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Biology-Botany

Do silicon and selenium mitigate aluminum toxicity in *Pfaffia glomerata* (Spreng.) Pedersen?

O silício e o selênio mitigam a toxicidade do alumínio em *Pfaffia glomerata* (Spreng.) Pedersen?

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ABSTRACT

The use of medicinal plants is a common alternative for the population. However, many of these plants grow in tropical and subtropical soils around the world contaminated with toxic metals such as aluminum (Al). Excess Al accumulation in plant tissues can enter the food chain of animals and humans causing irreversible harm. A possible strategy is to use elements capable of mitigating the uptake or the effects of toxic metals. Thus, this study aimed to assess whether silicon (Si) and selenium (Se) mitigate Al toxicity on morphological and biochemical variables of *Pfaffia glomerata*. Plants were submitted to the following combinations of Al, Si and Se: 1) Control; 2) 1.85 mM Al; 3) 1.5 mM Si; 4) 1.85 mM Al + 1.5 mM Si; 5) 0.002 mM Se; 6) 1.85 mM Al + 0.002 mM Se. The experimental design was completely randomized with four replications. At the end of the period of exposure to the treatments, we analyzed the morphological variables (shoot and root dry weight, leaf area and root morphology) and biochemical variables (photosynthetic pigments, hydrogen peroxide content, lipid peroxidation and guaiacol peroxidase (POD) and superoxide dismutase (SOD) activity in plants). Aluminum toxicity affected the growth of *P. glomerata* and only Si was able to reverse the toxic action of Al, especially in shorter exposure periods. However, Se did not show potential to significantly inhibit the toxic effects of Al.

Keywords: Antioxidant enzymes; Beneficial elements; Medicinal plants; Toxic metals

RESUMO

O uso de plantas medicinais é uma alternativa comum à população. No entanto, muitas dessas plantas crescem em solos tropicais e subtropicais ao redor do mundo contaminados com metais tóxicos como o alumínio (Al). O acúmulo excessivo de Al nos tecidos vegetais pode entrar na cadeia alimentar de animais e humanos causando danos irreversíveis. Uma estratégia possível é utilizar elementos capazes

de mitigar a absorção ou os efeitos de metais tóxicos. Assim, este estudo teve como objetivo avaliar se o silício (Si) e o selênio (Se) mitigam a toxicidade do Al sobre variáveis morfológicas e bioquímicas de *Pfaffia glomerata*. As plantas foram submetidas às seguintes combinações de Al, Si e Se: 1) Controle; 2) Al 1,85 mM; 3) Si 1,5 mM; 4) Al 1,85 mM + Si 1,5 mM; 5) Se 0,002 mM; 6) Al 1,85 mM + Se 0,002 mM. O delineamento experimental foi inteiramente casualizado com quatro repetições. Ao final do período de exposição aos tratamentos foram analisadas as variáveis morfológicas (massa seca da parte aérea e das raízes, área foliar e morfologia das raízes) e bioquímicas (pigmentos fotossintéticos, teor de peróxido de hidrogênio, peroxidação lipídica e guaiacol peroxidase (POD) e atividade da superóxido dismutase (SOD) em plantas). A toxicidade do alumínio afetou o crescimento de *P. glomerata* e apenas o Si foi capaz de reverter a ação tóxica do Al, principalmente em períodos mais curtos de exposição. No entanto, o Se não mostrou potencial para inibir significativamente os efeitos tóxicos do Al.

Palavras-chave: Enzimas antioxidantes; Elementos benéficos; Plantas medicinais; Metais tóxicos

1 INTRODUCTION

Medicinal plants are used worldwide to treat numerous diseases and are an important source of raw materials in the pharmaceutical industry to produce phytopharmaceuticals and food supplements (Luo et al., 2021). However, medicinal plants grown in tropical and subtropical regions of the world, due to soils being characteristically acidic, may present high accumulation of toxic metals (Chen et al., 2021), including aluminum (Al), generating a potential threat to plant growth and human health when such plants enter the food chain (Maldaner et al., 2015).

Soil acidification has increased worldwide as a result of human activities, atmospheric release of industrial contaminants (Shetty et al., 2021) and continued use of fertilizers containing ammonia and amide (Nava et al., 2022). In these soils, Al toxicity is one of the greatest limitations to plant productivity, as toxic forms of Al (especially Al^{3+}) become soluble and are absorbed by plants, resulting in reduced growth, negative effects on plant development and low productivity (Zhang et al., 2022).

Aluminum toxicity inhibits both cell division and root tip elongation, subsequently affecting the length (Du et al., 2020) and the volume of the root system (Maldaner et al., 2015). Therefore, there is damage to the plasma membrane and a consequent reduction in water and nutrient uptake (Sun et al., 2020). Furthermore, excess Al in

plants may cause an increase in hydrogen peroxide content, resulting in increased membrane lipid peroxidation, thus contributing to biomass reduction (Wei et al., 2021).

Thus, it is necessary to develop strategies that result in lower Al uptake, improving the use of natural resources and the production of safe foods. This is especially the case of a medicinal plant such as *Pfaffia glomerata* (Spreng.) Pedersen, which shows a reasonable degree of tolerance to aluminum (Nishimoto et al., 1987). This species, known as 'Brazilian ginseng', belongs to the Amaranthaceae family (Siqueira, 1988). It is of commercial interest in the production of herbal medicines and food supplements due to its antitumor, antidiabetic, tonic and stimulant properties as well as its effect against gastric and rheumatic disorders (Nishimoto et al., 1987).

Among the alternative strategies to solve problems caused by Al on plant growth is the use of beneficial elements which when used in low concentrations can mitigate the harmful effects of toxic metals (Dorneles et al., 2019). Among these elements is silicon (Si) and selenium (Se), which act to decrease the damage caused by phytotoxic elements such as heavy metals and reduce the symptoms of toxicity (Huang et al., 2020).

Therefore, Si is beneficial to growth, development, yield and disease resistance in a wide variety of plant species (Kovács et al., 2022), while Se is considered favorable to plants due to its effectiveness in increasing plant tolerance to environmental stress and toxicity of some metals, as reported in the literature (Trippe & Pilon-Smits 2021).

Our hypothesis is that Si and Se reduce Al toxicity in *P. glomerata* through reduced root uptake of this toxic element and increased enzymatic activity, contributing to increased biomass gain. Therefore, the aim of this study was to assess whether Si and Se mitigate Al toxicity on morphological and biochemical variables of *P. glomerata* plants exposed to Al.

2 MATERIAL AND METHODS

2.1 Plant material and growing conditions

The plant material used in this study included seedlings of *Pfaffia glomerata* (Spreng.) Pedersen accession GD obtained from the collection of medicinal plants of the Universidade Federal de Grande Dourados (UFGD). An exsicata of the specie *Pfaffia glomerata* is deposited in the SMDB Herbarium of the Botanical Garden of the Federal University of Santa Maria - UFSM (SMDB 7606), and can also be found in Flora do Brasil (http://floradobrasil.jbrj.gov.br).

