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## Salicylic acid alleviates the water stress on photochemical apparatus and quality of *Schinus terebinthifolia* seedlings<sup>1</sup>

O ácido salicílico alivia o estresse hídrico no aparato fotoquímico e qualidade das mudas de *Schinus terebinthifolia*

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### HIGHLIGHTS:

Water restriction impairs the synthesis of photosynthetic pigments and the growth of *Schinus terebinthifolia* seedlings.

Chlorophyll a fluorescence is an indicator of stress in *S. terebinthifolia* seedlings.

Salicylic acid helps *S. terebinthifolia* endure stressful conditions for a longer duration without adversely affecting seedling quality.

**ABSTRACT:** Water deficit adversely affects photosynthetic pigment synthesis, photochemical processes, and seedling quality. Exogenous foliar application of salicylic acid (SA) is hypothesized to contribute to photochemical regulation and increase stress tolerance. This study aimed to evaluate the effect of SA in alleviating the effects of water deficit stress on the photosynthetic photochemical activities and quality of *Schinus terebinthifolia* Raddi. seedlings. The seedlings were subjected to four regimes of water deficit: 0, 4, 8, and 12 days of water restriction (DWR), associated with the application of four concentrations of SA: 0, 50, 100, and 200 mg L<sup>-1</sup> (via foliar spray; 10 mL per plant) during 48 days. Growth of *S. terebinthifolia* seedlings was adversely affected by longer duration of water restriction in absence of SA treatment. However, application of 200 mg L<sup>-1</sup> of SA increased photosynthetic pigment levels, photochemical quantum efficiency in photosystem II, and absorbed energy conversion efficiency in seedlings under 12 DWR. The seedling quality was better at 7 and 12 DWR when associated with application of 100 and 200 mg L<sup>-1</sup> SA, respectively. The exogenous application of SA contributed positively to pigment concentration and photochemical stability of photosynthesis, and it improved the quality of *S. terebinthifolia* seedlings subjected to long water restriction periods.

**Key words:** chlorophyll a fluorescence, photosystem II, photosynthetic pigment, phytohormone, water deficit

**RESUMO:** O déficit hídrico afeta negativamente a síntese de pigmentos fotossintéticos, os processos fotoquímicos da fotossíntese e a qualidade das mudas. Supõe-se que a aplicação exógena foliar de ácido salicílico (AS) pode contribuir na regulação fotoquímica e aumenta a tolerância ao estresse. Objetivou-se avaliar o efeito do AS em aliviar o efeito estressante do déficit hídrico sobre as atividades fotoquímicas da fotossíntese e qualidade das mudas de *Schinus terebinthifolia* Raddi. As mudas foram submetidas a quatro regimes hídricos: 0, 4, 8 e 12 dias de restrição hídrica (DRH), associadas a quatro concentrações de AS: 0, 50, 100 e 200 mg L<sup>-1</sup> (via pulverização foliar; 10 mL por planta) durante 48 dias. O crescimento de *S. terebinthifolia* é afetado negativamente por maiores dias de restrição hídrica e sem tratamento de AS. Entretanto, a aplicação de 200 mg L<sup>-1</sup> de SA incrementou os pigmentos fotossintéticos, além da eficiência quântica fotoquímica no fotossistema II e de conversão de energia absorvida nas mudas sob 12 DRH. A qualidade das mudas foi maior aos 7 e 12 DRH com aplicação de 100 e 200 mg L<sup>-1</sup> de SA, respectivamente. A aplicação exógena de AS contribuiu positivamente nas concentrações de pigmentos, estabilidade fotoquímica da fotossíntese e qualidade das mudas de *S. terebinthifolia* submetidas à maiores períodos de restrição hídrica.

