

Original Article

Silicon as an attenuator of the toxic effects of aluminum in *Schinus terebinthifolius* plants

Silício como atenuador dos efeitos tóxicos do alumínio em plantas de *Schinus terebinthifolius*

M. V. M. Aguilár^{a*} , J. P. O. Mattos^a , G. S. Wertonge^a , F. C. R. Rosa^a , L. R. Lovato^a , D. V. Valsoler^a ,
T. D. Azevedo^a , F. T. Nicoloso^a  and L. A. Tabaldi^a 

^aUniversidade Federal de Santa Maria, Departamento de Biologia, Santa Maria, RS, Brasil

Abstract

Aluminum (Al) is highly toxic to plants, since it causes stress and inhibits plant growth. Silicon (Si) is known to mitigate the stress caused by Al in several plant species. Thus, the current study aims to investigate the soothing effects of Si on morphophysiological and photosynthetic variables, and the attributes associated with oxidative stress in *Schinus terebinthifolius* plants exposed to Al. Treatments have followed a completely randomized design, with three repetitions based on the following Al/Si combinations (in mM): Treatment 1: 0 Al + 0 Si; Treatment 2: 0 Al + 2.5 Si; Treatment 3: 1.85 Al + 0 Si; Treatment 4: 1.85 Al + 2.5 Si; Treatment 5: 3.71 Al + 0 Si; Treatment 6: 3.71 Al + 2.5 Si. Each sampling unit consisted of a tray with 15 plants, totaling forty-five per treatment. Shoot and root morphological variables, photosynthetic variables, photosynthetic pigments, hydrogen peroxide concentration, lipid peroxidation (MDA), guaiacol peroxidase (POD) and superoxide dismutase (SOD) enzymes, and non-enzymatic antioxidant such as Ascorbic acid (AsA) and non-protein thiol (NPSH) concentration were assessed. Root growth inhibition followed by changes in root morphological variables have negatively affected root and shoot biomass production in plants only subjected to Al. However, adding 2.5 mM Si to the treatment has mitigated the toxic effects caused by 1.85 mM of aluminum on *S. terebinthifolius* plants.

Keywords: antioxidant system, beneficial element, metal toxicity, morphophysiological variables, oxidative stress.

Resumo

O alumínio (Al) é altamente tóxico para as plantas, pois causa estresse e inibe o crescimento vegetal. O silício (Si) é conhecido por atenuar o estresse causado pelo Al em diversas espécies vegetais. Assim, o presente estudo tem como objetivo investigar os efeitos suavizantes do Si sobre variáveis morfofisiológicas e fotossintéticas, e os atributos associados ao estresse oxidativo em plantas de *Schinus terebinthifolius* expostas ao Al. Os tratamentos seguiram um delineamento inteiramente casualizado, com três repetições baseadas nas seguintes combinações Al/Si (em mM): Tratamento 1: 0 Al + 0 Si; Tratamento 2: 0 Al + 2,5 Si; Tratamento 3: 1,85 Al + 0 Si; Tratamento 4: 1,85 Al + 2,5 Si; Tratamento 5: 3,71 Al + 0 Si; Tratamento 6: 3,71 Al + 2,5 Si. Cada unidade amostral constou de uma bandeja com 15 plantas, totalizando quarenta e cinco plantas por tratamento. Variáveis morfológicas da parte aérea e da raiz, variáveis fotossintéticas, pigmentos fotossintéticos, concentração de peróxido de hidrogênio, peroxidação lipídica (MDA), enzimas guaiacol peroxidase (POD) e superóxido dismutase (SOD) e antioxidantes não enzimáticos como ácido ascórbico (AsA) e grupos tióis não protéicos (NPSH) foram avaliadas. A inibição do crescimento radicular seguida de alterações nas variáveis morfológicas radiculares afetaram negativamente a produção de biomassa radicular e aérea em plantas submetidas apenas ao Al. No entanto, a adição de 2,5 mM de Si ao tratamento atenuou os efeitos tóxicos causados por 1,85 mM de alumínio em plantas de *S. terebinthifolius*.

Palavras-chave: sistema antioxidante, elemento benéfico, toxicidade de metais, variáveis morfofisiológicas, estresse oxidativo.

1. Introduction

Aluminum (Al) toxicity, and phosphorus, magnesium, and calcium deficiency are common nutritional restrictions for crop yield in acidic soils (Rahman et al., 2018) widely distributed in tropical and subtropical regions. Furthermore, acidic soils account for approximately 50% of the world's

arable land. Most of these acidic soils are in developing countries (Yan et al., 2022). Therefore, Al toxicity and nutrient deficiency limit plant yield in areas where food security is weaker, a fact that leads to significant pressure on natural resources (Shetty et al., 2021).