Firstly, the plant material was sent to the Universidade Federal de Santa Maria (UFSM), city of Santa Maria, southern Brazil. Then, the nodal segments (1.0 cm long) were micropropagated in MS (Murashige & Skoog 1962) medium, supplemented with 30 g L^{-1}sucrose, 0.1 g L^{-1}myo-inositol and 6 g L^{-1} agar in a climate-controlled environment, according to Nicoloso (2001). After micropropagation, the seedlings were kept at 28ºC with a light/dark photoperiod of 16h/8h and 35 µmol m^{-2} $s^{-1}$of irradiance for 21 days in an *in vitro* culture chamber.

Afterwards, the seedlings were selected according to the uniformity of shoot and root growth and sent for acclimatization in the greenhouse of the Department of Biology at UFSM (29°42'56.35"S and 53°43'12.64"W) for seven days. The greenhouse had controlled temperature and humidity of 25ºC and 60%, respectively.

After this acclimatization period, the seedlings were removed from the test tubes and the culture medium was removed from the root system. The seedlings were then placed directly in 5L plastic pots containing 5 kg of coarse sand as substrate and left to acclimatize for a further seven days. During this period, the seedlings were irrigated daily until the experiment was installed.

After this period, the treatments were arranged in a completely randomized design with four replications, which consisted of the following combinations of Al, Si and Se: 1) Control (full-strength Hoagland &Arnon's nutrient solution (1950)); 2) 1.85 mM Al;

3) 1.5 mM Si; 4) 1.85 mM Al + 1.5 mM Si; 5) 0.002 mM Se; 6) 1.85 mM Al + 0.002 mM Se. Silicon was applied as sodium silicate ($NaSiO_3$), Se as sodium selenate (Na_2SeO_4) and Al as $AlCl_3$. Concentrations of Al, Se and Si 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 (Dorneles et al., 2016, Pereira et al., 2018, Tabaldi et al., 2007, 2009). Each experimental unit consisted of five plants, four replicates per treatment and two evaluation periods (30 and 60 days), totaling 120 pots.

During cultivation, the sand was maintained at 70% water holding capacity (WHC) using the direct gravimetric method. The sand in all the treatments was saturated with water and drained for a period of 48 h with the pot surface covered to stop evaporation, after which the pots were weighed again (Casaroli & Van Lier 2008, Mello, 2006). Thus, the WHC of sand was obtained by the difference in weight. To determine WHC of 70%, the following formula was used.

$$PW\ 70\% = (PWWHC - PWdry) * 0.70 + PWdry \quad (1)$$

Where:

PWWHC is the water holding capacity of sand;

PWdry is the weight of a pot filled with dry sand.

For the irrigation of the seedlings, we used Hoagland & Arnon's full-strength nutrient solution (1950). The solution had the following composition in mg L^{-1}: 85.31 N; 7.54 P; 11.54 S; 97.64 Ca; 23.68 Mg; 104.75 K; 176.76 Cl; 0.27 B; 0.05 Mo; 0.01 Ni; 0.13 Zn; 0.03 Cu; 0.11 Mn and 2.68 Fe. Concentrations of and Al, Si and Se were added to this nutrient solution. Irrigation was carried out daily, replacing the evaporated and/or transpired solution, calculated by weighing the pots. The pH of the solution was adjusted to 4.5 ± 0.1 with HCl or NaOH solution (1 M).

2.2 Morphological variables

At 30 and 60 days after transplanting, six plants of each treatment were collected, washed with running water and then distilled water to determine the morphological variables. The seedlings were separated into shoots and roots, placed in Kraft paper bags and dried in a forced air circulation oven at 65ºC. Afterwards, they were weighed on a precision scale (0.0001g) until constant weight was reached. With the results, shoot dry weight (SDW) and root dry weight (RDW) were calculated.

Root morphology, measured by total length, volume and diameter, was determined by washing the roots, placing them on sheets of Germitest® paper and then putting them in plastic bags in a refrigerator (4ºC). After that, the roots were placed in a transparent acrylic tray (30 × 40 cm^2) and 0.5 cm of water was added to the tray, making sure not to overlap them. Finally, they were scanned at 600 DPI in a scanner (EPSON Expression 11000 equipped with additional TPU light) and analyzed using the WinRHIZO® Pro 2007 software (Regent Instruments, Quebec, Canada).

To determine leaf area, leaf samples were also scanned in an EPSON Expression 11000 scanner equipped with additional light (TPU), but with a resolution of 200 DPI, and subsequently analyzed with the WinRHIZO® Pro 2007 software (Regent Instruments, Quebec, Canada).

2.3 Biochemical variables

At the time of collection (30 and 60 days of exposure), four plants from each treatment were separated into shoots and roots, washed with distilled water, placed in aluminum foil envelopes, and immediately frozen with liquid N to prevent sample degradation. Samples were kept in an ultra-freezer at -80°C until preparation for analysis, which was carried out through the process of manual maceration with liquid N, with each sample being macerated to a fine powder.

2.3.1 Pigment content

For the extraction of carotenoids and total chlorophylls, we used the Hiscox & Israelstan (1979) method and for estimation the Lichtenthaler equation (1987). The samples were previously weighed in 15 mL falcon tubes and arranged in grids, then 5 mL of dimethyl sulfoxide (DMSO) was added. The tubes were incubated at 65ºC for approximately 1.5 h until the pigments were completely released, resulting in a dark green solution. Afterwards, this solution was separated into two replicates of 2 mL each and solution absorbance was measured in a UV-visible spectrophotometer (1105, Bel Photonics).

2.3.2 Lipid peroxidation

Lipid peroxidation was determined according to the method by El-Moshaty *et al*. (1993). Plant samples macerated in liquid N were homogenized in 4 mL of sodium citrate buffer (pH 6.5) containing 0.5% Triton X-100. The homogenate was centrifuged at 20,000 x g for 15 min at 4°C. Then, one mL of the supernatant was added to 1 mL of 20% (w/v) trichloroacetic acid (TCA) containing 0.5% (w/v) thiobarbituric acid (TBA). The mixture was heated at 95°C for 40 min, then cooled in an ice bath for 15 min and centrifuged at 10,000 x g for 15 min. The absorbance of the supernatant was read at 532 and 600 nm (to correct for non-specific turbidity). Lipid peroxidation was expressed as nmol of malondialdehyde (MDA) mg^{-1} of protein.

2.3.4 Hydrogen peroxide content

Hydrogen peroxide (H_2O_2) content was determined according to Loreto & Velikova (2001) in which 0.3 g of roots and shoots were homogenized with 3 mL of 0.1% trichloroacetic acid (TCA) (w/v). Subsequently, the homogenate was centrifuged at 12,000 x g for 15 min at 4°C and 0.5 mL of the supernatant was added to 0.5 mL of potassium phosphate buffer (10 mM) (pH 7.0) and 1 mL of KI (1M). H_2O_2 concentration of the supernatant was analyzed by comparing its

absorbances at 390 nm with a standard calibration curve. H_2O_2 concentration was expressed as μmol g^{-1} fresh weight.

2.3.5 Antioxidant enzyme activity

To determine antioxidant enzyme activity, we used samples of leaves and roots macerated in liquid N. After maceration, 0.5 g samples were homogenized in 3.0 mL of 0.05 M sodium phosphate buffer (pH 7.8) containing 1 mM EDTA and 2% (w/v) polyvinylpyrrolidone (PVP) (Zhu et al., 2004). Afterwards, the homogenate was centrifuged at 13,000 x g for 20 min at 4°C and the supernatant was used to determine enzymatic activity.