**Palavras-chave:** fluorescência da clorofila a, fotossistema II, pigmentos fotossintéticos, fitohormônio, déficit hídrico

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## INTRODUCTION

In the context of the global climate change, water deficit stress is a major problem, as it adversely affects plant productivity and silvicultural activities. Under stress conditions, the intercellular CO<sub>2</sub> concentration in the substomatal chamber increases (Beltramin et al., 2020; Zandi & Schung, 2022), causing oxidative damage to chloroplasts and reducing photosynthetic pigment synthesis, thereby impairing the activities of photosystem II (PSII) and plant growth (Santos et al., 2021; Xia et al., 2022).

Therefore, research to explore substances that can alleviate the effects of water deficit stress on the photochemical apparatus has intensified to support the establishment of nursery practices and ex situ cultivation. Among the promising techniques, salicylic acid (SA), a phytohormone, effectively enhances plant tolerance to abiotic stresses (Ribeiro et al., 2020; Silva et al., 2022). It decreases oxidative stress and stabilizes photochemical metabolism and seedling quality by increasing the activity of antioxidant enzymes that act on the thylakoid membranes and inhibit D<sub>1</sub> protein degradation (Parashar et al., 2014; Hou et al., 2019).

*Schinus terebinthifolia* Raddi (Brazilian pink pepper, belonging to the Anacardiaceae family) is a tree species, found in several phytophysiognomic regions worldwide, that can reach up to 10 m height in its adult phase. It is fast growing and bears small reddish fruits that are attractive to wildlife (Lorenzi, 2008), and thereby it is a useful species for programs to restore degraded areas and agroforestry systems. However, water restriction is a limiting factor for the growth and physiology of this species (Beltramin et al., 2020).

It is hypothesized that water deficit conditions inhibit the photochemical processes of *S. terebinthifolia*, affecting the quality of its seedlings; the exogenous application of SA may positively regulate photosynthetic pigment concentration and the activity of PSII. Thus, this study aimed to evaluate the effect of different concentrations of salicylic acid in alleviating the effects of water restriction stress on the photochemical activities and quality of *S. terebinthifolia* seedlings.

## MATERIAL AND METHODS

The experiments were conducted in plastic pots of 1.5 L capacity and dimensions of 12 × 20 cm, at the School of Agricultural Sciences (22°11'43.7"S and 54°56'08.5"W; altitude: 452 m), at the Federal University of Grande Dourados (UFGD, acronym in Portuguese), Dourados, Mato Grosso do Sul, Brazil. The species was identified and an exsiccate was deposited in the DDMS Herbarium at UFGD under identification number 5688.

Initially, *S. terebinthifolia* matrices plants in the UFGD, Medicinal Plants Garden (HPM, acronym in Portuguese) were selected, considering adequate vigor patterns and phytosanitary aspects, and ripe fruits were collected from the selected plants (Access Register No. A9CDAAE - CGEN-MMA). After manual processing, the seeds were sown in 128-cell polyethylene trays filled with commercial substrate Tropstrato®, kept in 50% shade, and irrigated daily for 60 days after sowing (seedling emergence). The chemical characteristics

of Tropstrato® are: pH CaCl<sub>2</sub>, 5.75; P, 65.70 mg dm<sup>-3</sup>; K, 1.60 cmol<sub>c</sub> dm<sup>-3</sup>; Ca, 23.80 cmol<sub>c</sub> dm<sup>-3</sup>; Mg, 12.40 cmol<sub>c</sub> dm<sup>-3</sup>; Al, 0.00 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al, 4.20 cmol<sub>c</sub> dm<sup>-3</sup>; sum of bases, 39.80 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity, 42.10 cmol<sub>c</sub> dm<sup>-3</sup>; and base saturation (V%), 64.80.

