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Silicon mitigates oxidative stress and has positive effects in *Eucalyptus platyphylla* under aluminium toxicity

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ABSTRACT

The tolerance to metal toxicity, such as aluminium, can be induced by the formation of Al-Si complexes. Therefore, the aim of this research is to determine the contribution of Si on nutrient concentrations, compounds linked to oxidative stress, photosynthetic pigments and gas exchange and to determine if Si can improve the tolerance mechanism of young *Eucalyptus platyphylla* plants exposed to Al toxicity. The experimental setup was completely randomized with four treatments (0, 1.6 mmol/L Al, 2.0 mmol/L Si and 1.6 mmol/L Al + 2.0 mmol/L Si; being described as the control, Al toxicity, Si and Al toxicity + Si, respectively). The treatment with Si attenuated the negative effects of Al on nutrient concentrations by reducing superoxide, hydrogen peroxide, malondialdehyde and electrolyte leakage. Pigments and gas exchange exhibited beneficial effects after Si application, with positive interactions also being detected between Si-Al. Therefore, this study demonstrates that Si reduced the oxidative stress and improved the tolerance mechanism of young *E. platyphylla* plants exposed to Al toxicity.

Keywords: metal; nutrition; photosynthesis; reactive oxygen species; toxic element

Positive interactions to silicon (Si) were reported in *Solanum nigrum* plants (Liu et al. 2013) exposed to toxic cadmium (Cd) levels, while Kidd et al. (2001) also described that Si mitigates the toxicity by aluminium (Al) in *Zea mays*. In addition, the protective action of Si was observed in *Oryza sativa* under high levels of zinc (Zn) (Song et al. 2014), *Hordeum vulgare* under high concentrations of chromium (Cr) (Ali et al. 2013) and *Cucumis sativus* exposed to manganese (Mn) toxicity (Shi et al. 2005).

The tolerance to metal toxicity can be induced by the compartmentalization, chelation and/or immobilization of metal ions into the vacuole, cytoplasm, apoplast or/and cell wall. In addition, Britez et al. (2002) described that Si decreased Al toxicity due to the formation of Al-Si complexes in the apoplast, which are non-toxic forms. Based on this overview, our hypothesis is that Si must reduce the negative impacts on metabolism provoked by the action of Al in *Eucalyptus platyphylla* plants. The aim of this research is to determine the Si contribution on nutrient concentrations, compounds linked to oxidative stress, photosynthetic pigments and gas exchange, and determine if Si can improve the tolerance mechanism of young *E. platyphylla* plants exposed to Al toxicity.

MATERIAL AND METHODS

Plants, containers and acclimation. Young *Eucalyptus platyphylla* plants sixty-day-old that presented similar aspects and sizes were selected and placed in 1.2 L containers. The solution con-

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tained 500 mL of nutritive solution. The ionic force started at 25%, and it was modified to 50% and 100% at regular intervals over three days. After these periods, the nutritive solution remained with the total ionic force. Subsequently, the 75-day-old young plants were submitted to toxicity aluminium.

Experimental design. The experiment was set up using a completely randomized design with four treatments (0, 1.6 mmol/L Al; 2.0 mmol/L Si and 1.6 mmol/L Al + 2.0 mmol/L Si; being described as a control, Al toxicity, Si and Al toxicity + Si, respectively). The experiment was assembled with five replicates for a total of 20 experimental units, with one plant in each unit.

Plant conduction and Al and Si treatments. During plant conduction, one young plant was placed in each pot. The treatments received macronutrients and micronutrients from the nutritive solution of Hoagland and Arnon (1950) modified containing 6 mmol/L KNO_3, 5 mmol/L $Ca(NO_3)_2 \cdot 4\ H_2O$, 2 mmol/L $NH_4H_2PO_4$, 1 mmol/L $MgSO_4 \cdot 7\ H_2O$, 62.50 µmol/L KCl, 31.25 µmol H_3BO_3, 2.50 µmol/L $MnSO_4 \cdot H_2O$, 2.50 µmol/L $ZnSO_4 \cdot 7\ H_2O$, 0.63 µmol/L $CuSO_4 \cdot 5\ H_2O$, 0.63 µmol/L $NaMoO_4 \cdot 5\ H_2O$, and 200.0 µmol/L NaEDTAFe·3 H_2O. Based on the preliminary studies, to simulate Al toxicity, $AlCl_3$ was used at concentration of 1.6 mmol/L Al. For Si treatment, 2.0 mmol/L $Na_2SiO_3 \cdot 9\ H_2O$ was used, and for Al toxicity + Si treatment 1.6 mmol/L Al + 2.0 mmol/L Si was added. All reagents used in this study were obtained from Sigma-Aldrich™. The Al concentration was applied to young plants for 30 days. During the cultivation, the solutions were changed at 07:00 h over 5-day intervals and their pH value was adjusted to 4.5 in treatments under Al toxicity and Si + Al toxicity (Wang et al. 2004), and to 5.5 in other treatments, using HCl or NaOH. All plants were physiologically measured on the 30^{th} day after the treatments, and leaf tissue was harvested for biochemical and chemical analysis.