Guaiacol peroxidase (POD) activity was determined according to Zeraik et al., (2008), using guaiacol as substrate. The reaction mixture contained 1.0 mL of potassium phosphate buffer (100 mM, pH 6.5), 1.0 mL of guaiacol (15 mM) and 1.0 mL of H_2O_2 (3.0 mM). After homogenization and centrifugation, 50 μL of the supernatant was added to the solution. Enzymatic activity was measured through the oxidation of guaiacol to tetraguaiacol by reading its absorbance at 470nm. Results were expressed as U mg^{-1} protein.

Superoxide Dismutase (SOD) activity was evaluated according to the spectrophotometric method described by Giannopolitis & Ries (1977). For the reaction, 3 ml of the mix containing 50 mM potassium phosphate buffer solution (pH 7.8), 13 mM methionine, 0.1 μM EDTA, 75 μM NBT and riboflavin (2 μM) was added to a test tube. Then, the tubes were incubated in a 15-Watt fluorescent lamp for 15 min and the absorbance was read at 560 nm. We used this method to determine the inhibition of NBT (ρ-nitro blue tetrazolium) reduction by the enzymatic extract, thus avoiding the formation of the chromophore. For calculation purposes, the reaction blank was considered to be the tubes that did not contain extract, exposed and unexposed to light. A unit of enzymatic activity (U) of SOD was defined as the amount of enzyme required to obtain 50% inhibition of NBT reduction by SOD contained in the enzymatic

extract. Activity was determined by calculating the amount of extract that inhibits 50% of the NBT reaction and expressed in U mg^{-1} protein.

2.3.6 Statistical analysis

The normality of the error distribution was checked using the Shapiro-Wilk test and the homogeneity of the error variances using the Bartlett's test (Storck et al., 2016) for all experiment variables. When these assumptions were met, analysis of variance and the Tukey's test at 5% error probability were carried out using Sisvar statistical software (Ferreira, 2019).

3 RESULTS

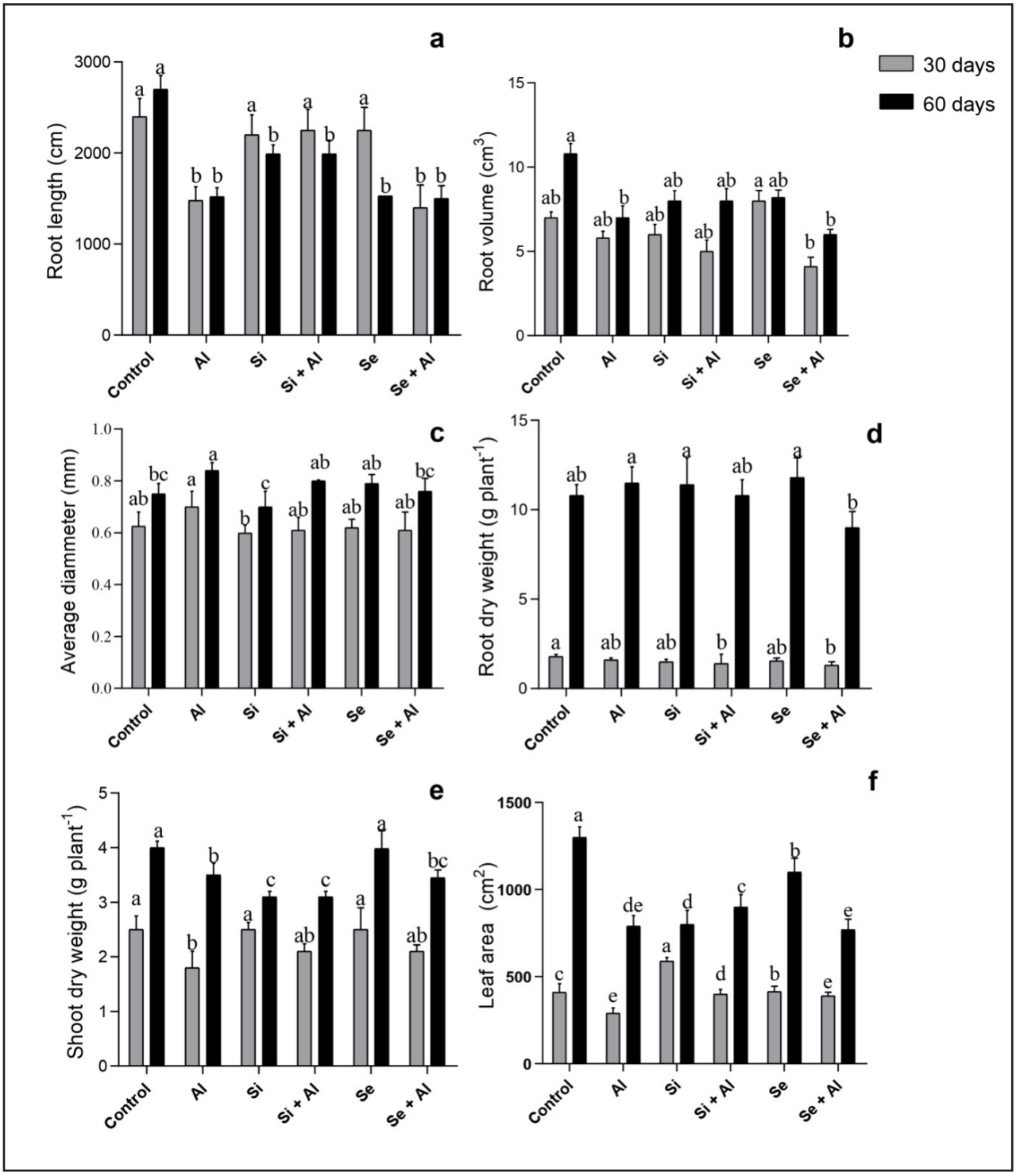
3.1 Morphological variables

According to the results of the analysis of variance, a significant effect ($p \leq 0.05$) was found for the evaluation factors (different combinations of Al with Si and Se) for the morphological variables.

At 30 days of exposure to the treatments, Al reduced total root length of *P. glomerata* seedlings exposed to the Al and Al + Se treatments in comparison to the control (Fig. 1a). However, Si mitigated the toxic effects of Al on total root length after 30 days of exposure, but Se did not. At 60 days, higher values for total root length were found only in the control (Fig. 1a).

There was no significant difference root volume among treatments at 30 days of exposure compared to the control (Fig. 1b). However, after 60 days, *P. glomerata* plants exposed to Al alone and Al + Se showed lower values of root volume (Fig. 1b).

Figure 1 – Mean values recorded for root length (a), root volume (b), average root diameter (c), shoot dry weight (d) and root dry weight (e) leaf area (f) in *Pfaffia glomerata* plants at 30 and 60 days of exposure to Al, Si and Se in a hydroponic system. Different letters between treatments represent statistical difference by the Tukey test. Bars representmean ± standard deviation



Source: Authors (2023)

For the average root diameter at 30 days of exposure, the highest values were found in the Al treatment, differing only from Si alone (Fig. 1c). At 60 days, we also found the highest values in the Al treatment, but it differed from the control, Si and Al + Se treatments (Fig. 1c).

