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Original Paper

Salicylic acid mitigating damage to the photosynthetic apparatus and quality of *Eugenia myrcianthes* seedlings under water deficit

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Abstract

This study aimed to evaluate the effect of exogenous application of salicylic acid on the photosynthetic metabolism and quality of *Eugenia myrcianthes* seedlings under water deficit and their recovery potential after rehydration. Two water regimes were evaluated, as follows: control - plants irrigated daily (I) and water deficit (S), with and without the application of 400 mg L^{-1} of salicylic acid (SA), totaling four treatments. Seedlings were evaluated at three times: at the beginning of the experiment (T0), that is, when the irrigation was interrupted; when the photosynthetic rate reached values close to zero (P0 - 15 days after irrigation interruption), that is, when irrigation restarted; and when the photosynthetic rate was recovered (REC). *Eugenia myrcianthes* seedlings were negatively affected when subjected to water restriction; salicylic acid attenuated the damage to the photosynthetic apparatus by acting positively on the relative water content in the leaves, SPAD index, photosynthetic metabolism, superoxide dismutase and peroxidase enzyme activity, and seedling growth in P0. *Eugenia myrchiantes* presented potential for recovery after resumption irrigation. The application of SA contributed to the maintenance of gas exchanges, photochemical processes and quality of *E. myrcianthes* seedlings during and after water deficit, suggesting the promotion of plant resistance induction.

Key words: antioxidant enzymes, chlorophyll *a* fluorescence, ecophysiology, gas exchange, phytohormone.

Resumo

Este trabalho teve como objetivo avaliar o efeito da aplicação exógena de ácido salicílico sobre o metabolismo fotossintético e a qualidade de mudas de *Eugenia myrcianthes* sob deficiência hídrica e seu potencial de recuperação após reidratação. Foram avaliados dois regimes hídricos: controle - plantas irrigadas diariamente (I) e déficit hídrico (S), com e sem a aplicação de 400 mg L^{-1} de ácido salicílico (AS), totalizando quatro tratamentos. As mudas foram avaliadas em três momentos: no início do experimento (T0) quando a irrigação foi interrompida; quando a taxa fotossintética atingiu valores próximos a zero (F0 - 15 dias após a suspensão hídrica), quando a irrigação foi reiniciada; e quando a taxa fotossintética foi recuperada (REC). Mudas de *E. myrcianthes* foram afetadas negativamente quando submetidas à restrição hídrica; o ácido salicílico atenuou os danos ao aparato fotossintético por agir positivamente sobre o conteúdo relativo de água nas folhas, índice SPAD, metabolismo fotossintético, atividade das enzimas superóxido dismutase e peroxidase e crescimento das mudas na F0. *Eugenia myrchiantes* apresentou potencial de recuperação após a retomada da irrigação. A aplicação de AS contribuiu na manutenção das trocas gasosas, processos fotoquímicas e qualidade de mudas de *E. myrcianthes* durante e após o deficit hídrico, sugerindo a promoção da indução de resistência de plantas.

Palavras-chave: enzimas antioxidantes, fluorescência da clorofila *a*, ecofisiologia, trocas gasosas, fitohormônio.

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Introduction

Native fruit species represent a genetic heritage of great value and potential for economic and ecological expression, such as *Eugenia myrcianthes* Nied. (Myrtaceae), a pioneer, deciduous tree species popularly known as wild peach, whose fruits and leaves present antibacterial activity (Dexheimer & Pozzobon 2017; Santos *et al*. 2014) and leaf infusion is rich in antioxidants and phenolic compounds (Takao *et al*. 2015). This species has a wide distribution in Brazil in the states of São Paulo, Mato Grosso do Sul, Minas Gerais, Goiás, and Rio Grande do Sul, where it is found in forest formations in the Atlantic complex, in addition to semi-deciduous seasonal forests and the Cerrados (Santos *et al*. 2014; Oliveira *et al*. 2015).

In the Cerrado, *E. myrcianthes* is found in different forms of vegetation, including forest (Cerradão) and savanna (Cerrado stricto sensu) phytophysiognomies (Oliveira *et al*. 2015), suggesting its physiological plasticity and good adaptability to different water conditions. Thus, it is necessary to understand the responses of plants under conditions of water deficit, as climate change can reduce water availability in some regions (Kim *et al*. 2011). This knowledge can favor the implementation of projects for the restoration of degraded areas and the use of these plants in biodiversified systems, such as agroforestry, adding economic and environmental value to the farmer.