*e-mail: aguilarmarcos2009@hotmail.com

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Aluminum is the third most abundant chemical element on the Earth's crust, and its presence in acidic soils is a stressing factor limiting plant production. Thus, Al at pH lower than 5.5 is in its trivalent aluminum (Al^{3+}) form, which is toxic to vegetables, since it reduces plant growth and, consequently, leads to low plant yield (Kuinchtner et al., 2021).

Root growth inhibition is the first symptom triggered by Al phytotoxicity, which reduces water and nutrient absorption by plants. It is also one of the main symptoms leading to decreased yield in crops affected by Al (Mota et al., 2020). In addition, Al toxicity accounts for several adverse effects, such as increased oxidative stress, limitations in gas exchange, reduced photosynthetic pigment synthesis, and nutritional imbalance. These effects show that Al is a limiting factor in plant metabolism (Cunha Neto et al., 2020).

The use of beneficial elements capable of mitigating the harmful effects of toxic metals, when used at low concentrations stands out among the alternatives used to solve plant growth issues caused by Al (Dorneles et al., 2019). Among these elements, one can mention silicon (Si), which helps reduce damage caused by phytotoxic elements such as Al and relieves symptoms of Al toxicity (Hodson and Evans, 2020).

Due to its strong affinity for other ions, Silicon (Si) is the second most abundant element on Earth's crust. It is often found in the form of silicic acid (H_4SiO_4), silicate ($xM_{12}OySiO_2$) and silica (SiO_2) (Khan et al., 2020). Silicon benefits some plants since it effectively reduces membrane lipid peroxidation (Etesami and Jeong, 2018). It happens due to the Si effect on plants' enzymatic and non-enzymatic antioxidant systems. Thus, Si can potentially relieve stress caused by toxic metals, mainly by Al (Haynes, 2017). This potential is also supported by high Si affinity for several metals, which leads to the coprecipitation of Si metal complexes in plants' cell walls (Pereira et al., 2018).

Considering that aluminum (Al) toxicity is one of the common nutritional restrictions for crop yield in acid soils widely distributed in tropical and subtropical regions, it is important to analyze the effect of the interaction between aluminum and silicon in forest species. Thus, understanding the mechanisms developed by plants tolerant and resistant to toxic metals becomes necessary to support the choice of suitable species for use in contaminated areas.

Given the high acidic soil availability in food producing regions, it is essential taking into consideration plant species naturally found in these soils, such as *Schinus terebinthifolius* Raddi, which is popularly known as broad-leaf pepper tree and Brazilian Peppertree. This species has timber potential and is recommended for degraded area restoration processes (Lorenzi, 2014). Given its rustic and pioneering nature, the species can even establish itself in soils presenting chemical, physical, and water restrictions. Besides, since *S. terebinthifolius* is an arboreal species not at risk of contamination via the food chain, it can be used as an alternative to remedy soils contaminated with Al.

Thus, if one considers that Al found in acidic soils is a phytotoxic element capable of damaging plants and that Si can reduce the harmful effects of abiotic stress, it is essential to conduct further research based on these factors to help

better understand the metabolic processes involved in this process. We hypothesized that Si reduces Al toxicity in *S. terebinthifolius* seedlings by increasing enzymatic activity, contributing to the reduction of lipid peroxidation levels. Therefore, the current study aimed to investigate the softening effects of Si on plants' morphophysiological and photosynthetic variables and attributes associated with oxidative stress in *Schinus terebinthifolius* plants exposed to Al. With the present study, it will be possible to analyze important questions about how plant growth in acidic soils distributed in different parts of the world can be related to Al tolerance/sensitivity. It will also be possible to prove the alleviating benefits of Si on morphological, photosynthetic, and biochemical variables that still await clarification, mainly in forest species, since work on the effects of Si on these species is still incipient.

2. Materials and Methods

2.1. Study site and experiment conduction

The study was conducted in a greenhouse at the Biology Department of the Federal University of Santa Maria (UFSM) - Santa Maria Campus - RS, under a controlled temperature of approximately 25°C, and mean humidity of 60%. Analyses were conducted out at the Plant Physiology and Nutrition Laboratory of the Biology Department.