The highest values for root dry weight (RDW) at 30 days were found in the control, differing only in the Al + Se and Al + Si treatments (Fig. 1d). However, at 60 days there was no difference for RDW between the control and the other treatments (Fig. 1d). On the other hand, the lowest value for shoot dry weight (SDW) at 30 days was found in the Al treatment, but it did not differ from the Al + Se and Al + Si treatments (Fig. 1e). At 60 days, the highest value for SDW was found in the control, but it was statistically equal to the Se treatment (Fig. 1e).

There was a significant reduction in leaf area in all treatments at 60 days of exposure compared to the control (Fig. 1f). However, the combination of Al + Si showed greater leaf area compared to Al alone, showing the potential of Si to mitigate the toxic effects of Al (Fig. 1f).

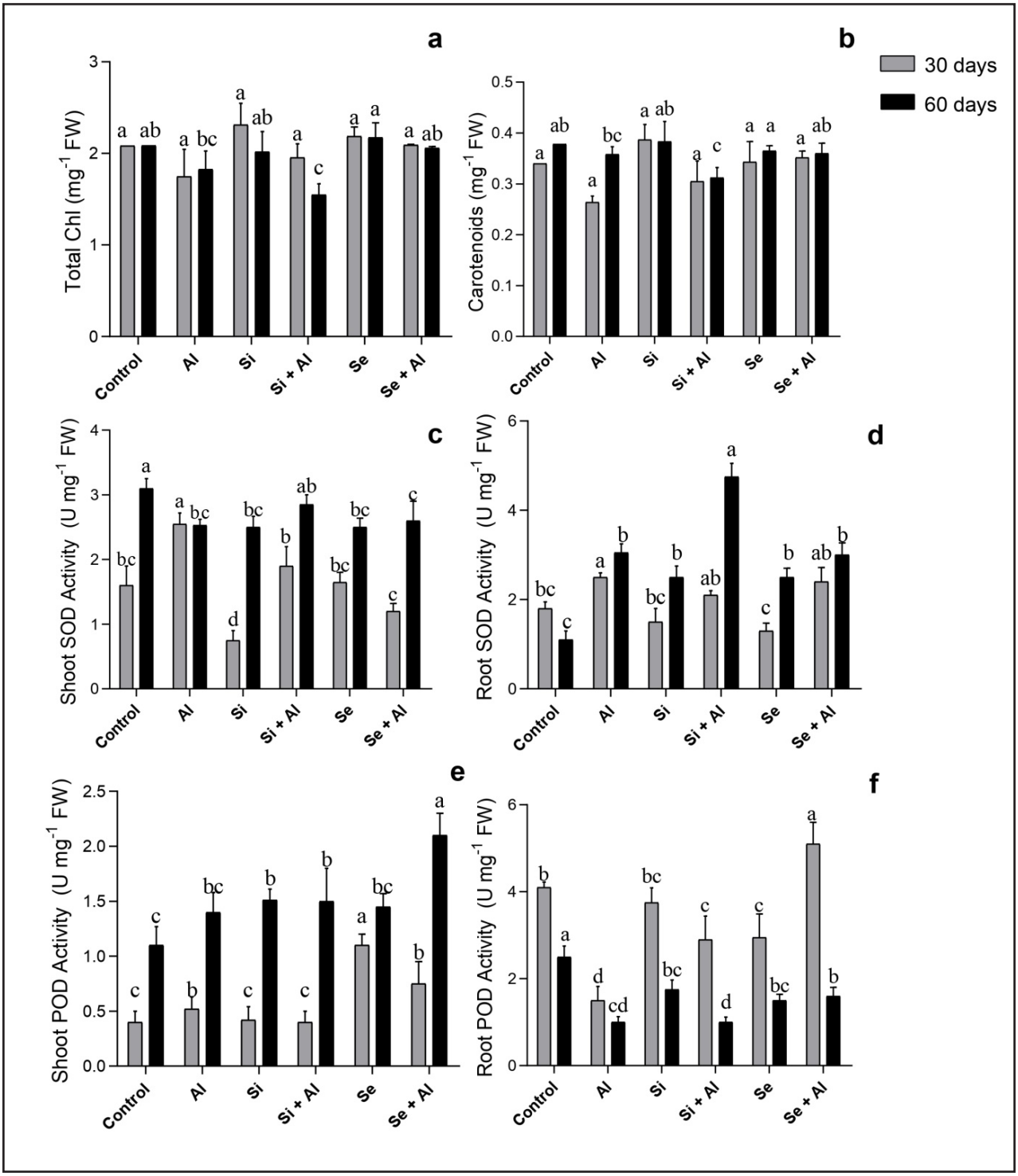
3.2 Biochemical variables

A significant effect ($p \leq 0.05$) of the different combinations of Al with Si and Se was found for the biochemical variables assessed in this study (Fig. 2 and Fig. 3).

However, for total Chl content at 30 days and for carotenoid content at 60 days, there was no significant difference, regardless of the treatment (Fig. 2a and Fig. 2b). Although a trend towards a reduction in total Chl was observed at 60 days with Al, the only treatment that differed from the control was Si + Al (Fig. 2a).

Superoxide Dismutase (SOD) activity in leaves was higher in plants exposed to Al alone at 30 days, indicating that Al promoted oxidative stress (Fig. 2c). However, at 60 days, leaf SOD activity was lower in the Al + Se and Al, Si and Se treatments compared to the control (Fig. 2c). Leaf SOD activity in the Al + Si treatment was statistically equal to the control and higher than the Al treatment (Fig. 2c).

Figure 2 – Mean values recorded for total chlorophyll (Total Chl) (a) and carotenoids (b), superoxide dismutase activity in shoots (Shoot SOD) (c) and roots (Root SOD) (d), guaiacol peroxidase activity in shoots (Shoot POD) (e) and in roots (Root POD) (f) in Pfaffia glomerata plants at 30 and 60 days of exposure to Al, Si and Se in a hydroponic system. Different letters between treatments represent statistical difference by the



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For SOD activity in roots at 60 days, the highest value was found in Al + Si, differing significantly from the other treatments (Fig. 2d). The highest root SOD activity at 30 days was observed in the Al treatment, which did not differ from Al + Se and Al + Si (Fig. 2d).