The pots were filled with Oxisols of clayey texture and coarse sand (3:1, v/v) with the following chemical attributes without correction soil: pH CaCl<sub>2</sub>, 5.67; P, 27.92 mg dm<sup>-3</sup>; K, 0.63 cmol<sub>c</sub> dm<sup>-3</sup>; Ca, 8.55 cmol<sub>c</sub> dm<sup>-3</sup>; Mg, 2.04 cmol<sub>c</sub> dm<sup>-3</sup>; Al, 0.00 cmol<sub>c</sub> dm<sup>-3</sup>; H + Al, 2.37 cmol<sub>c</sub> dm<sup>-3</sup>; sum of bases, 11.22 cmol<sub>c</sub> dm<sup>-3</sup>; cation exchange capacity, 13.59 cmol<sub>c</sub> dm<sup>-3</sup>; S, 4.22 mg dm<sup>-3</sup>; B, 0.48 mg dm<sup>-3</sup>; Fe, 52.13 mg dm<sup>-3</sup>; Cu, 3.90 mg dm<sup>-3</sup>; Mn, 78.60 mg dm<sup>-3</sup>; Zn, 1.75 mg dm<sup>-3</sup>; organic matter, 20.82 g dm<sup>-3</sup> and base saturation (V%), 82.6.

When the seedlings achieved an average height of 7.0 cm, they were transplanted into pots and placed in the nursery of the experimental site covered with a 30% shaded black nylon screen and additional upper and lateral plastic coverings of 150 μm thickness, to protect them from rainfall. Thirty days after transplanting (DAT), the seedlings were irrigated daily to maintain 70% water retention capacity (WRC) in the substrate according to Souza et al. (2000), characterizing the acclimatization period; at 15 DAT, nitrogen fertilization was applied (50 mg kg<sup>-1</sup> soil) using urea (45% N).

Following acclimatization period, salicylic acid (SA) was applied to the seedlings at concentrations: 0, 50, 100, and 200 mg L<sup>-1</sup> (based on the work of Mazzuchelli et al., 2014), via spraying on the abaxial and adaxial sides of the leaf limbs, up to the dripping point, in the morning at 24 hours intervals, for 10 days before the seedlings were subjected to the restriction periods (20–30 DAT), characterizing the seedlings' priming. Then, the seedlings were subjected to four water restriction periods, based on irrigation regimes of: 0 [daily irrigation to maintain 70% of water retention capacity (WRC)], 4, 8, and 12 days of water restriction (DWR), based on previous unpublished results for this experiment. At the end of each water restriction period, irrigation by one day was resumed for the corresponding seedlings, maintaining 100% of the WRC (Souza et al., 2000), followed by DWR, totaling 0, 12, 6, and 4 cycles of irrigation restriction/resumption for each period.

The experimental design adopted was a randomized block in a subplot scheme, with restriction periods in the plots and SA application in the subplots, with three replicates. The experimental unit consisted of four pots, each with one seedling.

At 48 days after the water restriction initiation (78 DAT), when the seedlings had an average height of 20.0 cm, the photosynthetic and seedling characteristics were evaluated.

For photosynthetic pigments analysis, fully expanded leaves were collected, and 1 g of leaf sample was macerated in 8 mL of acetone (80 %), thereafter the solutions were stored in test tubes wrapped with laminated paper at a temperature of 4 °C for 24 hours. After 24 hours, the samples were centrifuged for 10 min at 3000 rpm, and the absorbance of the supernatant was measured using a spectrophotometer at wavelengths of 470, 645, and 663 nm. Chlorophyll a, b, and total (a + b) (Arnon, 1949) and carotenoid (Lichtenthaler & Welburn, 1983) contents were calculated.

Chlorophyll a fluorescence and PSII activities were estimated by selecting fully expanded leaves of two plants from each plot and subjecting them to dark conditions for 30 min between 8:00 and 10:00 am by using leaf clips. Under a flash of  $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ , using a portable fluorometer (OS-30p; Opti-Sciences Chlorophyll Fluorometer, Hudson, NY, USA), the initial ( $F_0$ ) and maximum ( $F_m$ ) chlorophyll-a fluorescence emissions were recorded. The variable fluorescence ( $F_v = F_m - F_0$ ), the potential photochemical quantum efficiency of PSII ( $F_v/F_m$ ), and the absorbed energy conversion efficiency ( $F_v/F_0$ ) were determined.