Treatments have followed a completely randomized design, with three repetitions, which comprised the following Al/Si combinations (in mM): Treatment 1: 0 Al + 0 Si; Treatment 2: 0 Al + 2.5 Si; Treatment 3: 1.85 Al + 0 Si; Treatment 4: 1.85 Al + 2.5 Si; Treatment 5: 3.71 Al + 0 Si; Treatment 6: 3.71 Al + 2.5 Si. Aluminum (Al) and Si concentrations were defined based on previous experiments carried out by the research group of the Plant Physiology and Nutrition Laboratory of the Biology Department at UFSM. Each sample unit comprised a tray with 15 plants and 18 experimental units.

Seeds purchased in the Forest Research Center - DDPA of Santa Maria - produced *Schinus terebinthifolius* seedlings. Seeds were sown in Carolina Soil® commercial substrate composed of *Sphagnum* sp. and vermiculite. Plastic trays (38 cm x 56 cm) were used as cultivation containers for seedling germination and initial growth.

Seedlings were irrigated with a complete nutrient solution at pH 4.5 ± 0.1 weekly from the 15th day after sowing (DAS). The nutrient solution was formed by 6090.5 µM of nitrogen; 974.3 µM of magnesium; 4986.76 µM of chlorine; 2679.2 µM of potassium; 2436.2 µM of calcium; 359.9 µM of sulfur; 243,592 µM of phosphorus; 0.47 µM of copper; 2.00 µM of manganese; 1.99 µM of zinc; 0.17 µM of nickel; 24.97 µM of boron; 0.52 µM of molybdenum and 47.99 µM of Iron ($FeSO_4/Na$ -EDTA).

Thirty (30) days after sowing, when seedlings were approximately 10 cm tall, they were carefully removed from the trays' substrate and transferred to the hydroponic system. Then, each seedling was placed in a 16 L tray filled with Hoagland complete nutrient solution. A polystyrene blade with fifteen central holes was added to the surface of each tray to allow plants to pass through it. The polystyrene

blade allowed plants to be fixed and reduced solution evaporation in each tray.

Seedlings were left to acclimate for seven days in Hoagland's nutrient solution at 100% of their initial concentration. The nutrient solution in each tray was aerated through PVC microtubes connected to an air compressor. Its original form comprised the following concentrations: $\text{NO}_3^- = 196 \text{ mg L}^{-1}$; $\text{NH}_4^+ = 14 \text{ mg L}^{-1}$; $\text{P} = 31 \text{ mg L}^{-1}$; $\text{K} = 234 \text{ mg L}^{-1}$; $\text{Ca} = 160 \text{ mg L}^{-1}$; $\text{Mg} = 48.6 \text{ mg L}^{-1}$; $\text{S} = 70 \text{ mg L}^{-1}$; $\text{Fe-EDTA} = 5 \text{ mg L}^{-1}$; $\text{Cu} = 0.02 \text{ mg L}^{-1}$; $\text{Zn} = 0.15 \text{ mg L}^{-1}$; $\text{Mn} = 0.5 \text{ mg L}^{-1}$; $\text{B} = 0.5 \text{ mg L}^{-1}$; $\text{Mo} = 0.01 \text{ mg L}^{-1}$.

After the acclimation period, seedlings were subjected to treatments based on different Al availability conditions for 21 days, thus totaling 28 days in a hydroponic system. Plants were collected when metal toxicity symptoms were observed, mainly at the highest Al concentrations. The nutrient solution in each tray was replaced twice a week, and its pH was adjusted daily to 4.5 ± 0.1 by using $1.0 \text{ mol L}^{-1} \text{ HCl}$ or $1.0 \text{ mol L}^{-1} \text{ NaOH}$.

2.2. Determining the growth variables

Five plants from each experimental unit were collected for growth assessment purposes. Collected plants were separated into shoot and roots. Then, separated samples were subjected to determine the growth variables.

The seedlings' shoot height and root length were measured with a millimeter ruler. Measurements were taken before and after treatment application and plant growth corresponded to increased values recorded within that period.

Leaf area was measured with the aid of the WinRhizo 2013 system. Samples were digitized in a professional scanner (EPSON Expression 11000), and images in TIFF format were analyzed. Plants were harvested, separated into shoot and root systems, washed in running water and kiln dried under forced air circulation at approximately $65 \text{ }^\circ\text{C}$ until they reached constant weight to determine dry shoot weight (SDW) (g plant^{-1}) and root dry weight (RDW) (g plant^{-1}).

Roots were subjected to morphological features based on digitized images. This procedure was conducted in WinRhizo Pro 2013 software coupled to EPSON Expression 11000 scanner equipped with additional light (TPU), at 600 DPI resolution. Root length (cm plant^{-1}), mean root diameter (mm) and number of branches were measured.