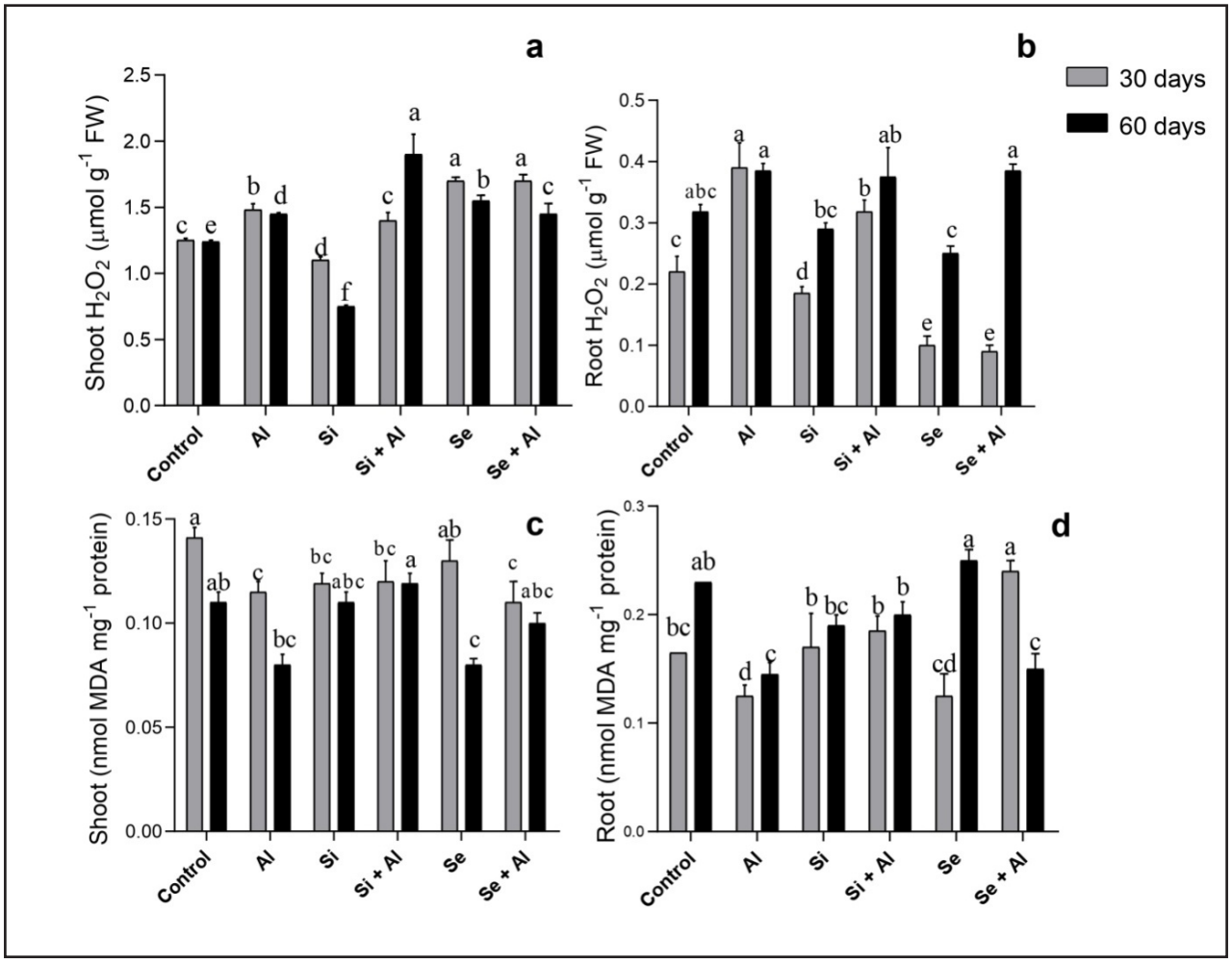
Guaiacol peroxidase (POD) activity in the leaves, at 30 days, was higher in the Al, Se and Al + Se treatments (Fig. 2e). However, leaf POD activity in Al + Si was statistically equal to the control and higher than the Al treatment. When analyzed at 60 days, Al did not significantly affect leaf POD activity compared to the control (Fig. 2e).

Aluminum promoted a reduction in root POD activity at 30 days, while there was higher POD activity in the Al + Se treatment in comparison to Al alone (Fig. 2f). Still, there was a reduction in root POD activity at 60 days for all the treatments compared to the control (Fig. 2f).

There was a reduction in hydrogen peroxide (H_2O_2) content in leaves in Al + Si in comparison to Al alone at 30 days (Fig. 3a). On the other hand, in the Al and Al + Se treatments, there was an increase in leaf H_2O_2 production at 30 and 60 days (Fig. 3a). In roots, there was no reduction in H_2O_2 content in Al + Se at 30 days in comparison to Al alone (Fig. 3b). Furthermore, the highest values for root H_2O_2 content were found in the Si treatment, significantly differing from the other treatments (Fig. 3b).

For lipid peroxidation (MDA) levels in leaves at 30 days, the highest values were found in the control, which did not differ from the Se treatment (Fig. 3c). At 60 days, leaf MDA content was only different between the control and Se treatments. However, the highest values in roots at 30 days were found in Al + Se, while lower values for root MDA levels were observed in the Al and Al + Se treatments at 60 days (Fig. 3d).

Figure 3 – Mean values recorded for hydrogen peroxide (H_2O_2) concentration in shoots (a) and in roots (b), membrane lipid peroxidation in shoots (c) and in roots (d) in *Pfaffia glomerata* plants at 30 and 60 days of exposure to Al, Si and Se in a hydroponic system. Different letters between treatments represent statistical difference by the Tukey test. Bars represent mean ± standard deviation



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4 DISCUSSION

At 30 days of exposure to the treatments, Al reduced root length and volume of *P. glomerata* in the Al and Al + Se treatments in comparison to the control (Fig. 1a and Fig. 1b). This occurred because Al strongly binds to negatively charged carboxyl groups in the cell wall of cortical and epidermal root cells, changing the binding and distribution of ions in the apoplast, which directly influences organ growth (Dorneles et al., 2016).

In contrast, the addition of Si mitigated the negative effects of Al, resulting in an increase in total root length, while Se did not have this effect (Fig. 1a). This suggests that the addition of Si under Al toxicity increases the rate of Si accumulation in the roots and consequently Si may have caused a decrease in Al toxicity by the co-deposition of Al and Si in the root epidermal cell walls (Pontigo et al., 2017). Thus, the formation of Al-Si complexes in the root apoplast is a possible Al-detoxification mechanism in plants. In addition to benefitting the root system, the formation of Al-Si complexes in the roots can also reduce the translocation of Al from the roots to other organs (e.g., leaves and stems) (Jesus et al., 2017).

There was no significant difference for root diameter at 30 days of exposure to the different treatments (Fig. 1c). On the other hand, with the increase in the exposure period (60 days) to Al, there was an increase in root diameter (Fig. 1c). However, Se mitigated the effect of Al on root diameter at 60 days, as the average diameter in Al + Se was smaller than in the Al treatment (Fig. 1c). The increase in root diameter is a typical symptom of Al toxicity, because when present in high concentrations in the soil Al paralyzes growth in root length, causing an increase in diameter (Reis et al., 2018). Thus, in the presence of Al, the roots of *P. glomerata* became shorter and larger in diameter (Fig. 1c). This effect of Al on root growth can negatively affect water and nutrient uptake, resulting in lower biomass production (Zhang et al., 2022).

The lowest values for leaf area were found in the Al treatment, but we did notice that Si mitigated the toxic effects of Al at 30 and 60 days in the Al + Si treatment (Fig. 1f). These results show that the addition of Si protects the leaf structures, possibly increasing the length of leaf epidermal cells and consequently increasing cell wall extensibility (Singh et al., 2011). Thus, the isolated presence of Al possibly decreased the length of the leaf epidermal cells, while the addition of Si in combination with the concentration of Al prevented it, resulting in increased leaf area. This result may also be related to the lower Al translocation to the shoots with the presence of Si, which is significantly relevant for the plant, as leaf area is directly related to photosynthetic rates and biomass production.

The determination of pigments involved in photosynthesis, such as total chlorophylls and carotenoids, makes it possible to observe plant photosynthetic performance, because pigments are responsible for capturing light energy necessary for photosynthesis (Cunha Neto et al., 2020). Thus, chlorophyll levels directly influence the photosynthetic capacity and growth of plants (Dorneles et al., 2019).