Low soil water availability can cause metabolic changes in several forest species, such as stomatal closure increased activity of antioxidant enzymes, reductions in stomatic conductance, photosynthesis, transpiration in addition to damage to the photosynthetic apparatus, which leads to a decline in the growth rate (Reis *et al*. 2020). In addition, water deficit is one of the limiting environmental factors for the initial establishment of most species, especially native trees, because it destabilizes photochemical activities in photosystem II - PS II (Santos *et al*. 2021), promoting tissue dehydration, and limiting the production of photoassimilates (Beltramin *et al*. 2020; Saracho *et al*. 2021).

In this context, the use of some phytohormones has stimulated mechanisms of tolerance of plants to adverse environmental conditions (Fermiano *et al*. 2018). Salicylic acid (SA) is a phenolic compound that acts as a signal and messenger for molecules that defend plants against abiotic stresses, participates in the regulation of physiological processes, and plays a fundamental role in tolerating water stress (Azooz & Youssef 2010; Fermiano *et al*. 2018; Brito *et al*. 2019).

Salicylic acid acts in the regulation of transpiration and stomatal conductance, favoring the maintenance of cell turgor even under water deficit conditions, directly affecting plant growth, and assisting in the pos-stress recovery (Hayat *et al*. 2012). Exogenous stimuli with SA alter of SA favored the sínteses of pigments photosynthetic, and improved the accumulation of osmolytes such as proline and antioxidant enzyme activity, alleviating the damage to the photosynthetic apparatus, but its effects depend on the species, concentration, application method, and plant exposure time to SA (Nivedithadevi *et al*. 2012; Sharma *et al*. 2017; Janah *et al*. 2021). However, there are not studies in the literature evaluating the potential of the application do SA in seedlings of this species during and after the period of deficit.

Considering the wide distribution of *E. myrcianthes* and the scarcity of information on this species, besides the good results found through SA application for attenuating water stress in other species, this study seeks to prove that salicylic acid can contribute to the stability of the processes in PS II and gas exchange, potentiating enzymatic activity, favoring *E. myrcianthes* growth under water deficit, and assisting in the recovery of seedlings after the stress is interrupted.

Thus, this study aimed to evaluate the effect of exogenous application of salicylic acid on *E. myrcianthes* seedlings under water deficit and their potential for recovering the gas exchange, chlorophyll *a* fluorescence, enzymatic activity and growth during and after rehydration.

Material and Methods

Ripe *E. myrcianthes* fruits were collected in a remaining Cerrado area and manually processed to extract the seeds that were sown in 54-cell trays filled with the commercial substrate Carolina®. The seedlings were transplanted at 60 days after emergence (15 cm in height) into 7.0-liter pots filled with dystrophic Red Latosol + sand (1:1, v/v), where they remained for 60 days for acclimatization and beginning of the experiment.

The experiment was carried out at the School of Agricultural Sciences (22°11’43.7”S and 54°56’08.5”W, 452 m) of the Federal University of Grande Dourados (UFGD), Dourados, MS, Brazil, in a nursery under 30% shading with additional and superior protection of a 150-micron plastic cover, aiming to avoid precipitations.

Two water regimes were evaluated: control - plants irrigated daily (I), with 75% water retention capacity throughout the experimental cycle, and water deficit - stressed plants (S), which were subjected to irrigation restrictions after the beginning of the experiment at zero time (T0) until the plants reached photosynthesis close to zero (P0) when irrigation was restarted. Plants in both water regimes received (+) or not (–) exogenous application of 400 mg L^{-1} of salicylic acid (SA), totaling four treatments: 1) irrigation with SA application (I+SA), 2) irrigation without SA application (I–SA), 3) water stress with SA (S+SA), and 4) water stress without SA (S–SA). Three SA applications were performed within a three-day interval, the first being carried out at the beginning of the experiment (T0). The SA dilutet in distilled water and applied in the morning via foliar spraying onto the abaxial and adaxial surfaces until drip point (Saracho *et al.* 2021).