2.3. Photosynthetic variables

The third fully expanded leaf of plants was used to evaluate photosynthetic variables in an infrared gas analyzer [Infra red gas analyzer (IRGA), Mod. Li-COR® 6400 XT] at $1,500 \text{ } \mu\text{mol m}^{-2} \text{ photosynthetic radiation}^{-2} \text{ s}^{-1}$ and CO_2 concentration $400 \text{ } \mu\text{mol mol}^{-1}$. Measurements were carried out in the morning, between 8:00am and 10:00 am, before plants were collected for growth analysis. The following variables were determined at that time: net CO_2 assimilation rate (A), transpiratory rate (E), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), Rubisco's instant carboxylation efficiency (A/Ci – based on the ratio between photosynthetic rate and intercellular CO_2

concentration), and water use efficiency (WUE – based on the ratio between photosynthetic and transpiratory rates).

2.4. Determining biochemical variables

Thirty (30) plants from each treatment were collected and subjected to analysis of biochemical variables. Then, plants were separated into shoots and roots, washed in distilled water, placed in aluminum foil envelopes, and frozen with liquid nitrogen to avoid sample degradation. They were kept in an ultra-freezer at -80°C until pre-prepared for analysis. Sample preparation was carried out through manual maceration with liquid nitrogen and each sample was macerated into a fine powder.

Thus, chlorophyll a, b, and carotenoid concentrations in leaves were determined based on the method by Hiscox and Israelstam (1979) and estimated through the Lichtenthaler equation (Lichtenthaler, 1987). Superoxide dismutase (SOD) activity was determined based on the spectrophotometric method described by Giannopolitis and Ries (1977), whereas guaiacol peroxidase (POD) activity was determined based on Zeraik et al. (2008); guaiacol was used as substrate.

Hydrogen peroxide concentration (H_2O_2) was determined based on Loreto and Velikova (2001). Results were expressed as $\mu\text{mol g}^{-1}$ fresh weight. The membrane lipid peroxidation degree was estimated based on the method by El-Moshaty et al. (1993). Results were expressed as nmol MDA mg^{-1} protein.

Ascorbic acid (AsA) concentration was determined based on the methodology described by Jacques-Silva et al. (2001), whereas non-protein thiol concentration was measured based on Ellman (1959). AsA concentration was expressed as $\mu\text{g g}^{-1}$ fresh weight, whereas non-protein thiol concentration was expressed as $\mu\text{mol g}^{-1}$ fresh weight.

2.5. Statistical data analysis

Error distribution normality was investigated through the Shapiro-Wilk test, whereas error variance homogeneity was investigated through the Bartlett test; both tests were applied to all experimental variables. Analysis of variance and the Scott-Knott test were applied to all treatments, at a 5% probability of error, whenever these assumptions were met. All tests were conducted using Sisvar statistical software (Ferreira, 2019).

3. Results and Discussion

Based on the analysis of variance, the evaluated factors (different Al/Si combinations) have a significant effect ($p \leq 0.05$) on morphological growth variables. Growth restriction is the most common plant response to stress conditions, such as the one caused by toxic metals (Mota al. 2020). Thus, aluminum (Al) used in the current study has reduced the increase in taproot length and decreased biomass production in *Schinus terebinthifolius* seedlings (Table 1). On the other hand, Si (2.5 mM) addition to the treatment has mitigated the adverse effects induced by 1.85 mM Al and enabled increased root growth (Table 1). This outcome suggests that Si addition under Al toxicity

increases the Si accumulation rate in the roots. In addition, the beneficial effect of Si on Al toxicity in plants can be attributed to decreased phytotoxic Al availability in the nutrient solution. Thus, there may have been decrease in Al availability due to Si addition to the nutrient solution, and it may be associated with the formation of biologically inactive hydroxyaluminum silicate complexes in the external solution (Singh et al., 2011).

Moreover, Si (2.5 mM) addition has also mitigated adverse effects induced by 1.85 mM Al on all root morphological variables (Table 1). This response may have happened because Si mitigates Al toxicity due to Al and Si co-deposition in epidermal root walls (Pontigo et al., 2017). Thus, Al-Si complexes' formation in root apoplast is a likely Al-detoxification mechanism triggered by plants to reduce Al translocation from the root to other organs, such as leaves and stems (Jesus et al., 2017).