The Dickson quality index (DQI) was calculated using growth and dry biomass data, according to Dickson et al. (1960).

The data were subjected to analysis of variance and regression analysis to evaluate the linear and quadratic effects ( $p \leq 0.05$ ) using SISVAR software.

## RESULTS AND DISCUSSION

The concentrations of photosynthetic pigments in *S. terebinthifolia* seedlings were influenced by the interaction between the various concentrations of salicylic acid (SA) and duration of water restriction; the exogenous application of SA contributed to an increase in chlorophyll a content, with a maximum content ( $27.12 \mu\text{g cm}^{-2}$ ) in seedlings developed at 6 DWR regime when treated with  $200 \text{ mg L}^{-1}$  of SA (Table 1). However, without SA application, with a mean chlorophyll a value of  $11.57 \mu\text{g cm}^{-2}$ , which was lower than that of the seedlings treated with SA, the data did not fit the regression models tested.

The highest chlorophyll b content was  $12.02 \mu\text{g cm}^{-2}$  in the seedlings without SA application, whereas in all the other treatments, it was comparatively less (Table 1). Under stress conditions of 12 DWR, an increase in chlorophyll b by conversion of chlorophyll a through the enzyme chlorophyll a

**Table 1.** Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids concentrations in leaves of *Schinus terebinthifolia* Raddi. seedlings produced under days of water restriction (DWR) with salicylic acid (SA) concentrations

| Salicylic acid ( $\text{mg L}^{-1}$ )       |   | C.V. (%) |
|---|---|----------|
| Chlorophyll a ( $\mu\text{g cm}^{-2}$ )     |   |          |
| 0   | $\hat{y} = \hat{y} = 11.57$   | 14.19    |
| 50  | $\hat{y} = 22.0275 - 2.0193 \cdot \text{DWR} + 0.1585 \cdot \text{DWR}^2; R^2 = 0.61$ |          |
| 100   | $\hat{y} = 24.0401 - 2.0902 \cdot \text{DWR} + 0.1208 \cdot \text{DWR}^2; R^2 = 0.80$ |          |
| 200   | $\hat{y} = 23.2193 + 1.1935 \cdot \text{DWR} - 0.0905 \cdot \text{DWR}^2; R^2 = 0.94$ |          |
| Chlorophyll b ( $\mu\text{g cm}^{-2}$ )     |   |          |
| 0   | $\hat{y} = 5.5850 + 0.5354 \cdot \text{DWR}; R^2 = 0.95$                              | 10.05    |
| 50  | $\hat{y} = 8.4650 + 0.7662 \cdot \text{DWR} - 0.0630 \cdot \text{DWR}^2; R^2 = 0.66$  |          |
| 100   | $\hat{y} = 10.5570 - 0.2170 \cdot \text{DWR}; R^2 = 0.61$                             |          |
| 200   | $\hat{y} = 11.7136 - 0.6649 \cdot \text{DWR}; R^2 = 0.81$                             |          |
| Total chlorophyll ( $\mu\text{g cm}^{-2}$ ) |   |          |
| 0   | $\hat{y} = \hat{y} = 20.58$   | 12.23    |
| 50  | $\hat{y} = 30.4998 - 1.2543 \cdot \text{DWR} + 0.0956 \cdot \text{DWR}^2; R^2 = 0.97$ |          |
| 100   | $\hat{y} = 32.6740 - 0.8565 \cdot \text{DWR}; R^2 = 0.80$                             |          |
| 200   | $\hat{y} = 34.7844 + 0.5919 \cdot \text{DWR} - 0.0958 \cdot \text{DWR}^2; R^2 = 0.94$ |          |
| Carotenoids ( $\mu\text{g cm}^{-2}$ )       |   |          |
| 0   | $\hat{y} = 3.6033 - 0.2170 \cdot \text{DWR}; R^2 = 0.72$                              | 8.46     |
| 50  | $\hat{y} = 5.6172 - 0.7944 \cdot \text{DWR} + 0.0586 \cdot \text{DWR}^2; R^2 = 0.65$  |          |
| 100   | $\hat{y} = 4.5502 - 0.7002 \cdot \text{DWR} + 0.0476 \cdot \text{DWR}^2; R^2 = 0.86$  |          |
| 200   | $\hat{y} = 4.0113 + 0.1749 \cdot \text{DWR}; R^2 = 0.81$                              |          |

