM, Nerling D (2018) Revisiting carotenoids and their role in plant stress responses: from biosynthesis to plant signaling mechanisms during stress. In: Gupta D, Palma J, Corpas F (eds) Antioxidants and antioxidant enzymes in higher plants. Springer, Cham. https://doi.org/10.1007/978-3-319-75088-0_10
82. Valentovic P, Luxova M, Kolarovic L, Gasparikova O (2006) Effect of osmotic stress on compatible solutes content, membrane stability and water relations in two maize cultivars. Plant Soil Environ 52:186–191. https://doi.org/10.17221/3364-PSE
83. Vandegeer RK, Zhao C, Cibils-Stewart X, Wuhrer R, Hall CR, Hartley SE, Tissue DT, Johnson SN (2020) Silicon deposition on guard cells increases stomatal sensitivity as mediated by K^+ efflux and consequently reduces stomatal conductance. Physiol Plant 171:358–370. https://doi.org/10.1111/ppl.13202
84. Wang M, Wang R, Mur LAJ, Ruan J, Shen Q, Guo S (2021) Functions of silicon in plant drought stress responses. Hortic Res 8:254. https://doi.org/10.1038/s41438-021-00681-1
85. Weatherley PE (1950) Studies in the water relations of the cotton plant. I. The field measurement of water deficit in leaves. New Phytol 49:81–97. https://doi.org/10.1111/j.1469-8137.1950.tb05146.x
86. Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144:307–313. https://doi.org/10.1016/S0176-1617(11)81192-2
87. Yemm EW, Willis AJ (1954) The estimation of carbohydrates in plant extracts by anthrone. Biochem J 57:508–514. https://doi.org/10.1042/bj0570508
88. Zhang W, Yu X, Li M, Lang D, Zhang X, Xie Z (2018) Silicon promotes growth and root yield of *Glycyrrhiza uralensis* under salt and drought stresses through enhancing osmotic adjustment and regulating antioxidant metabolism. Crop Prot 107:1–11. https://doi.org/10.1016/j.cropro.2018.01.005
89. Zhu Y-X, Gong H-J, Yin J-L (2019) Role of silicon in mediating slat tolerance in plants: a review. Plants 8:147. https://doi.org/10.3390/plants8060147