Evaluating gas exchange. The stomatal conductance (g_s), net photosynthetic rate (P_n) and transpiration rate (E) were evaluated by using an infrared gas analyser (ADC BioScientific, model LCPro+, Hoddesdon, UK). These parameters were measured at the ad axial surface of fully expanded leaves, in the middle region of the plant. The water use efficiency (WUE) was calculated by the formula P_n/E. The gas exchange was evaluated in all plants between 9:00 h and 12:00 h. The irradiance was maintained at 900 µmol/m^2/s during the measurements.

Superoxide and non-enzymatic determinations. Superoxide was extracted from leaf and root tissues as per the method of Badawi et al. (2004) and the determination was done using the procedures described by Elstner and Heupel (1976). Non-enzymatic compounds (H_2O_2 and MDA) were extracted as described by Wu et al. (2006). To determine H_2O_2 methodology described by Velikova et al. (2000) was used; MDA determination was done using the method of Cakmak and Horst (1991).

Electrolyte leakage. Electrolyte leakage was measured according to the method described by Gong et al. (1998) using leaf and root tissues. The percentage of electrolyte leakage was calculated using the formula:

$$EL\ (\%) = (EC_1/EC_2) \times 100.$$

Determining photosynthetic pigments. Chlorophyll and carotenoid determination was performed with 40 mg of leaf tissue, in agreement with methodology of Lichtenthaler and Buschmann (2001).

Extraction and Si determination. Samples containing 100 mg of dry leaf matter were placed in a muffle furnace and kept for 3 h at 500°C. For Si determination the method described by Ma et al. (2004) was used.

Digestion and determination of chemical elements. Samples were treated according to Batista et al. (2014). The determination of Al, Si, P, K, Ca, Mg, B, Mn, Fe, Cu, Zn and Ni was carried out using an inductively coupled plasma mass spectrometer (ICP-MS, Agilent 7900, Santa Clara, USA).

Data analysis. The data were subjected to an analysis of variance and significant differences between the means were determined by a Scott-Knott test at a probability level of 5%. Standard deviations were calculated for each treatment. The statistical analyses were performed with the software Assistat (Campina Grande, Brazil).

RESULTS AND DISCUSSION

Si increases the nutrient concentrations in *E. platyphylla* subjected to Al toxicity. The increase in Si concentrations corroborates that the plant absorbed and accumulated this element. For Al toxicity + Si treatment, there was a decrease in the Al concentration. This result can be explained

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Table 1. Concentrations of aluminium (Al), silicon (Si), phosphorus, potassium, calcium and magnesium in young *Eucalyptus platyphylla* plants exposed to Al toxicity and Si

Treatment	Concentrations in leaf					
	Al (μg/g DM)	Si	P	K	Ca	Mg
		(mg/g DM)				
Control	8.19 ± 0.61^{c}	11.65 ± 1.10^{c}	2.74 ± 0.18^{a}	7.16 ± 0.58^{a}	0.38 ± 0.02^{a}	2.55 ± 0.22^{a}
Al toxicity	52.87 ± 5.25^{a}	9.21 ± 0.53^{c}	1.28 ± 0.11^{c}	3.49 ± 0.31^{b}	0.15 ± 0.01^{c}	1.35 ± 0.12^{c}
Si	8.22 ± 0.50^{c}	46.39 ± 2.45^{a}	2.88 ± 0.26^{a}	6.96 ± 0.46^{a}	0.36 ± 0.03^{a}	2.54 ± 0.20^{a}
Al toxicity + Si	28.53 ± 1.40^{b}	41.17 ± 2.59^{b}	2.41 ± 0.20^{b}	6.43 ± 0.39^{a}	0.26 ± 0.02^{b}	2.01 ± 0.18^{b}

Columns with different letters next to treatments indicate significant differences from the Scott-Knott test ($P < 0.05$). Values described correspond to means and standard deviations from five repetitions. DM – dry matter

by the formation of the Al-Si complexes in leaf tissue, which are non-toxic forms, contributing to Al detoxification (Britez et al. 2002). Similar results were reported by Wang et al. (2004), demonstrating that Si is efficient in the Al detoxification of *Zea mays* plants subjected to Al toxicity + Si.