Root growth inhibition followed by changes in root morphological variables has negatively affected root and shoot biomass production in plants only exposed to Al (Table 1). Consequently, Al transported from roots to the shoot may have negatively influenced the formation and growth of these organs, a fact that reduced photosynthetic

rates (Table 2) and resulted in lower biomass production in these plants (Guo et al., 2018).

However, Si has significantly mitigated biomass reduction induced by Al (Table 1), as well as reduction of photosynthetic variables (Table 2) in *S. terebinthifolius* seedlings subjected to the lowest Al concentration, since plants subjected to 1.85 mM Al combined to Si (2.5 mM) addition have significantly increased their growth. It led to increased shoot dry weight (SDW) and root dry weight (RDW) (Table 1), in comparison to treatments with Al.

Increased biomass production in *S. terebinthifolius* seedlings can be attributed, at least in part, to the beneficial Si effect on leaf area (Table 1). Anatomical variations produced by silica deposition on epidermal cell walls kept leaves in a perpendicular position, which improved light absorption, stimulated photosynthesis, and promoted higher dry biomass accumulation (Dorneles et al., 2019). In addition, Si improved the mineral absorption of plants, increased the availability of nutrients such as nitrate and iron and increased biomass production (Gottardi et al., 2012).

There was a significant effect ($p \leq 0.05$) of different Al/Si combinations on physiological variables analyzed

Table 1. Mean values of increment in taproot length (cm), leaf area (cm²), shoot dry weight (SDW) (g plant⁻¹), root dry weight (RDW) (g plant⁻¹), root length (cm), root surface area (cm²), root volume (cm³) and root diameter (mm) in *Schinus terebinthifolius* seedlings exposed to Al and Si.

Variables	0 Al + 0 Si	0 Al + 2.5 Si	1.85Al + 0Si	1.85 Al + 2.5 Si	3.71Al+0Si	3.71Al +2.5Si
Taproot length	15± 1 a	11±1 a	0.8±0.1 c	8±1 b	0.77±0.43 c	0.66±0.46 c
Leaf area	360± 10 a	350±7.63 a	180±5 c	195±1.0 b	120±10 c	110±7.63 d
SDW	1.25±0.08 a	1.1±0.03 a	0.7±0.07 c	0.88±0.1 b	0.6±0.04 d	0.4±0.07 d
RDW	0.33±0.04 a	0.28±0.03 b	0.21±0.0 c	0.25±0.0 b	0.14±0.01 d	0.15±0.02 d
Root length	2,300±10 a	2,300±12 a	500±0.08 c	1,500±15 b	200±0.05 c	250±0.05 c
Surface area	380±1.5 a	320±1.5 b	100±10 d	210±5 c	70±5 e	80±5 d
Root volume	4.5±0.3 a	3.8±0.16 b	1.8±0.07 d	2.4±0.13 c	1.5±0.1 e	1.73±0.07 d
Root diameter	1.9±0.05 a	1.51±0.11 b	0.6±0.02 d	1±0.02 c	0.3±0.02 e	0.4±0.03 e

Different letters on the lines between treatments represent statistical difference in the Scott-Knott test, at 5% probability of error ($p \leq 0.05$). Data represent the mean ± standard deviation.

Table 2. Average values of the liquid assimilation rate (A) ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), instant carboxylation efficiency (by Rubisco) (A/Ci) A/Ci ($\mu\text{mol m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$), stomatal conductance (Gs) Gs ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), transpiration (E) ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$), chlorophyll a ($\text{mg}^{-1} \text{ FW}$) (a) chlorophyll b ($\text{mg}^{-1} \text{ FW}$) (b), total chlorophyll ($\text{mg}^{-1} \text{ FW}$) (c) and carotenoids ($\text{mg}^{-1} \text{ FW}$) in *Schinus terebinthifolius* seedlings exposed to Al and Si.

Variables	0 Al + 0 Si	0 Al + 2.5 Si	1.85 Al + 0 Si	1.85 Al + 2.5 Si	3.71 Al + 0 Si	3.71 Al + 2.5 Si
A	17±1.5 a	12±1.5 b	13.9±1.5 a	14.1±1.5 a	9±1.5 c	9.9±1.26 c
A/Ci	0.6±0.02 a	0.4±0.02 b	0.52±0.02 a	0.02 a	0.39±0.06 b	0.395±0.01 b
GS	0.395±0.036 a	0.3±0 a	0.295±0.10 a	0.25±0.05 b	0.18±0 c	0.19±0 c
E	7.75±0.55 a	6.25±0.4 a	6.25±0.354 a	5.8±0.03 b	4.1±0.26 b	4±0.11 b
Chl a	1.8±0.05 b	2.3±0.08 a	1.82±0.04 b	1.65±0.07 c	1.64±0.05 c	1.63±0.02 c
Chl b	0.56±0.02 c	0.75±0.06 a	0.61±0.03 b	0.59±0.01 c	0.499±0.01 d	0.562±0.04 c
Total chl	2.4±0.1 b	2.99±0.02 a	2.4±0.1 b	2±0.03 c	1.99±0.04 c	1.97±0.1 c
Carotenoids	0.79±0.04 a	0.81±0.02 a	0.65±0.03 b	0.6±0.015 b	0.61±0.02 b	0.58±0.01 b