The evaluations were carried out in three periods: at the beginning of the experiment - zero time (T0); when the plants showed photosynthesis close to zero - zero photosynthesis (P0) (average photosynthetic rate of 0.35 µmol m^{-2} s^{-1}), to avoid irreversible damage; and when the plants reached 85% of the photosynthetic rate values of the control plants for their respective treatments - recovery (REC).

The treatments were arranged in a completely randomized design in a split plot scheme, being allocated in the plots the treatments of water regimes associated with the presence or not of SA, and in the subplots the evaluation periods, with three replications, in which each experimental unit was composed of a pot with two plants. In each period, the following characteristics were evaluated:

- Relative water content in the leaves (RWC, %): determined using four leaves of each treatment according Slavick (1979), which were collected between 7:00 and 10:00 am. For this, discs of the known area were cut, weighed the fresh mass, and were placed in Petri dishes with distilled water for 24 h for saturation. After weighing, the saturated discs were dried in an air-circulation oven to determine the dry mas.

- Chlorophyll index: determined using the SPAD 502 MINOLTA portable chlorophyll meter.

Chlorophyll *a* fluorescence and gas exchanges were determined between 8:00 and 11:00 am on the second pair of fully expanded leaves.

- Gas exchanges: measured using an LCIPro-SD portable photosynthesis meter (ADC BioScientific Ltd.). The photosynthetic rate (A, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration (E, mmol H_2O m^{-2} s^{-1}), internal CO_2 concentration (C_i, µmol CO_2 mol^{-1}), intrinsic carboxylation efficiency (A/C_i, µmol m^{-2} s^{-1}/µmol mol), and water use efficiency - *WUE* (A/E, µmol CO_2/mmol H_2O).

- Chlorophyll *a* fluorescence: measured with an OS-30p portable chlorophyll fluorometer (Opti-Sciences, Hudson, USA). The initial fluorescence (F_0), potential quantum efficiency of photosystem II (F_v/F_m), effective conversion efficiency of absorbed energy (F_v/F_0), and basal quantum yield of non-photochemical processes in PS II (F_0/F_m) were measured. The leaves were subjected to a period of 30 minutes of adaptation to the dark using adapter clips to allow all the reaction centers in this leaf region to reach the open condition (Bolhàr-Nordenkampf *et al.* 1989), that is, complete oxidation of the photosynthetic electron transport system.

- Growth: the plants were harvested, and the leaf area (LA, cm^2) was determined using an LI-3100 Area Meter area integrator. The roots were evaluated for length (RL, cm) using a graduated ruler. The seedling quality index (DQI) was calculated according to Dickson *et al.* (1960) from the data of growth and dry mass production.

- Activity of antioxidant enzymes: evaluated in leaves used to quantify gas exchange and chlorophyll-*a* fluorescence and roots of each treatment using tissues frozen in liquid nitrogen. An amount of 1 g from each sample was weighed and macerated in a 6-mL solution containing 0.3 g polyvinylpyrrolidone diluted in 100 mL potassium phosphate buffer (0.2 M). Subsequently, the samples were centrifuged at 4,000 rpm for 10 minutes and the supernatant was used as an enzyme extract to determine the superoxide dismutase (SOD, µg FM^{-1}) and peroxidase activity (POD, µmol mg^{-1} protein min^{-1}) (Broetto 2014).

Total leaf proteins (mg): performed through quantitative determination by the Bradford (1976) method.

The data were subjected to analysis of variance (ANOVA), and when the results were significant (F test, $p < 0.05$), and the means were subjected Tukey test ($p < 0.05$) for water regimes in combination with SA and evaluation periods, using SISVAR software.

Results

The relative water content decreased in plants grown under water deficit (S) in P0, with and without SA (+ or – SA). However, the RWC values of S+SA seedlings remained higher than the S–SA treatment, with averages of 56.17 and 45.05%, respectively. The S+SA seedlings showed an RWC higher than that of S–SA seedlings at REC. RWC reduced by 41.85% under water deficit in S–SA seedlings compared to I-SA. On the other hand, the RWC in S+SA seedlings was 19.79% higher than in S–SA seedlings (Fig. 1a).