\*- Significant at  $p \leq 0.05$  by F test

oxygenase is desirable because it is a photoprotective accessory pigment which maintains the integrity of photochemical processes (Li et al., 2021).

Total chlorophyll content was the lowest ( $3.73 \mu\text{g cm}^{-2}$ ) at 12 DWR with  $200 \text{ mg L}^{-1}$  of SA, and the maximum concentration ( $34.54 \mu\text{g cm}^{-2}$ ) was found in seedlings grown under 6 DWR with  $200 \text{ mg L}^{-1}$  of SA. The mean value of total chlorophyll was  $20.58 \mu\text{g cm}^{-2}$  without SA application and this data did not fit the regression models tested. In the present study, higher concentrations were found, especially of chlorophyll a under these same conditions, indicating that daily irrigation or water restriction decreased the concentration of photosynthetic pigments, although SA inhibited its degradation.

In case of water deficit, similar findings of decreased chlorophyll content under lower water availability have been reported in seedlings of several tree species (Santos et al., 2021; Zuazo et al., 2021). SA application, Ribeiro et al. (2020) observed increase in chlorophyll content with exogenous application of SA, which favored higher photochemical yields, results similar to those observed in this study with *S. terebinthifolia*.

The seedlings had the highest carotenoid content ( $6.11 \mu\text{g cm}^{-2}$ ) when grown under 12 DWR with  $200 \text{ mg L}^{-1}$  of SA, while the lowest content ( $0.99 \mu\text{g cm}^{-2}$ ) was found in the same treatment without exogenous application of SA. The increase in carotenoid pigment content favored a higher chlorophyll a content at the same SA concentration. Considering the protective effect of carotenoids on photo-oxidation and dissipation of excess heat (Zielewicz et al., 2020), its observed increase in *S. terebinthifolia*, indicates that SA enhances antioxidant activities (Hou et al., 2019), which contributed to alleviating oxidative stress in *S. terebinthifolia* seedlings. Zuazo et al. (2021) reported that *Mangifera indica* L. exhibited low growth, chlorophyll a, and increased carotenoids in response to water deficit.

The decrease in chlorophyll under low water availability in the growth substrate creates a stress condition, possibly by generation of reactive oxygen species (ROS), which result in collapse of the thylakoid and chloroplast membranes (Zhu, 2016), and degradation of photosynthetic pigments, as observed in our study. Therefore, for *S. terebinthifolia* seedlings, the tested SA concentrations, especially  $200 \text{ mg L}^{-1}$ , contributed to an increase in chlorophylls and carotenoids even under water deficit stress conditions for this species, which demonstrate the mitigating potential of the phytohormone. Similarly, Silva et al. (2022) verified that foliar application of SA attenuated the stress effects on photosynthetic pigments in *Annona muricata* L. seedlings.

However, high SA concentrations can compromise chloroplast structure and pigment concentration. For example, Poór et al. (2019) found that SA concentrations starting from 1 mM led to thylakoid depletion, and lumen deformation, which affected total chlorophyll content.