Different letters on the lines between treatments represent statistical difference in the Scott-Knott test, at 5% probability of error ($p \leq 0.05$). Data represent the mean ± standard deviation.

in the present study. Leaves are the main organs used by plants for the photosynthetic assimilation of CO₂ and leaf area determines light absorption and affects plants' photosynthetic performance and photosynthetic product accumulation (Verma et al., 2020). Si addition to a nutrient solution comprising 1.85 mM Al resulted in leaf area expansion (Table 1), compared to treatments only comprising Al. These results have shown that Si addition to the nutrient solution, protected leaf structures, increased the length of leaf epidermal cells, and increased cell wall extensibility (Singh et al., 2011). However, treatments that were only based on Al may have decreased the length of leaf epidermal cells. In contrast, Si addition, combined with an Al concentration of 1.85 mM, prevented decreased leaf epidermal cell length and increased leaf area (Table 1).

Thus, increased plant biomass observed in treatment 1.85 Al + 2.5 Si was associated with beneficial effects caused by Si addition to the nutrient solution since it contributed to improving leaf architecture, enabled better light absorption and, consequently, enabled higher liquid assimilation rate (A), and Rubisco's instant carboxylation efficiency (A/Ci) (Table 2). Thus, Si has likely mitigated the adverse effects of Al due to its neutralization and improved Rubisco's activity efficiency (Jesus et al., 2017).

However, decreased stomatal conductance and transpiratory rate were observed in plants exposed to 1.85 Al + 2.5 Si (Table 2). Such a decrease in stomatal conductance may have happened to ensure the appropriate balance in CO₂ assimilation during photosynthesis to control the internal carbon amount, and meet the carboxylation and regeneration capacity of ribulose 1.5 biphosphate, as well as to reduce water loss through transpiration (Lawson et al., 2014). Thus, at least partially, Si can decrease the stomatal conductance rate to protect the stomatal complex from destruction (Verma et al., 2020). Such a decrease in stomatal conductance may also be associated with wax layer thickening in leaves treated with Si, which covers the stomata, affects the photosynthetic process, controls the gas exchange necessary for vital cell activities, and reduces plant sweating.

Decreased transpiration (Table 2) may have happened due to Si deposition as silicate crystals in epidermal tissues. These crystals acted as obstacles to transpiration through stomata and cuticles, helped reduce Al toxicity, and simultaneously prevented excessive water loss into the atmosphere (Khan et al., 2020).

There was a significant effect ($p \leq 0.05$) of different Al/Si combinations on biochemical variables analyzed in the present study. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll recorded the highest means in plants only subjected to Si in nutrient solution (Table 2). Photosynthetic pigments are the main photosynthesis components and can be used as growth indicators in plants under stress conditions. However, seedlings grown under the 1.85 Al + 2.5 Si condition recorded lower means for Chl *a*, Chl *b*, and total Chl, than plants subjected to the treatment only based on the lowest Al concentration (1.85 mM) (Table 2). Despite the reduced photosynthetic pigment concentration (Table 2) promoted by Si, combined with Al, there was no evidence of decreased biomass production in *S. terebinthifolius* seedlings (Table 1). This outcome indicates

that plants exposed to Si have somehow compensated for the photosynthetic activity decrease caused by Al to avoid damage in biomass production.

The treatment based on Si has increased Chl *a*, Chl *b*, and total Chl concentration in plants compared to the control treatment (Table 2). This response has indicated that the double effects caused by Si, either separately or in combination with other metals, may be associated with regulation in the uptake and redistribution of essential elements, such as Mn, Fe and even Mg, which play a crucial role in the structure of chlorophyll molecules (Hu et al., 2017). However, plants subjected to the treatment based on Si have shown carotenoid concentrations similar to that of the control. In contrast, plants subjected to treatments with Al recorded a carotenoid concentration lower than the control's (Table 2). Thus, it is possible that Al was more effective in reducing carotenoid concentration in plants exposed to it, whereas Si was incapable of reversing this effect.