The treatment of Al toxicity + Si induced significant increases of 88, 84, 73 and 48% (Table 1) in concentrations of the macronutrients P, K, Ca and Mg, respectively, compared with Al toxicity. The values verified to P, K and Mg of the Al toxicity + Si treatment are in agreement with reference values describe by Judd et al. (1996) and Herbert (1996) in *Eucalyptus*. Plants exposed to Al toxicity + Si presented increases in concentrations of B, Mn, Fe, Cu, Zn and Ni at 26, 67, 86, 159, and 64%, respectively, compared to treatment under Al toxicity (Table 2). The increase in P, K, Ca and Mg concentrations in plants subjected to the treatment of Al toxicity + Si is related to the alkalizing power promoted by Si, which induces Al non-solubilisation and consequently neutralisation of this metal (Gutierrez et al. 2011). This Si action presents repercussions on availability and assimilation of these nutrients, increasing their concentrations in tissues. In addition, the Si mitigated the negative effects induced by Al toxicity on B, Mn, Fe, Cu, Zn and Ni concentrations. Salvador et al. (2000) working with young *Psidium guajava* plants grown under Al toxicity found significant reductions of 56% and 69% to Fe and Mn concentrations, respectively.

Overproduction of ROS promoted by Al stress. The O_2^- production in plants under Al toxicity + Si was significantly reduced by 44% and 45%, respectively, in comparison with Al toxicity treatment (Figure 1a,b). For H_2O_2 production, plants subjected to Al toxicity + Si resulted in significant reductions of 21% and 37%, respectively, compared with plants exposed to Al toxicity (Figure 1c,d). Si attenuated the O_2^- and H_2O_2 accumulations in plants of the Al toxicity +

Table 2. Concentrations of boron, manganese, iron, copper, zinc and nickel in young *Eucalyptus platyphylla* plants exposed to aluminium (Al) toxicity and silicon (Si)

Treatment	Concentrations in leaf (μg/g DM)					
	B	Mn	Fe	Cu	Zn	Ni
Control	41.26 ± 1.43^{a}	303.49 ± 23.16^{a}	41.32 ± 3.80^{a}	3.44 ± 0.29^{a}	13.10 ± 0.92^{a}	1.34 ± 0.09^{a}
Al toxicity	28.87 ± 2.35^{c}	148.10 ± 4.67^{c}	15.61 ± 1.43^{c}	1.51 ± 0.12^{c}	4.03 ± 0.39^{c}	0.56 ± 0.02^{d}
Si	42.63 ± 1.83^{a}	296.26 ± 9.57^{a}	43.65 ± 3.52^{a}	3.46 ± 0.21^{a}	13.29 ± 1.06^{a}	1.19 ± 0.10^{b}
Al toxicity + Si	36.31 ± 3.04^{b}	247.57 ± 21.11^{b}	29.12 ± 1.74^{b}	2.58 ± 0.20^{b}	10.47 ± 0.92^{b}	0.92 ± 0.07^{c}

Columns with different letters next to treatments indicate significant differences from the Scott-Knott test ($P < 0.05$). Values described corresponding to means and standard deviations from five repetitions. DM – dry matter