The duration of water restriction affected the initial ( $F_0$ ), maximum ( $F_m$ ), and variable ( $F_v$ ) chlorophyll a fluorescence (Table 2), where the highest values were 0.240, 0.554, and 0.432 electrons per quantum at 6, 7, and 12 DWR, respectively. Lower values of  $F_0$  and  $F_m$  were observed in seedlings that were

**Table 2.** Initial ( $F_0$ ), maximum ( $F_m$ ) and variable ( $F_v$ ) chlorophyll a fluorescence in leaves of *Schinus terebinthifolia* Raddi. seedlings in function of days of water restriction (DWR)

| $F_0$ – Initial fluorescence (electrons per quantum)                                    |
|---|
| $\hat{y} = 0.1284 + 0.1367 \cdot \text{DWR} - 0.0030 \cdot \text{DWR}^2$ ; $R^2 = 0.96$ |
| C.V. (%) = 9.76   |
| $F_m$ – Maximum fluorescence (electrons per quantum)                                    |
| $\hat{y} = 0.4994 + 0.0163 \cdot \text{DWR} - 0.0012 \cdot \text{DWR}^2$ ; $R^2 = 0.62$ |
| C.V. (%) = 15.21  |
| $F_v$ – Variable fluorescence (electrons per quantum)                                   |
| $\hat{y} = 0.3709 + 0.0204 \cdot \text{DWR} - 0.0017 \cdot \text{DWR}^2$ ; $R^2 = 0.94$ |
| C.V. (%) = 12.78  |

\*- Significant at  $p \leq 0.05$  by F test

irrigated daily (0 DWR) or subjected to 12 DWR whereas  $F_v$  was the lowest in seedlings under 6 DWR.

As the restriction duration extended,  $F_0$  increased starting at 6 DWR, and then decreased at 12 DWR. The increase under 6 DWR may be caused by stomatal closure and elevated intercellular carbon concentration. However, the species exhibited the ability to adapt to water deficit because of the increased electron transfer capacity associated with  $F_v$  on PSII. Therefore, the plant may have inherent mechanisms to overcome stress and resume photochemical metabolism. The higher  $F_v$  starting at 7 DWR contributed to the decrease in  $F_0$  as they presented similar responses during the same period.

The decrease in  $F_m$  and  $F_v$  indicates disruption of the photochemical activities in PSII and electron transport, a phenomenon observed both under daily irrigation or low water availability in the substrate, characterized by 0 water restriction and 12 DWR, respectively, reinforcing that water deficit leads to impairment of the reaction centers, although the disruptions are reversible and do not compromise the photosynthetic apparatus.

$F_v/F_m$  and  $F_v/F_0$  were influenced by the interaction between the SA concentrations and DWR, with lower photochemical activities in PSII of seedlings developed under 12 DWR without SA application (Table 3). The  $F_v/F_m$  ratio values at 12 DWR without SA application and with 50 mg L<sup>-1</sup> of SA were 0.602 and 0.617 electrons per quantum, respectively. With the exogenous application of 100 and 200 mg L<sup>-1</sup> SA, an increase in photochemical stability was observed due to the influence of the photochemical quantum efficiency of PSII ( $F_v/F_m$ ), and the highest values were 0.755 and 0.771 electrons per quantum in the seedlings produced under 12 DWR, respectively (Table 3).

The reduction in  $F_v/F_m$  without SA under 12 DWR (Table 3) was a result of the decreased contents of chlorophylls and carotenoids (Table 1) and subsequent interference with the production and transport of electrons, indicating the stressed condition for *S. terebinthifolia* seedlings. Values below 0.750 are considered critical for this characteristic (Maxwell & Johnson, 2000), leading to photoinhibition by damage to the photosynthetic apparatus (Campelo et al., 2015), resulting in lower production of photo-assimilates and biomass. This condition promotes impaired electron energy transport between acceptors in the photosystems, increasing the maximum basal yield of non-photochemical processes.