No significant difference was observed between the Al treatment and the control for total Chl content (Fig. 2a). This response may have occurred because Al stress did not inhibit enzymes related to the synthesis of these pigments. Furthermore, the presence of Al may not have resulted in the photodestruction of photosynthetic pigments, a phenomenon generally referred to as photooxidation, and consequently pigment concentration was not affected (Mota et al., 2020). There was also no significant difference for carotenoid content at 60 days, regardless of the treatment (Fig. 2b). Carotenoids not only function as antenna pigments for absorbing light, but also act as photoprotective pigments for the photosynthetic system. In addition to protecting chlorophylls by preventing the formation of singlet oxygen (a reactive oxygen species - ROS), they are non-enzymatic antioxidants because of their ability to suppress ROS and free radicals (Xu et al., 2020).

However, excess heavy metals stimulate plants to produce more ROS, which can react with lipids, proteins, nucleic acids and other substances, causing lipid peroxidation, membrane damage and thus affecting cell performance and viability (Zhao et al., 2021). To deal with the stress caused by ROS, one of the possible strategies used by plants is the activation of the antioxidant enzymes superoxide dismutase (SOD) and guaiacol peroxidase (POD) (Shiyab, 2019).

SOD and POD are considered the main antioxidant enzymes, playing critical roles in the elimination of ROS and acting in the maintenance of homeostasis in plant cells (Schmitt et al., 2020). SOD catalyzes the dismutation of the superoxide radical into O_2 and H_2O_2 (Lima et al., 2021), while POD acts in the conversion of H_2O_2 into water and oxygen by H_2O_2 dissociation, playing an essential role in providing tolerance to unfavorable conditions in plants (Bernardy et al., 2020).

We observed that SOD activity in leaves was higher in plants exposed to Al alone at 30 days. This result, in combination with those of the growth variables, indicates that Al promoted oxidative stress (Fig. 2c). However, the lowest SOD activity in leaves was observed when Al was applied together with the beneficial elements (Si and Se). In such cases, SOD activity was equal to that of control plants (Fig. 2c), that is, Si and Se were able to revert SOD activity to normal levels in that organ, even with the presence of Al. This response may be related to the occurrence of lower Al uptake with the presence of Si and Se and to the reduction of oxidative damage in plants through the modulation of antioxidant defense systems promoted by these elements (Zhu & Gong, 2014, Kim et al., 2017).

However, at 60 days, lower values for SOD activity in leaves were observed in the Al, Si, Se and Al + Se treatments compared to the control, while SOD activity in Al + Si was statistically equal to that of the control plants (Fig. 2c). This may be associated to the ability of Si to activate genes involved in the production of secondary compounds of metabolism (polyphenols) and enzymes (antioxidant) related to plant defense mechanisms, that is, Si mitigated the phytotoxic effect of Al on leaf SOD activity (Wang et al., 2017). Dorneles et al. (2019) found a difference in the mitigation of enzymatic activity according to exposure time to Si, thus justifying the reason it had an influence on the metal at 30 days and no decrease in enzymatic activity at 60 days.

In roots, treatments that showed an increase in SOD activity (Al, Si, Si + Al, Se and Se + Al) had a reduction in POD activity. These results justify the greater accumulation of H_2O_2 (Figure 3b) in roots in these treatments, as greater SOD activity promotes greater production of H_2O_2, which is not being converted into non-toxic compounds for the cell, since POD activity was reduced. Furthermore, Si was efficient in mitigating this effect of Al on SOD activity only after 30 days of exposure (Fig. 2). Thus, it can be inferred that Si in the medium did not significantly interfere with the toxic effect of Al on SOD activity at 60 days, which could be linked to the time of exposure to the treatment.

In leaves of *P. glomerata*, POD activity enzyme was higher in the presence of Al, Se and Al + Se treatments at 30 days (Fig. 2e). In the combination of Al + Si, POD activity in leaves was statistically equal to the control at 30 days (Fig. 2e). Thus, Si mitigated the toxic effect of Al on leaf POD activity, which means it has the potential to reverse the toxic effects of this metal through the antioxidant system.

However, for leaf POD activity at 60 days, Al did not have the same effect as that observed at 30 days, as it was statistically equal to the control in the presence of Al (Fig. 2e). There was only one activation of the enzyme in the presence of Si, Si + Al and Se + Al. This was due to Si- and Se-induced oxidative protection associated with improved antioxidant defense, suggesting a positive regulatory role for Si and Se in ROS detoxification under Al stress conditions (Mostofa et al., 2021, Shetty et al., 2021).

In roots, Al caused a reduction in POD activity and so did the Si + Al and Se treatments at 30 and 60 days (Fig. 2f). This decrease in enzyme activity can be explained by the fact that Si also acts to prevent contact between the enzyme and its phenolic substrate, or even by the removal of free monophenols as a consequence of the formation of Si-phenol complexes (Maksimovic et al., 2012).

We observed that Al caused an increase in H_2O_2 content both in leaves and in roots in both evaluation periods (Fig. 3a and Fig. 3b). It should be noted that the action of the beneficial elements was different according to the element and exposure period analyzed (Fig. 3a and Fig. 3b). In the Al + Se and Al treatments, there was an increase in the production of H_2O_2 in leaves and in POD enzyme which increases activity to detoxify H_2O_2 (Fig. 3a). However, there was a reduction in leaf H_2O_2 content in the Si + Al treatment at 30 days compared to Al alone (Fig. 3a). This was because Si inhibits the toxic effects associated with toxic metals in plants (Parrotta et al., 2015), stimulates the antioxidant system and has the ability to complex the metal ion.

H_2O_2 plays a dual role in vascular plants, inducing oxidative damage or acting as a signaling molecule in various physiological processes, including senescence, photorespiration, photosynthesis, growth and development (Pontigo et al., 2017).

However, excessive accumulation of H_2O_2 can be one of the extremely harmful factors that lead to lipid peroxidation and increased permeability of the plasma membrane.

The plasma membrane is considered one of the main targets of the phytotoxic effects of Al, as Al is able to bind to phospholipids and/or change the fatty acid composition of the plasma membrane, reducing its fluidity and increasing its permeability, and consequently resulting in increased malondialdehyde (MDA) levels (Bose et al., 2015). MDA is the product of the fragmentation of polyunsaturated fatty acids present in the membrane and is used to indicate oxidative damage in these organelles (Cai et al., 2018).

The concentration of MDA in leaves with Al + Si at 60 days and in roots at 30 and 60 days was statistically equal to that of the plants grown in the control (Fig. 3a). This likely happened because Si may be acting more significantly in alleviating Al-confined toxicity, as it stimulates antioxidant activity (Dorneles et al., 2016). The negative charge of Si complexes in the cell wall can lead to Al binding and thus inhibit Al transport in the apoplast and plant xylem. Furthermore, antioxidant enzyme activity in plants treated with Si seems to create conditions of tolerance to this type of stress, ensuring the preservation of the cell wall (Pereira et al., 2018). Thus, lower concentrations of Si lead to a reduction in the phytotoxic effects of Al.