Toxic metals affect redox homeostasis by stimulating the excessive generation of reactive oxygen species (ROS), such as superoxide anion (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH⁻) and singlet oxygen (¹O₂) (Pontigo et al., 2017). Plants use several antioxidant enzymes to eliminate ROS, and can trigger oxidative damage. Superoxide dismutase (SOD) is the first enzyme involved in detoxification since it quickly converts superoxide radicals into H₂O₂ (Kuinchtner et al., 2021). Guaiacol peroxidase (POD), which mainly fights free radicals in the cell wall, is another antioxidant enzyme (Pereira et al., 2018).

Thus, a gradual SOD activity increase was observed in the shoot and roots of *S. terebinthifolius* seedlings (Table 3). This outcome suggests that SOD's ability to detoxify ROS is positively regulated. Thus, it is possible that Si had a beneficial effect on Al toxicity reduction, since it increased SOD activity, and this outcome confirms our initial hypothesis. Silicon can increase antioxidant enzyme activity and protect cells and tissues from oxidative damage caused by stress. In addition, antioxidant enzyme activity in plants treated with Si appears to have created conditions enabling plants to tolerate this stress type to preserve cell walls (Ali et al., 2016). Therefore, SOD activity results have suggested that Si could mitigate Al toxicity by activating this antioxidant enzyme.

The SOD/POD combined action is essential to help mitigate the effects of oxidative stress caused by metals, since these enzymes play complementary roles in cell metabolism. However, POD activity in the roots of *S. terebinthifolius* seedlings has decreased (Table 3). It happened because Si has suppressed the Al-induced POD activity increase and led to fast suppression of its activity under excessive Al. The fast decrease in POD activity can be explained by Si's effectiveness in preventing contact between the enzyme and its phenolic substrate or removing free monophenols due to the formation of Si-phenol complexes (Maksimović et al., 2012).

There was increased H₂O₂ concentration in the shoot and roots of plants subjected to the Al/Si combination (1.85 Al + 2.5 Si) (Table 3). The significant increase in H₂O₂ production may be associated with reduced POD activity observed in plants simultaneously treated with Al and Si (Table 3) since

POD acts to convert H₂O₂ into water and oxygen through H₂O₂ dissociation, and this process plays an essential role in giving plants' tolerance to unfavorable conditions (Kuinchtner et al., 2021). However, this process did not have adverse effects, like lipid peroxidation.

H₂O₂ plays dual role in vascular plants by inducing oxidative damage or acting as a signaling molecule in several physiological processes, such as senescence, photorespiration, photosynthesis, plant growth and development (Pontigo et al., 2017; Silva et al., 2024). However, excessive H₂O₂ accumulation can be among the highly harmful factors leading to lipid peroxidation and increased plasma membrane permeability.

The plasma membrane is one of the main targets of Al phytotoxic effects, since this metal can bind to phospholipids and/or change fatty acid composition in the plasma membrane, a fact that can reduce its fluidity, increase its permeability and, consequently, result in lipid peroxidation (Bose et al., 2015). Malondialdehyde (MDA) results from lipid peroxidation in cells and this product is still used as an essential oxidative stress indicator in several studies conducted with plants (Chen et al., 2017; Feng et al., 2024). Si combined with Al has decreased MDA concentration in plants' shoots and roots (Table 3) compared to treatments based only on Al. Thus, reduced membrane lipid peroxidation caused by Si may have happened due to the stimulation of enzymatic and non-enzymatic antioxidants (Shi et al., 2005), such as NPSH and ascorbic acid.

Aluminum did not affect NPSH production in the shoot (Table 4). However, the concentration of this non-enzymatic antioxidant in the roots has increased as Al concentration also increased (Table 4). Thus, the treatment comprising 3.71 mM Al has shown the highest NPSH production in the roots, which evidenced the interconnection of this antioxidant in metal detoxification and ROS elimination processes (Noctor et al., 2012). Studies have associated NPSH production increase with different stress types, such as metal exposure, and desiccation tolerance. The likely explanation for the higher concentration of this non-enzymatic antioxidant in plants exposed to metals is that higher NPSH levels lead to a more efficient metal sequestration (Zagorchev et al., 2013).