The chlorophyll index of S–SA seedlings in P0 was lower than the other treatments and evaluation periods. Moreover, SA application contributed to increasing the chlorophyll index in P0 of S+SA seedlings. Under irrigation, seedlings without SA (I–SA) showed an increase in the chlorophyll index in P0, with similar values in the recovery phase, but S–SA seedlings did not recover the values after re-irrigation (Fig. 1b).

The photosynthetic rate (*A*) decreased in S–SA seedlings, reaching values close to zero (0.34 µmol m^{-2} s^{-1}) (P0) at 15 days after irrigation interruption. During this period, S+SA seedlings maintained higher values (3.79 µmol m^{-2} s^{-1}), reinforcing the mitigating effect of SA. In general, plants under water deficit in the recovery period increased the photosynthetic rate, reaching values that did not vary from the control (Fig. 2a).

The S–SA seedlings reduced transpiration and *gs* in P0, while S+SA and I–SA maintained high values during the same period (Fig. 2b,c). However, I–SA and S+SA seedlings showed similar *gs* values, with only a 4.43% difference (Fig. 2c). On the other hand, C_i was higher in plants with –SA in P0, being higher than in S+SA seedlings (Fig. 2d).

Water use efficiency (*WUE*) decreased in P0, being lower in S–SA seedlings (Fig. 2e), which maintained average values lower than those of the other treatments even after rehydration (Fig. 2e). The values of instantaneous carboxylation efficiency (A/C_i) were practically null in S–SA seedlings in P0, being lower than in S+SA seedlings and irrigated daily, but the average values increased after rehydration in REC (Fig. 2f).

The quantum efficiency of photosystem II (F_v/F_m) varied only according to the use of SA and S–SA seedlings showed an average value of 0.64, which is 8.95% lower than the value observed in S+SA seedlings (Fig. 3a). The initial fluorescence (F_0) of S–SA seedlings was 45.32% higher than that of S+SA in P0, demonstrating the mitigating effect of SA on the damage in the reaction centers. We emphasize that the values in these seedlings were lower in both P0 and REC (Fig. 3b). P0 presented a reduction in F_v/F_0 in S–SA seedlings while S+SA showed values close to those under continuous irrigation (I) (Fig. 3c). All plants increased their values in REC under all water regimes, not differing significantly from each other during this period. Regarding F_0/F_m, S–SA seedlings showed a higher value in P0 than the others although they recovered after re-irrigation (Fig. 3d).

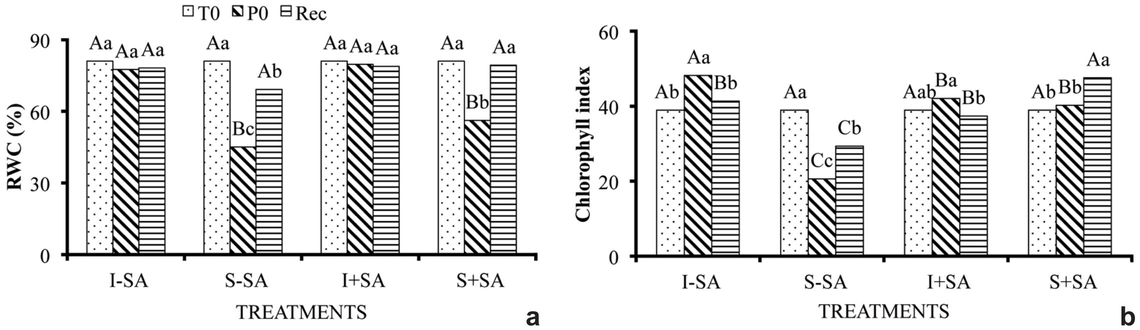

Figure 1 – a-b. *Eugenia myrcianthes* seedlings under different water regimes and salicylic acid applications: irrigation with (I+SA) or without SA (I–SA) and water stress with (S+SA) or without SA (S–SA), in the different evaluation periods: zero time (T0), zero photosynthesis (P0), and recovery (REC) – a. relative water content - RWC; b. chlorophyll index - SPAD. Means followed by the same uppercase letters (evaluation period within different water regimes and salicylic acid application) and lowercase letters (water regime within different evaluation periods) do not differ from each other by the Tukey test ($p > 0.05$).