In general, the application of Si showed greater capacity to mitigate Al toxicity, helping in the activity of antioxidant enzymes on the toxic effects of Al on *P. glomerata*, while Se was unable to mitigate Al stress. In addition, we observed that *P. glomerata* plants needed to activate the enzymatic antioxidant system at 30 days of exposure to the treatments and there was a reduction in enzymatic activity at 60 days. Therefore, we can assume that *P. glomerata* plants were able to trigger their own defense mechanisms at 60 days, not requiring the aid and/or intervention of mitigating elements to combat the effect of Al stress. Thus, changes in antioxidant activity vary according to plant species, exposure time, silicon and/or selenium concentrations and experimental conditions (Wu et al., 2017).

5 CONCLUSION

The effects of Al toxicity were significant on the growth of *Pffafia glomerata*, but Si was able to reverse the toxic action of Al, especially in shorter exposure periods, while Se did not show the potential to significantly inhibit the toxic effects of Al.

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REFERENCES

Bernardy, K., Farias, J.G., Pereira, A.S., Dorneles, A.O.S., Bernardy, D., Tabaldi, L.A., Neves, V.M., Dressler, V.L. & Nicoloso, F.T. (2020). Plants' genetic variation approach applied to zinc contamination: secondary metabolites and enzymes of the antioxidant system in Pfaffia glomerata accessions. *Chemosphere, 253*: 01-11.

Bose, J., Babourina, O., Ma, Y., Zhou, M., Shabala, S. & Rengel, Z. (2015). Specificity of Ion Uptake and Homeostasis Maintenance During Acid and Aluminium Stresses. In: Panda, S., Baluška, F. (eds) Aluminum Stress Adaptation in Plants. *Signaling and Communication in Plants, 24*.

Cai, J.-G., Luo, L.-M., Tang, H. & Zhou, L. (2018). Cytotoxicity of Malondialdehyde and Cytoprotective Effects of Taurine via Oxidative Stress and PGC-1α Signal Pathway in C2C12 Cells. *Molecular Biology, 52*: 532-542.

Casaroli, D. & Van Lier, Q.J. (2008). Critérios para determinação da capacidade de vaso. [Criteria for determining vessel capacity]. *Revista Brasileira de Ciência do Solo, 32*: 59-66.

Chen, Y.G., He, X.L.S., Huang, J.H., Luo, R., Ge, H.Z., Wołowicz, A. & Chen, S.H. (2021). Impacts of heavy metals and medicinal crops on ecological systems, environmental pollution, cultivation, and production processes in China. *Ecotoxicology and Environmental Safety, 219*: 01-17.

Cunha Neto, A.R., Ambrósio, A.S., Wolowski, M., Westin, T.B., Govêa, K.P., Carvalho, M. & Barbosa, S. (2020). Negative effects on photosynthesis and chloroplast pigments exposed to lead and aluminum: a meta-analysis. *Cerne, 26*: 232-237.

Dorneles, A.O.S., Pereira, A.S., Rossato, L.V., Possebom, G., Sasso, V.M., Bernardy, K., Sandri, R.Q., Nicoloso, F.T., Ferreira, P.A.A. & Tabaldi, L.A. (2016). Silicon reduces aluminum content in tissues and ameliorates its toxic effects on potato plant growth. *Ciência Rural, 46*: 506-512.

Dorneles, A.O.D., Pereira, P.S., Sasso, V.M., Possebom, G., Tarouco, C.P., Schor, M.R.W., Rossato, L., Ferreira, P.A.A. & Tabaldi, L.A. (2019). Aluminum stress tolerance in potato genotypes grown with silicon. *Bragantia, 78*: 12-25.

Du, H., Huang, Y., Qu, M., Li, Y., Hu, X., Yang, W., Li, H., He, W., Ding, J., Liu C., Gao, S., Cao, M., Lu, Y. & Zhang, S. (2020). A Maize ZmAT6 Gene Confers Aluminum Tolerance via Reactive Oxygen Species Scavenging. *Frontiers in Plant Science, 11*: 01-12.

El-Moshaty, F.I.B., Pike, S.M., Novacky, A.J. & Sehgal, O.P. (1993). Lipid peroxidation and superoxide productions in cowpea (Vigna unguicultata) leaves infected with tobacco rings virus or southern bean mosaic virus. *Journal Physiological and Molecular Plant Pathology, 43*: 109-119.

Ferreira, D.F. (2019). SISVAR: A computer analysis system to fixed effects Split plot type designs. *Revista Brasileira de Biometria, 37*: 529-535.

Giannopolitis, C. N. & Ries, S. K. (1977). Purification and quantitative relationship with water-soluble protein in seedlings. *Journal of Plant Physiology, 48*: 315-318.

Hiscox, J. D. & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany, 57*: 1332-1334.

Hoagland, D. R. & Arnon, D. I. *The waterculture method for growing plants without soil. Berkeley, CA: Agric. Exp. Stn., Univ. Of California.* (Circ. 347). 1950.

Huang, H., Li, M., Rizwan, M., Dai, Z., Yuan, Y., Hossain, M.M., Cao, M., Xiong, S. & Tu, S. (2020). Synergistic effect of silicon and selenium on the alleviation of cadmium toxicity in rice plants. *Journal of Hazardous Materials, 401*: 01-11.

Jesus, L.R., Batista, B.L. & Lobato, A.K.S. (2017). Silicon reduces aluminum accumulation and mitigates toxic effects in cowpea plants. *Acta Physiologiae Plantarum, 39*: 138-145.

Kim, Y.H., Khan, A. L., Waqas, M. & Lee, I. (2017). Silicon regulates antioxidant activities of crop plants under abiotic- induced oxidative stress: a review. *Frontiers in Plant Science, 8*: 01-07.

Kovács, S., Kutasy, E. & Csajbók, J. (2022). The multiple role of silicon nutrition in alleviating environmental stresses in sustainable crop production. *Plants, 11*: 01-22.

Lima, C.C., Gurgel, E.S.G. & Borges, E.E.L. (2021). Antioxidant enzyme activity in germination of Dalbergia spruceana seeds under different temperatures. *Journal of Seed Science, 43*: 01-10.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer L, Douce R (eds). *Methods in Enzimology, London: Academic Press, 148*: 350-381.

Loreto, F. & Velikova, V. (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology. 12*:1781-1787.

Luo, L., Wang, B., Jiang, J., Fitzgerald, M., Huang, Q., Yu, Z., Li, H., Zhang, J., Wei, J., Yang, C., Zhang, H., Dong, L. & Chen, S. (2021). Heavy metal contaminations in herbal medicines: Determination, comprehensive risk assessments, and solutions. *Frontiers in Pharmacology, 11*, 01-14.

Maksimovic, J.D., Mojović, M., Maksimović, V., Römheld, V. & Nikolic, M. (2012). Silicon amelio rates manganes e toxicity in cucumber by decreasing hydroxyl radical accumulation in the leaf apoplast. *Journal of Experimental Botany, 63*: 2411-2420.

Maldaner, J., Nicoloso, F.T., Tabaldi, L.A., Cargnelutti, D., Skrebsky, E.C., Rauber, R., Goncalves, J.F. & Rossato, L.V. (2015). Aluminum accumulation in two Pfaffia glomerata genotypes and its growth effects. *Ciencia Rural, 45*: 1013-1020.

Mello, R. P. (2006). *Water consumption of the Asian lily in vase with different substrata (Master's thesis)*. Universidade Federal de Santa Maria. https://repositorio.ufsm.br/handle/1/7490.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum, 15*: 473-497.