In general, with treatment between 4 and 8 DWR, the quadratic adjustment with minimum points for photosynthetic pigments and lower efficiency of photochemical yields in *S.*

**Table 3.** Potential photochemical quantum efficiency in PSII ( $F_v/F_m$ ), absorbed energy conversion efficiency ( $F_v/F_0$ ), and Dickson quality index (DQI) of *Schinus terebinthifolia* Raddi. seedlings produced under days of water restriction (DWR) with salicylic acid (SA) concentrations

| Salicylic acid (mg L <sup>-1</sup> ) |   | C.V. (%) |
|--------------------------------------|---|----------|
| $F_v/F_m$ (electrons per quantum)    |   |          |
| 0                                    | $\hat{y} = 0.7199 - 0.0619 \cdot \text{DWR} + 0.0050 \cdot \text{DWR}^2$ ; $R^2 = 0.98$ | 11.98    |
| 50                                   | $\hat{y} = 0.7656 - 0.0536 \cdot \text{DWR} + 0.0034 \cdot \text{DWR}^2$ ; $R^2 = 0.79$ |          |
| 100                                  | $\hat{y} = 0.7392 - 0.0577 \cdot \text{DWR} + 0.0049 \cdot \text{DWR}^2$ ; $R^2 = 0.97$ |          |
| 200                                  | $\hat{y} = 0.7426 - 0.0624 \cdot \text{DWR} + 0.0054 \cdot \text{DWR}^2$ ; $R^2 = 0.99$ |          |
| $F_v/F_0$ (electrons per quantum)    |   |          |
| 0                                    | $\hat{y} = 2.3971 - 0.3686 \cdot \text{DWR} + 0.0307 \cdot \text{DWR}^2$ ; $R^2 = 0.89$ | 17.23    |
| 50                                   | $\hat{y} = 3.3931 - 0.7258 \cdot \text{DWR} + 0.0597 \cdot \text{DWR}^2$ ; $R^2 = 0.98$ |          |
| 100                                  | $\hat{y} = 2.7964 - 0.5331 \cdot \text{DWR} + 0.0465 \cdot \text{DWR}^2$ ; $R^2 = 0.76$ |          |
| 200                                  | $\hat{y} = 2.3971 - 0.3686 \cdot \text{DWR} + 0.0307 \cdot \text{DWR}^2$ ; $R^2 = 0.98$ |          |
| DQI                                  |   |          |
| 0                                    | $\hat{y} = \hat{y} = 0.11$  | 12.27    |
| 50                                   | $\hat{y} = \hat{y} = 0.12$  |          |
| 100                                  | $\hat{y} = 0.0902 + 0.0161 \cdot \text{DWR} - 0.0011 \cdot \text{DWR}^2$ ; $R^2 = 0.85$ |          |
| 200                                  | $\hat{y} = 0.2025 - 0.0279 \cdot \text{DWR} + 0.0023 \cdot \text{DWR}^2$ ; $R^2 = 0.92$ |          |

\*- Significant at  $p \leq 0.05$  by F test

*terebinthifolia* is due to the increase in  $F_0$  in this period, and the higher  $F_v$  subsequently may be attributed to the physiological response of this species to SA application.

Degradation of photosynthetic pigments occurred under a longer duration of soil water restriction and no SA (Table 1). Since these pigments are responsible for light interception and electron transfer excitation processes, their degradation decreased the photochemical activity of PSII ( $F_v/F_m$ ). The decreased PSII activity leads to increased excitation energy, resulting in excessive energy, ROS production, and lipid peroxidation (Santos et al., 2021; Zhao et al., 2021), thus inhibiting NADPH oxidation and electron transfer to the reaction centers (Xiong et al., 2021).