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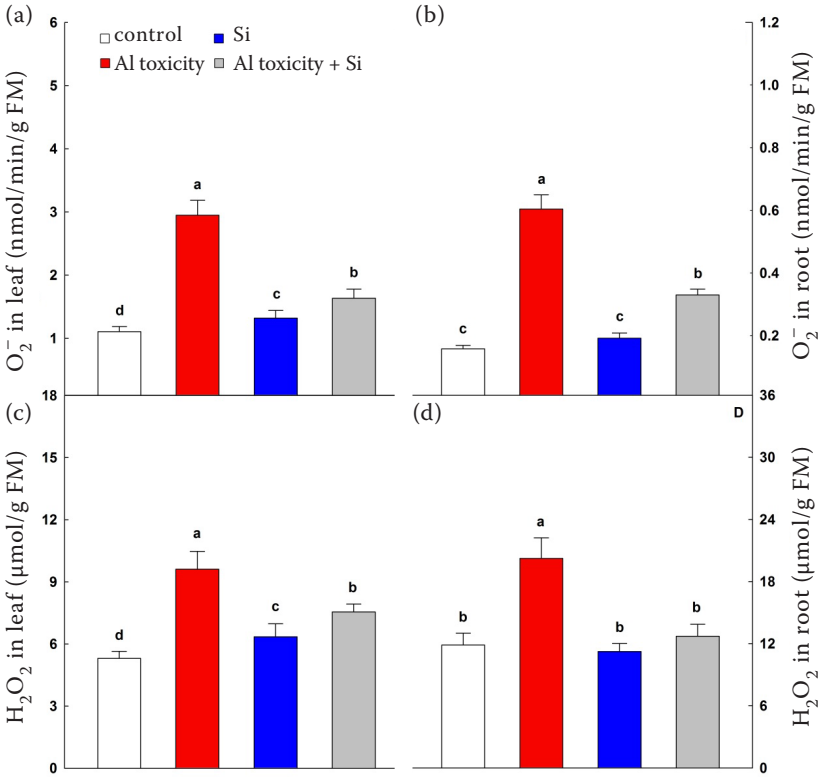

Figure 1. (a,b) Superoxide and (c,d) hydrogen peroxide in young *Eucalyptus platyphylla* plants exposed to aluminium (Al) toxicity and silicon (Si) . Bars with different letters indicate significant differences from the Scott-Knott test ($P < 0.05$). Bars represent the mean values and error bars represent the standard deviations from five repetitions. FM – fresh matter

Si treatment, suggesting that Si deposition in tissue interfered in O_2^- production (Liu et al. 2009). In addition, the reduction in O_2^- levels had beneficial consequences on H_2O_2 because a minor amount of H_2O_2 was also generated. Liu et al. (2013) reported that exogenous Si application induced reduction

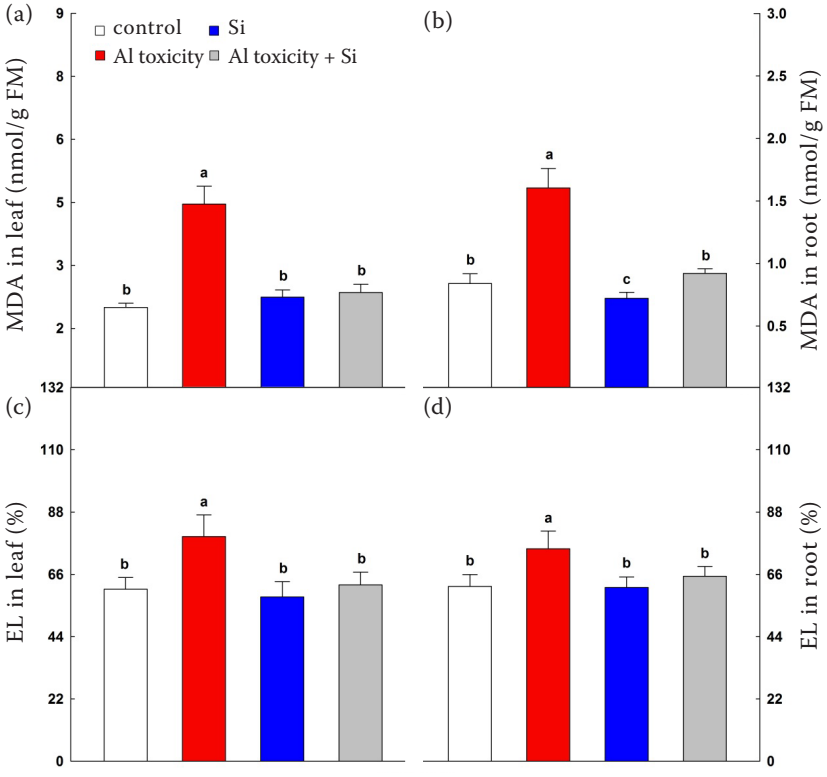

Figure 2. (a,b) Malondialdehyde and (c,d) electrolyte leakage in young *Eucalyptus platyphylla* plants exposed to aluminium (Al) toxicity and silicon (Si). Bars with different letters indicate significant differences from the Scott-Knott test ($P < 0.05$). Bars represent the mean values and error bars represent the standard deviations from five repetitions. FM – fresh matter; MDA – malondialdehyde; EL – electrolyte leakage

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in H_2O_2 concentration in *Solanum nigrum* plants grown under Cd toxicity, confirming thus our results.