Mostofa, M. G., Rahman, M., Ansary, M. U., Keya, S.S., Abdelrahman, M., Miah, G. & Tran, L. P. (2021). Silicon in mitigation of abiotic stress-induced oxidative damage in plants. *Critical Reviews in Biotechnology* 01-18.

Mota, L. H. S., Scalon, S. P. Q., Dresch, D. M. & Silva, C. J. (2020). Emergence and physiological behavior of provenances of pinhão manso in function of level of aluminum. *Bioscience Journal, 36*: 702-712.

Nava, G., Reisser Júnior, C., Parent, L.-É., Brunetto, G., Moura-Bueno, J.M., Navroski, R., Benati, J.A. & Barreto, C.F. (2022). Esmeralda peach (Prunus persica) fruit yield and quality response to nitrogen fertilization. *Plants, 11*: 01-17.

Nicoloso, F.T. (2001). Micropropagação do Ginseng Brasileiro [Pfaffia glomerata (Spreng.) Pedersen]. *Revista Brasileira de Plantas Medicinais, 03*: 11-18.

Parrotta, L., Guerriero, G., Sergeant, K., Cai, G. & Hausman, J. (2015). Target or barrier? The cell wall of early and later diverging plants vs cadmium toxicity: differences in the response mechanisms. *Frontiers Plant Science, 6*: 01-16.

Pereira, A.S., Dorneles, A.O.S., Bernardy, K., Sasso, V.M., Bernardy, D., Possebom, G., Rossato, L.V., Dressler, V.L. & Tabaldi, L.A. (2018). Selenium and silicon reduce cadmium uptake and mitigate cadmium toxicity in Pfaffia glomerata (Spreng.) Pedersen plants by activation antioxidant enzyme system. *Environmental Science and Pollution Research, 25*: 18548-18558.

Pontigo, S., Godoy, K., Jiménez, H., Gutiérrez-Moraga, A., Mora, M.L.L. & Cartes P. (2017). Silicon-mediated alleviation of aluminum toxicity by modulation of al/si uptake and antioxidant performance in ryegrass plants. *Frontiers in Plant Science, 08*: 01-15.

Reis, A.R., Lisboa, L.A.M., Reis, H.P.G., Barcelos, J.P. de Q., Santos, E.F., Santini, J.M.K. & Lavres, J. (2018). Depicting the physiological and ultrastructural responses of soybean plants to Al stress conditions. *Plant Physiology and Biochemistry, 130*: 377-390.

Schmitt, O.J., Brunetto, G., Chassot, T., Tiecher, T.L., Marchezan, C., Tarouco, C.P., De Conti, L., Lourenzi, C. R., Nicoloso, F.T., Kreutz, M.A. & Andriolo, J.L. (2020). Impact of Cu concentrations in nutrient solution on growth and physiological and biochemical parameters of beet and cabbage and human health risk assessment. *Scientia Horticulturae, 272*: 01-09.

Shetty, R., Vidya, C. S., Prakash, N. B., Lux, A. & Vaculík, M. (2021). Aluminum toxicity in plants and its possible mitigation in acid soils by biochar: A review. *Science of The Total Environment, 65*: 01-11.

Shiyab, S. (2019). Morphophysiological effects of chromium in sour orange (Citrus aurantium L.). *HORTSCIENCE, 54*: 829-834.

Singh, V.P., Tripathi, D.K., Kumar, D. & Chauhan, D.K. (2011). Influence of exogenous silicon addition on aluminium tolerance in rice seedlings. *Biological Trace Element Research, 144*: 1260-1274.

Storck, L., Garcia, D. C., Lopes, S. J. & Estefanel, V. *Experimentação vegetal. [Vegetable experimentation], 3. ed.,* Santa Maria, RS: Editora da UFSM. 2016. 198p

Sun, L., Zhang, M., Liu, X., Mao, Q., Shi, C., Kochian, L. V. & Liao, H. (2020). Aluminum is essential for root growth and development of tea plants (Camellia sinensis). *Journal of Integrative Plant Biology, 62*: 984-987.

Tabaldi, L.A., Cargnelutti, D., Gonçalves, J.F., Pereira, L.B., Castro, G.Y., Maldaner, J., Rauber, R., Rossato, L.V., Bisognin, D.A., Schetinger, M.R.C. & Nicoloso, F.T. (2009). Oxidative stress is an early symptom triggered by aluminum in al sensitive potato plantlets. *Chemosphere, 761*:1402-1409.

Tabaldi, L.A., Nicoloso, F.T., Castro, G.Y., Cargnelutti, D., Gonçalves, J.F., Rauber, R., Skrebsky, E.C., Schetinger, M.R.C., Morsch, V.M. & Bisognin, D.A. (2007). Physiological and oxidative stress responses of four potato clones to aluminum in nutrient solution. *Brazilian Journal of Plant Physiology, 19*: 211-222.

Trippe, R.C. & Pilon-Smits, E.A.H. (2021). Selenium transport and metabolism in plants: Phytoremediation and biofortification implications. *Journal of Hazardous Materials, 404*: 124-134.

Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q. & Guo, S. (2017). Role of Silicon on Plant-Pathogen Interactions. *Frontiers in Plant Science, 8*: 01-14.

Wei, Y., Han, R., Xie, Y., Jiang, C. & Yu, Y. (2021). Recent advances in understanding mechanisms of plant tolerance and response to aluminum toxicity. *Sustainability, 13*: 01-22.

Wu, Z., Liu, S., Zhao, J., Wang, F., Du, Y., Zou, S., Li, H., Wen, D. & Huang, Y. (2017). Comparative responses to silicon and selenium in relation to antioxidant enzyme system and the glutathione-ascorbate cycle in flowering Chinese cabbage (Brassica campestris L. ssp. chinensis var. utilis) under cadmium stress. *Environmental and Experimental Botany, 133*: 01-11.

Xu, P., Chukhutsina, V. U., Nawrocki, W. J., Schansker, G., Bielczynski, L. W, Lu, Y., Karcher, D., Bock, R. & Croce, R. (2020). Photosynthesis without b-carotene. *Elife* 01-14.

Zeraik, A. E., Souza, F. S. & Fatibello-Filho, O. (2008). Desenvolvimento de um spot test para o monitoramento da atividade da peroxidase em um procedimento de purificação [Spot test development to monitor peroxidase activity in purification procedure]. *Química Nova, 31*: 731-734.

Zhang, H., Li, Xy., Lin, M., Hu, P., Lai, N., Huang, Z. & Chen, L. (2022). The aluminum distribution and translocation in two citrus species difering in aluminum tolerance. *BMC Plant Biology, 22*: 01-11.

Zhao, H., Guan, J., Liang, Q., Zhang, X., Hu, H. & Zhang, J. (2021). Effects of cadmium stress on growth and physiological characteristics of sassafras seedlings. *Scientific Reports, 11*: 01-11.

Zhu, Y.X. & Gong, H.J. (2014). Beneficial effects of silicon on salt and drought tolerance in plants. *Agronomy for Sustainable Development, 34*: 455-472.

Zhu, Z., Wei, G., Li, J., Qian, Q. & Yu, J. (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (Cucumis sativus L.). *Plant Science, 167*: 527-533.

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