Ascorbate (AsA), in its turn, is a water-soluble antioxidant synthesized in mitochondria and can translocate to other cell compartments through different pathways. Frequently, AsA can directly eliminate ROS (¹O₂, O₂^{·-} and OH[·]) in cell (Kim et al., 2017). Moreover, Si combined with 1.85 mM Al has increased AsA production in roots (Table 4). This response may be explained by Si being associated with oxidative damage mitigation through an increase in non-enzymatic products such as AsA (Kim et al., 2017). However, an AsA increase in the shoot was not observed (Table 4). This outcome has indicated that the incidence of antioxidants in roots was more necessary than in the shoot, and this finding may be associated with more significant stress caused by Al in roots. It happened because roots are the initial target of and are mostly affected by damage caused by Al, which requires a more robust response from the antioxidant system.

Table 3. Average values of the activity of the superoxide dismutase (SOD) enzyme in the shoot and roots (U mg⁻¹ protein) and guaiacol peroxidase enzyme (POD) in the roots (U mg⁻¹ protein) and average values of the hydrogen peroxide content (H₂O₂) in shoot and roots (µmol g⁻¹ FW), peroxidation of membrane lipids in the shoot and roots (nmol MDA mg⁻¹ protein) in *Schinus terebinthifolius* seedlings exposed to Al and Si.

Variables	0 Al + 0 Si	0Al + 2.5 Si	1.85 Al + 0 Si	1.85 Al + 2.5 Si	3.71 Al + 0 Si	3.71 Al + 2.5 Si
SOD Shoot	1,470±1.5 a	600±7.6 b	850±5 b	1,480±2.51 a	840±10 b	1,460±1.5 a
SOD Root	10±0.015 e	10±0.02 e	280±1.21 b	150±5 d	240±1.5 c	360±10 a
POD Root	1.7±0.1 a	1.4±0.08 b	0.1±0.04 e	0.6±0.1 c	0.49±0.015 d	0.45±0.03 d
H ₂ O ₂ Shoot	0.45±0.01 c	0.47±0.0 c	0.6±0.02 b	0.9±0.01 a	0.47±0.02 c	0.56±0.04 b
H ₂ O ₂ Root	0.15±0.05 d	0.1±0.04 e	0.6±0.05 a	0.3±0.07 c	0.395±0.05 b	0.4±0.05 b
MDA Shoot	0.01±0 d	0.01±0 d	0.051±0 c	0.03±0.01 e	0.16±0.01 a	0.08±0.01 b
MDA Root	0.12±0.01 e	0.13±0.0 e	0.95±0.05 b	0.25±0.03 d	1.35±0.04 a	0.49±0.01 c

Different letters on the lines between treatments represent statistical difference in the Scott-Knott test, at 5% probability of error (p≤0.05). Data represent the mean ± standard deviation.

Table 4. Average values of non-protein thiols content (NPSH) in shoot and roots (µ mol SH g⁻¹) and ascorbic acid content (AsA) in shoot and roots (ug g⁻¹) in *Schinus terebinthifolius* seedlings exposed to Al and Si.

Variables	0 Al + 0 Si	0 Al + 2.5 Si	1.85 Al + 0 Si	1.85 Al + 2.5 Si	3.71 Al + 0 Si	3.71 Al + 2.5 Si
NPSH Shoot	1.1±0.04 b	1.25±0.02 a	1±0.02 b	1.1±0.01 b	0.96±0.02 b	0.98±0.01 b
NPSH Root	0.195±0.01 d	0.26±0.010 b	0.22±0.016 c	0.19±0.01 d	0.31±0.01 a	0.27±0.01 b
AsA Shoot	260±5 a	250±5.5 a	150±6 b	147±5.4 b	149±5 b	151±4.8 b
ASsA Root	36±2 d	60±1.98 a	40±2.2 c	45±2.3 b	46±1.8 b	48±2 b

Different letters on the lines between treatments represent statistical difference in the Scott-Knott test, at 5% probability of error (p≤0.05). Data represent the mean ± standard deviation.

4. Conclusion

Therefore, the present study clarified the biochemical/physiological behavior of the species *Schinus terebinthifolius* when exposed to aluminum. In addition, the importance of silicon as a mitigator of the toxic effects caused by aluminum in *S. terebinthifolius* plants was highlighted. Also, it was possible to analyze important questions about how plant growth is related to Al tolerance/sensitivity and the mitigating effects of Si on morphological, photosynthetic, and biochemical variables that still await clarification. Therefore, it was possible to state that *Schinus terebinthifolius* plants are sensitive to aluminum. However, the addition of 2.5 mM Si to has mitigated the toxic effects caused by 1.85 mM aluminum on *S. terebinthifolius* plants exposed to this metal.

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