Al toxicity attenuated by Si. MDA of plants grown in the presence of Al toxicity + Si induced significant reductions in leaf and root tissue of 47% and 42%, respectively, comparing with plants exposed to Al toxicity (Figure 2a,b). As for EL, plants treated with Al toxicity + Si application promoted a significant reduction of 21% and 12% in relation to leaf and root tissues, respectively, when compared to plants subjected to Al toxicity (Figure 2c,d). The lower MDA and EL levels in plants grown with Al toxicity + Si are responses linked to the protective role induced by Si on membranes and the stimulation of antioxidant enzymes. Si acts to prevent the structural and functional deterioration of membrane permeability against oxidative damages (Shahnaz et al. 2011). Additionally, Si also increases enzymatic activities linked to antioxidant defense systems aiming to eliminate ROS and reduce lipid peroxidation (Feng et al. 2009).

Benefits on pigments promoted by Si. The chl *a*, chl *b* and total chl levels linked to treatment under Al toxicity + Si promoted similar behaviours with significant increases of 25, 52 and 29%, compared with plants treated only with Al (Figure 3). However, the Al treatment induced a significant increase of 35% in the CAR (carotenoids) levels (Figure 3d), when compared to control plants. Toxicity + Si caused a significant reduction of 16% compared with plants treated with Al toxicity. Si alleviated the damaging effects on chl *a*, chl *b* and total chl of plants exposed to Al toxicity, and these responses are related to increase in Mg concentration and reduced membrane oxidation indicated by the MDA and EL, previously described in this research. Maintenance in CAR levels verified in plants subjected to Al toxicity + Si can be explained by lower oxidative stress. CAR is an accessory pigment and works as a protective mechanism during stress conditions. Beneficial effects promoted by Si on chlorophyll were verified by Bharwana et al. (2013) who studied *Gossypium herbaceum* plants exposed to lead toxicity.

Repercussion of Al toxicity and Si on gas exchange. In g_s and E of the treatment under Al toxicity presented significant increases of 40% and 24%, respectively, compared to control plants (Figure 4a,c). The g_s of Al toxicity + Si treatment showed a significant reduction of 14%, when compared with treatment under Al toxicity. The values to P_n and WUE were significantly decreased at 26% and 41%,

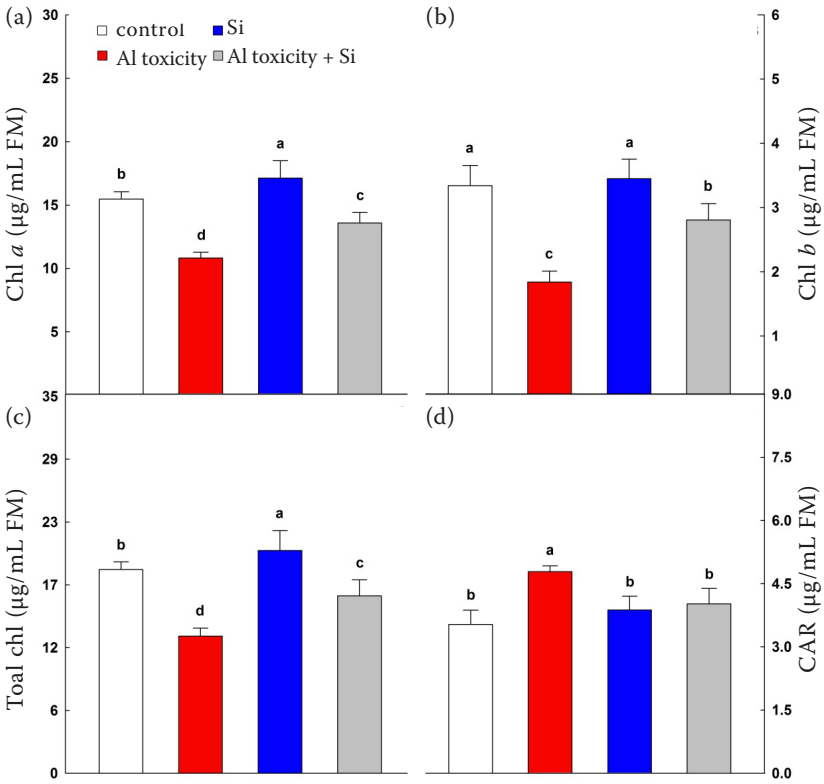

Figure 3. (a) Chlorophyll *a*; (b) chlorophyll *b*; (c) total chlorophyll and (d) carotenoids in young *Eucalyptus platyphylla* plants exposed to luminium (Al) toxicity and silicon (Si). Bars with different letters indicate significant differences from the Scott-Knott test ($P < 0.05$). Bars represent the mean values and error bars represent the standard deviations from five repetitions. FM – fresh matter; CAR – carotenoids