Figures

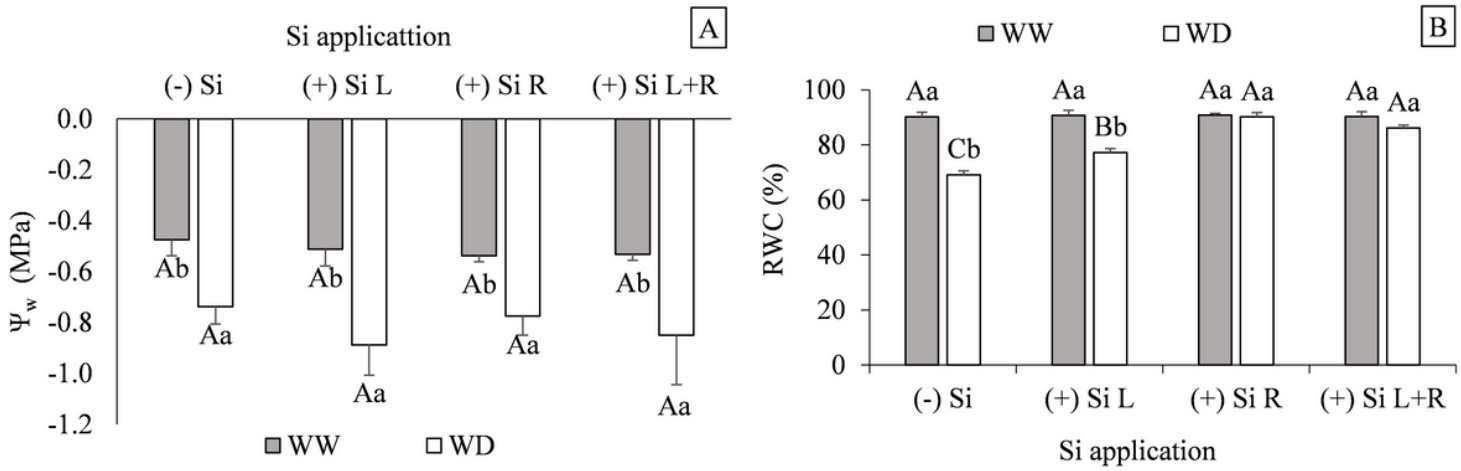

Figure 1

Leaf water potential (Ψ_w) [A] and relative water content (RWC) [B] in young *Eucalyptus urophylla* plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in the control treatment (without Si) and different Si application methods. Uppercase letters compare the control and the Si application methods in each irrigation regime, while lowercase letters compare irrigation regimes in the control or Si application methods (n = 5) using Tukey's test (p < 0.05).