In general, we verified continuous growth in *E. myrcianthes* seedlings, showing development during the experimental cycle. Root length was influenced only by evaluation periods, and we observed a progressive increase throughout the cultivation cycle, with a higher value in REC (Fig. 4a), showing an increment of 47.43% compared to the values in T0 (Fig. 4a). The leaf area also increased during the periods, with I+SA seedlings showing the highest LA compared to the others in REC. Although S–SA seedlings showed an increased LA in REC, the values were lower than the others (Fig. 4b).

The DQI values of S–SA and S+SA seedlings reached 0.30 in P0, increasing to 0.70 and 1.08 in REC, respectively (Fig. 4c). Although the increased DQI in REC occurred under all cultivation conditions, this increase was higher when the seedlings received SA application. The I+SA seedlings showed an increase of 11.58 times relative to P0, while I–SA seedlings had an increase of 8.41 (Fig. 4c).

The SOD activity in the leaf and root increased in seedlings under water deficit in P0, with the most pronounced increase in the root of S+SA seedlings (Fig. 5a-b). POD increased in P0, especially in S+SA seedlings, but we noticed a reduction in these values in all treatments after irrigation restart (Fig. 5c-d). S+SA seedlings showed a higher POD activity in leaves and roots (Fig. 5c-d).

Unlike the enzymatic activity, the protein content in leaves and roots decreased in S+SA seedlings in P0. This reduction was also significant in the roots of S–SA seedlings. The protein values recovered in the roots after irrigation restart, but not in the leaves (Fig. 5e-f).