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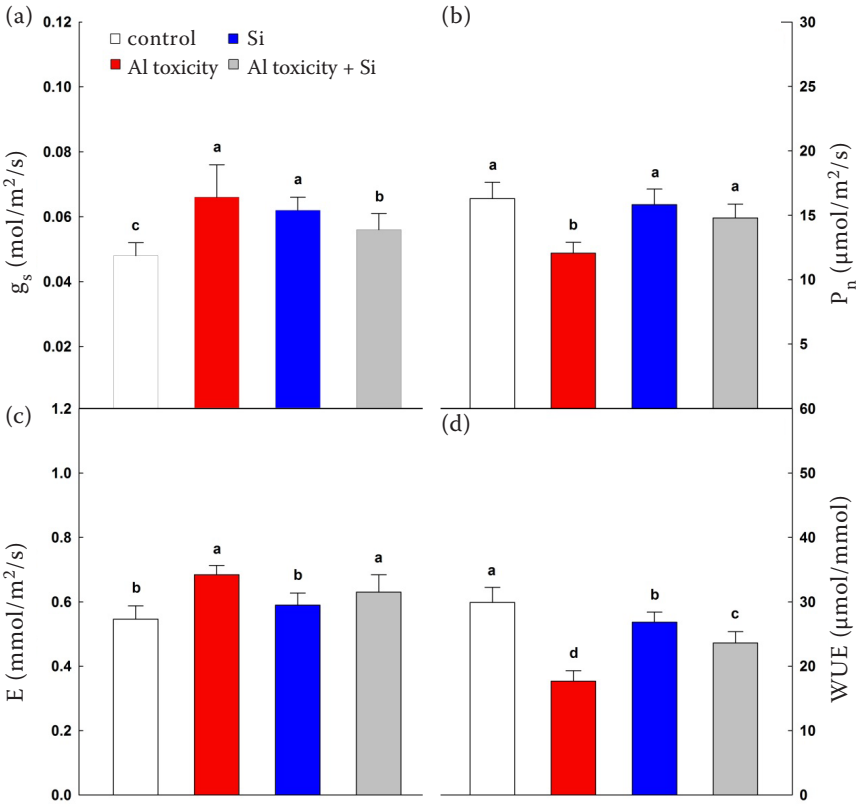

Figure 4. (a) Photosynthesis rate (g_s); (b) stomatal conductance (P_n); (c) transpiration rate (E) and (d) water use efficiency (WUE) in young *Eucalyptus platyphylla* plants exposed to aluminium (Al) toxicity and silicon (Si). Bars with different letters indicate significant differences from the Scott-Knott test ($P < 0.05$). Bars represent the mean values and error bars represent the standard deviations from five repetitions

respectively, after Al toxicity (Figure 4b,d), but the combined effect of Al toxicity + Si promoted significant increases of 22% and 33%, respectively, compared with plants treated with Al toxicity. Increases in g_s and E in plants subjected to Al toxicity revealed probable disruption of a stomatal mechanism. The Ca limitation can promote disorders and compromise the stomatal regulation mechanism because Ca^{2+} acts as a second messenger during the stomatal closing process. The increase in P_n of plants treated with Al toxicity + Si indicates that the damages caused by Al on the photosynthetic apparatus were attenuated by Si, behaviour linked to maintenance of the pigments verified in this study. The increase observed in WUE of plants subjected to Al + Si toxicity demonstrated that Si attenuated the toxicity of Al on this variable. These values are explained by the increase in P_n and the decrease in E verified in this study and induced by Si. Paula et al. (2015) working with *Zea mays* plants exposed to Si and Zn reported that Si increased the WUE by 9.8%, compared to plants under Zn toxicity, confirming that Si mitigates metal toxicity.

Treatment with Si attenuated the negative effects of Al on nutrient concentrations, thereby reducing superoxide, hydrogen peroxide, malondialdehyde and electrolyte leakage. Beneficial effects were observed on pigments and gas exchange after Si application. Positive interactions were also detected between Si and Al. Therefore, this study demonstrates that Si reduced the oxidative stress and improved the tolerance mechanism of young *E. platyphylla* plants exposed to Al toxicity.

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