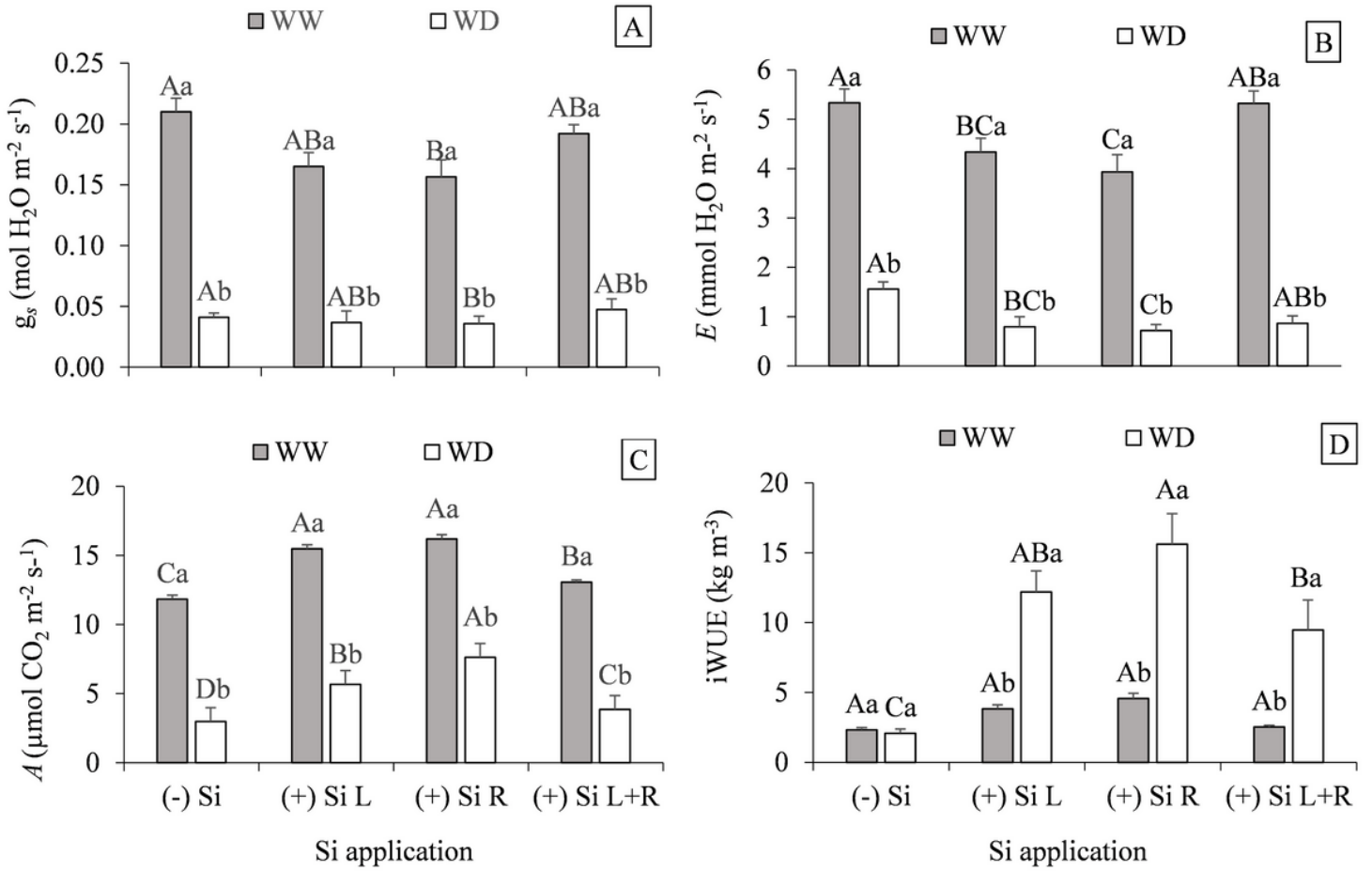

Figure 2

Stomatal conductance (g_s) [A], transpiration (E) [B], CO_2 assimilation (A) [C], and intrinsic water-use efficiency (iWUE) [D] in young *Eucalyptus urophylla* plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in the control treatment (without Si) and treatments with different Si application methods. Uppercase letters compare the control and Si application methods in each irrigation regime, while lowercase letters compare irrigation regimes in the control or Si application methods (n = 5) using Tukey's test ($p < 0.05$).

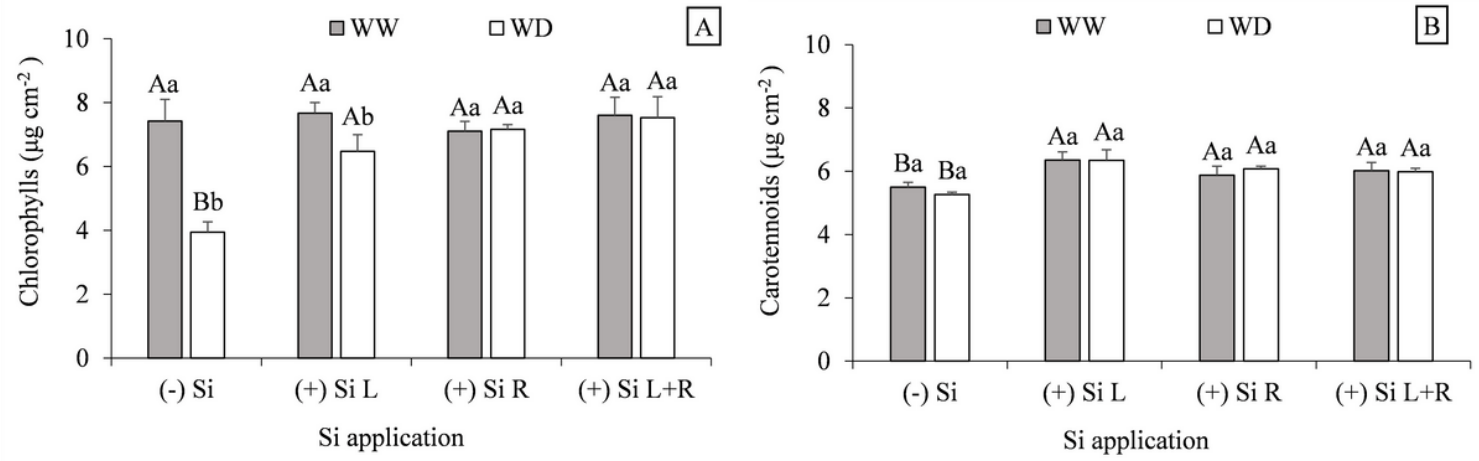

Figure 3

Total contents of chlorophylls [A] and carotenoids [B] in young *Eucalyptus urophylla*plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in control treatment (without Si) and Si application methods. Uppercase letters compare the control and Si application methods in each irrigation regime, while lowercase letters compare irrigation regimes in the control or each Si application method (n = 5) using Tukey's test ($p < 0.05$).