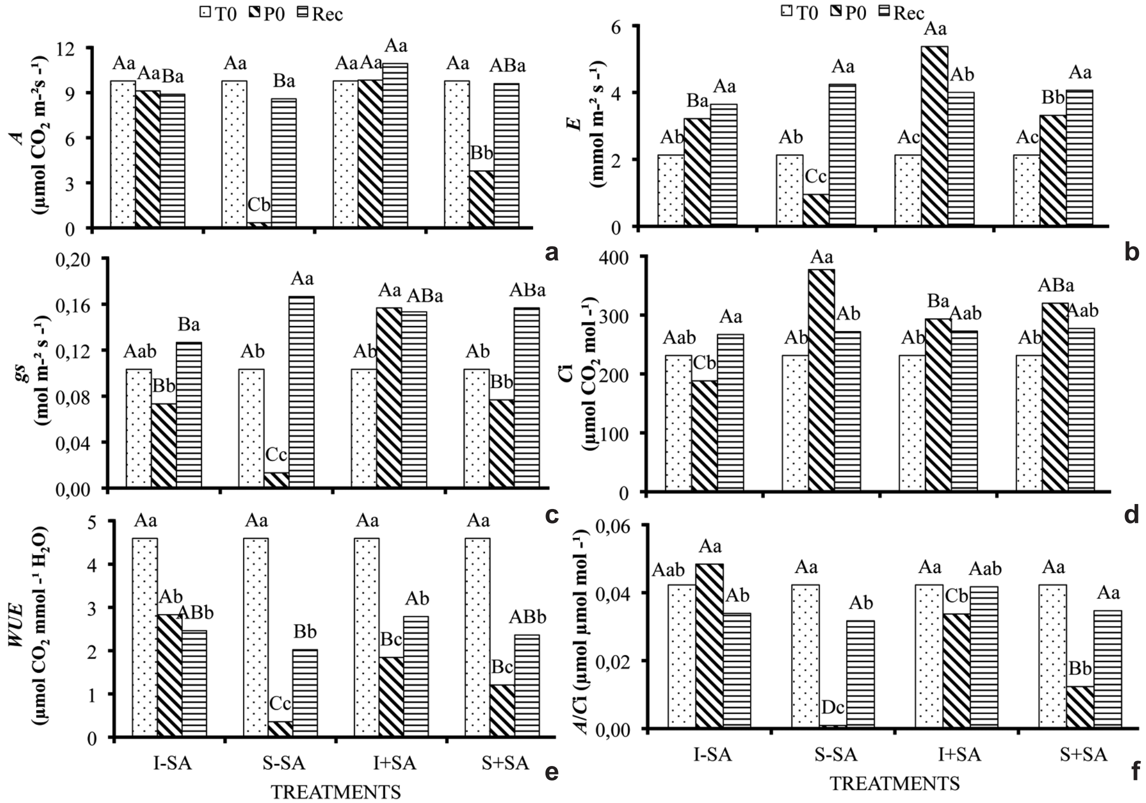

Figure 2 – a-f. *Eugenia myrcianthes* seedlings under different water regimes and salicylic acid applications: irrigation with (I+SA) or without SA (I–SA) and water stress with (S+SA) or without SA (S–SA), in the different evaluation periods: zero time (T0), zero photosynthesis (P0), and recovery (REC) – a. photosynthesis - *A*; b. transpiration - *E*; c. stomatal conductance - *gs*; d. intracellular CO$_2$ concentration - *C*; e. water use efficiency - *WUE*; f. intrinsic efficiency of carboxylation - A/C_i. Means followed by the same uppercase letters (evaluation period within different water regimes and salicylic acid application) and lowercase letters (water regime within different evaluation periods) do not differ from each other by the Tukey test ($p > 0.05$).

Discussion

In general, *E. myrcianthes* seedlings were negatively affected when subjected to water restriction. However, SA worked by reducing the damage to the photosynthetic apparatus of the seedlings, which recovered their metabolism after rehydration. Moreover, the responses of seedling growth characteristics to this phytohormone indicate its positive effects on seedling production of this species. Thus, the results found in *E. myrcianthes* prove the hypothesis of our study.

This behavior shows the beneficial effect of using SA in maintaining leaf turgor. The decreased RWC in the leaf due to water deficit is related to a reduction of soil moisture and the closure of stomata due to an increase in temperature, reducing water loss (Casson & Hetherington 2010). This response was also observed in the seedlings after re-irrigation evaluated in REC when S+SA seedlings recovered but S–SA seedlings failed to recover the values found at the beginning of the evaluations.

The SA effect on drought tolerance (Habibi 2012) has also been suggested in other species, such as *Vigna unguiculata* L. Walp. under four days of water restriction and SA application, with a gain of approximately 30% with rehydration after reaching RWC of around 70% (Silva *et al.* 2012). Plants of *Hordeum vulgare* L. sprayed with 500 μmol SA solution maintained higher RWC than plants not treated with SA (Habibi 2012).

The reduced SPAD index in S–SA seedlings of *E. myrcianthes* was already expected, as chlorophyll degradation occurs under less water availability because of the formation of reactive oxygen species, which damage, among other cellular structures, photosynthetic pigments (Sharma *et al.* 2012), and increase chlorophyllase, which degrades the molecules of this pigment (Freire *et al.* 2013).

However, S+SA plants showed an increase in the chlorophyll index, especially in REC, similar to that observed in *Ocimum basilicum* L. plants stressed with SA application, which had a significant increase in photosynthetic pigments compared to plants without SA (Kordi *et al.* 2013). SA application also assisted in gas exchange, in which plants treated with this phytohormone showed better results.

Similarly, plants of *H. vulgare* (Habibi 2012) treated with salicylic acid under adverse environmental conditions showed an increase in