Regarding the absorbed energy conversion efficiency ( $F_v/F_0$ ), the highest ratio was 4.04 electrons per quantum at 12 DWR with 200 mg L<sup>-1</sup> of SA, while in the same treatment regime, seedlings without SA had the lowest value (2.21 electrons per quantum) (Table 3). The beneficial effect of SA on  $F_v/F_m$  and  $F_v/F_0$  is associated with the delay/attenuation of D<sub>1</sub> protein degradation and functional damage to PSII, improved adenosine triphosphate (ATP) production (Hou et al., 2019) and enhanced activity of antioxidant enzymes, including superoxide dismutase and the amino acid proline (Parashar et al., 2014), which are important for preventing membrane damage by scavenging reactive oxygen species (Jini & Joseph, 2017).

The results of this study confirmed the hypothesis that exogenous application of SA mitigates oxidative damage in the photochemical processes of photosynthesis in *S. terebinthifolia* seedlings exposed to longer periods of water restriction stress. According to Ozfidan et al. (2013), the  $F_v/F_0$  ratio is an indicator of the potential for conversion of light energy into chemical energy in PSII, presenting conditions that can cause disturbances in photosynthetic photochemical reactions, and its decrease can compromise the functional efficiency of the photosynthetic apparatus, particularly decreased  $F_v/F_m$ , as observed in our study with *S. terebinthifolia* seedlings.

Overall, prolonged exposure of the seedlings to water deficit, characterized by 12 DWR, compromised the photochemical activities in PSII. When plants are subjected to water restriction conditions, energy loss occurs because the absorbed light energy is dissipated rather than used in photochemical reactions (Lawlor & Tereza, 2009; Santos et al., 2021).

However, even under low water availability conditions (12 DWR), the exogenous application of SA enhanced the chlorophyll a concentration, and a higher dose of SA contributed to tolerance to water restriction (Table 1), increasing the capture and excitation of electrons, maintaining stable transfer between acceptors, and regulating the photosynthetic processes of *S. terebinthifolia*.

Furthermore, the characteristics based on chlorophyll a fluorescence emission can be used as tools to investigate and understand the changes caused to the photosynthetic apparatus through the photochemical responses of PSII of *S. terebinthifolia* seedlings subjected to stress by water restriction.

The Dickson quality index (DQI) was influenced by the interaction between the factors in this study. *S. terebinthifolia* seedlings subjected to 7 and 12 DWR with 100 and 200 mg L<sup>-1</sup> of SA had a DQI of 0.15 and 0.19, respectively (Table 3). The increased DQI of *S. terebinthifolia* seedlings with SA application under water restriction demonstrates that SA contributed to the tolerance and increased photoassimilates in this species even under adverse conditions. DQI is an aggregation of the main biometric characteristics and photo assimilate production, which indicates that the seedling is adequately developed for transfer to the field and has sufficient potential for survival and development.

The DQI has been used to evaluate seedling standards of many species, especially those of forests and/or fruit trees. The DQI value obtained in this study for *S. terebinthifolia* treated with 200 mg L<sup>-1</sup> of SA is close to the minimum reference standards since Fonseca et al. (2002) described that the minimum value should be 0.20. However, these values can vary with species and their degree of development, and further studies should be conducted considering the post-transplantation development of seedlings in the field.

The results of our study demonstrate that SA has potential as a mitigating agent for water deficit stress effects on the physiology and growth of *S. terebinthifolia* seedlings, increasing their tolerance for possible introduction in degraded areas or integrated systems, which are characterized by limited irrigation systems and/or areas with irregular rainfall. SA treatment can contribute to the success of silvicultural activities, especially in the seedling stage. In addition, we suggest further studies aimed at determining the antioxidant enzyme activity and membrane integrity of this species in response to water deficit and priming with exogenous application of SA.

## CONCLUSIONS

1. Water restriction lead to decreased concentrations of total chlorophyll and chlorophyll a, impaired photochemical apparatus, and lower Dickson quality indices for *Schinus terebinthifolia* seedlings.

2. Foliar application of 200 mg L<sup>-1</sup> salicylic acid enhanced the photosynthetic pigment concentrations, photochemical

processes in photosystem II, and Dickson quality indices of seedlings subjected to 12 days of water restriction.

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