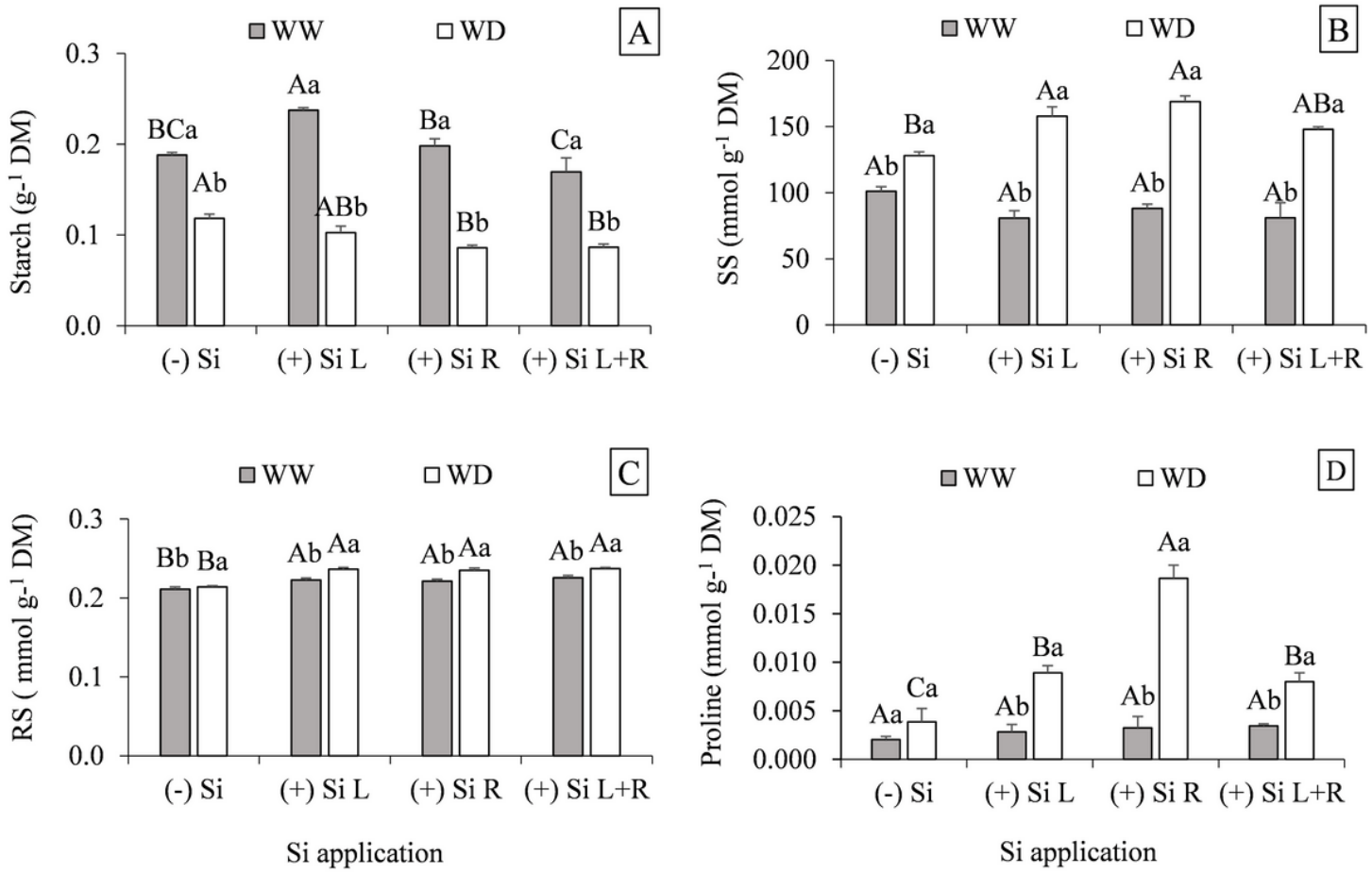

Figure 4

Contents of starch [A], soluble sugars (SS) [B], reducing sugars (RS) [C], and proline [D] in young *Eucalyptus urophylla* plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in the control treatment (without Si) and different Si application methods. Uppercase letters compare the control and Si application methods in each irrigation regime, while lowercase letters compare irrigation regimes in the control or each Si application method (n = 5) using Tukey's test ($p < 0.05$).

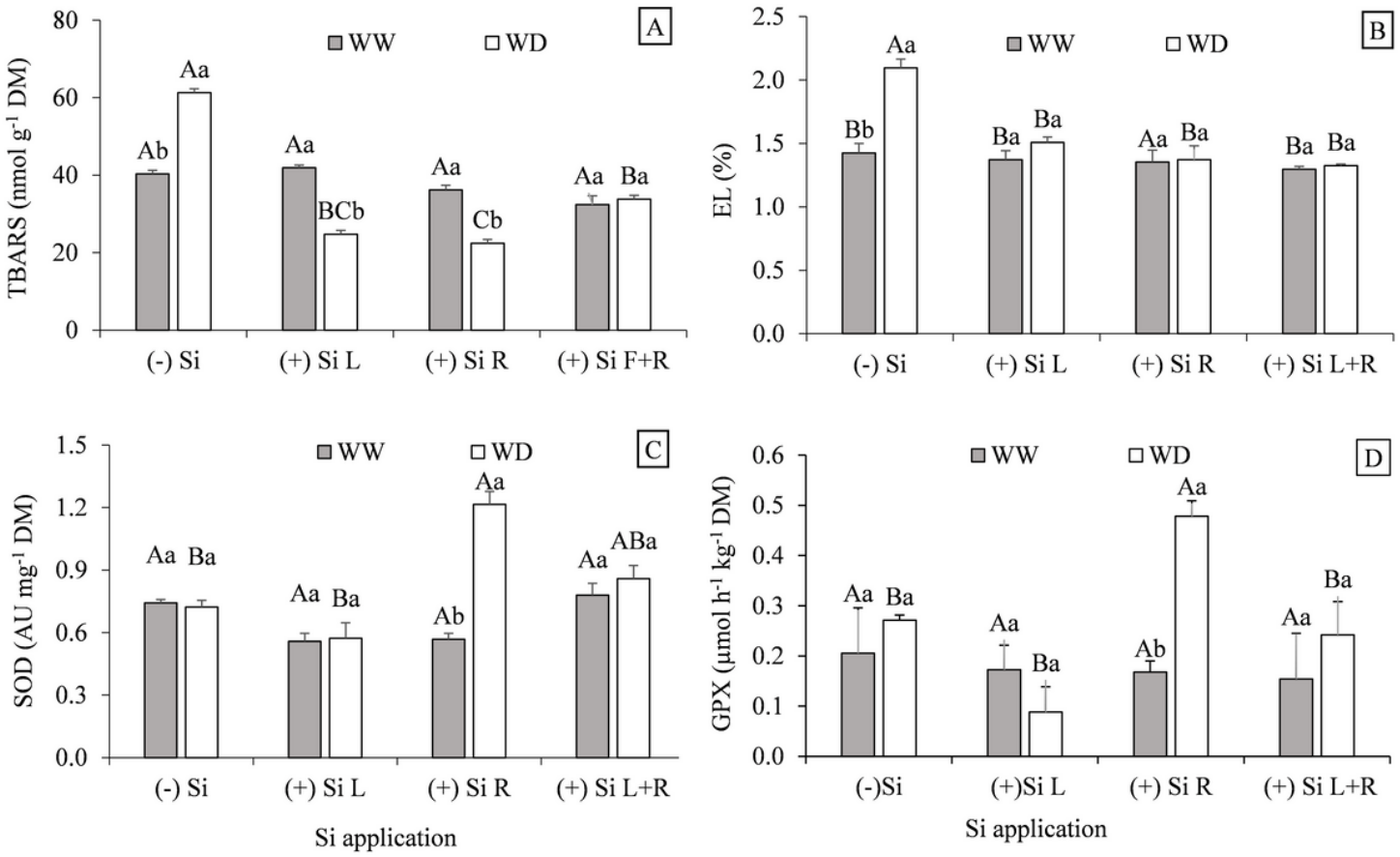

Figure 5

Thiobarbituric acid reactive substances (TBARS) content [A], electrolyte leakage (EL) [B], superoxide dismutase activity (SOD) [C], and guaiacol peroxidase activity (GPX) [D] in young *Eucalyptus urophylla* plants subjected to well-watered (WW) and water-deficit (WD) irrigation regimes, in control treatment (without Si) and different Si application methods. Uppercase letters compare the control and Si application methods in each irrigation regime, while lowercase letters compare the irrigation regime in the control or each Si application method ($n = 5$) using Tukey's test ($p < 0.05$).