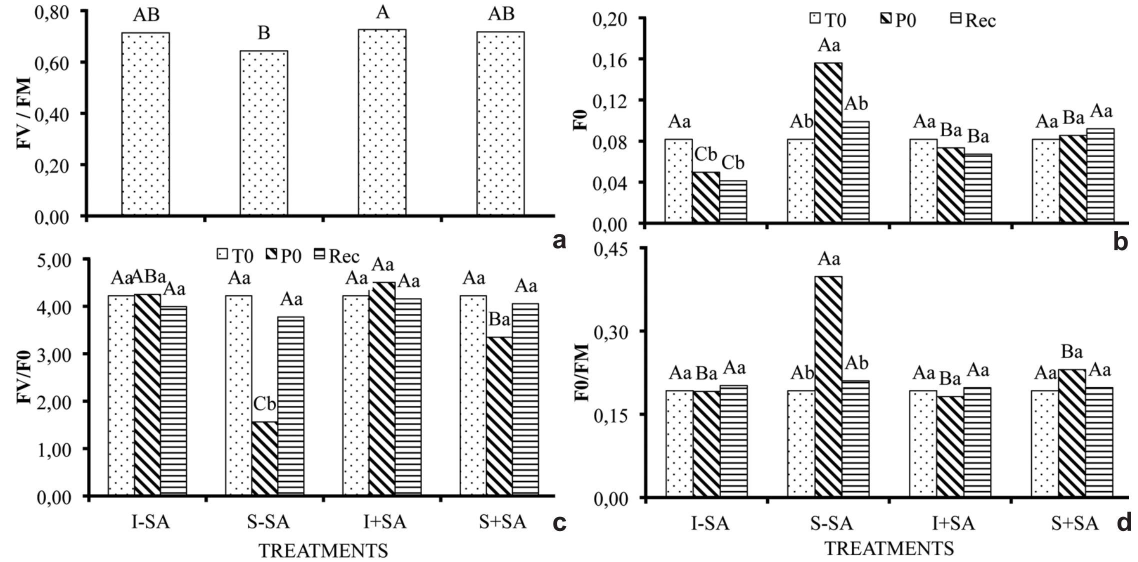

Figure 3 – a-d. *Eugenia myrcianthes* seedlings under different water regimes and salicylic acid applications: irrigation with (I+SA) or without SA (I–SA) and water stress with (S+SA) or without SA (S–SA), in the different evaluation periods: zero time (T0), zero photosynthesis (P0), and recovery (REC) – a. quantum efficiency on the photosystem II - F_v/F_m; b. initial fluorescence - F_0; c. absorbed energy conversion efficiency - F_v/F_0; d. maximum non-photochemical yield - F_0/F_m. Means followed by the same uppercase letters (evaluation period within different water regimes and salicylic acid application) and lowercase letters (water regime within different evaluation periods) do not differ from each other by the Tukey test ($p > 0.05$).

the photosynthetic rate, transpiration and water use efficiency with reduction in stomatic conductance. Thus, we observed in the literature that the responses of plants depend on environmental conditions, cultivar, time of application, dose and form of use (Nivedithadevi *et al.* 2012). Seedlings other species, such *Schinus terebinthifolia* Raddi (Saracho *et al.* 2021) also showed good physiological and growth responses regarding the application of SA. The authors report that this phytohormone improves the antioxidant defense system and reduces reactive oxygen species and water use eficiency, which contributes positively to the increase in biomass.

The reduced stomatal conductance in plants grown under less water availability is a strategic response to reduce water loss through transpiration, avoiding tissue dehydration (Campelo *et al.* 2015; Reis *et al.* 2020). This behavior was observed in S–SA seedlings of *E. myrcianthes* in P0, corroborating the results of RWC (Fig. 1a) and *A* (Fig. 2a). Thus, a reduction in stomatal conductance may represent an advantage to avoid immediate dehydration (Egea *et al.* 2011). The increase of g_s in REC compared to T0 due to the increase in growth gradually under better cultivation conditions, represented by water availability in the soil, whicj favored higher *A* under these same conditions.

On the other hand, C_i was higher in S–SA plants in P0, causing a decrease in the photosynthetic rate (*A*) mainly due to the non-stomatal limitation (Liu *et al.* 2020), that is, the lower carboxylation efficiency of Rubisco (A/C_i). This behavior has been observed in other species under these conditions, such as *C. brasiliense* (Reis *et al.* 2020). However, studies have shown reductions in the photosynthetic rates of plants under water deficiency even without alteration or increase in the internal CO_2 concentrations (Campelo *et al.* 2015), as observed in S+SA seedlings, in which the increase in C_i was not as pronounced as under S–SA.

The presence of SA reduced the harmful effect of water deficit, as observed in S–SA seedlings in P0, with high F_0 and F_0/F_m and low F_v/F_0, promoting a reduction in the potential quantum efficiency of photosystem II (F_v/F_m) (Fig. 3) and an increase in the photoinhibition processes (Lage-Pinto *et al.* 2012). Seedlings of *E. myrcianthes* under stress (S–SA) presented an average F_v/F_m value of 0.64, which is below that indicated for plants with an intact photosynthetic apparatus, and the standard values of this ratio should vary from 0.75 to 0.85 (Bolhàr-Nordenkampf *et al.* 1989). However, no significant difference was found between plants under water deficit in the presence of SA (S+SA), showing that SA did not act in the regulation of this characteristic. The significant decrease in F_v/F_m under water stress conditions is possibly due to a reduction in g_s and CO_2 restriction for photosynthesis, indicating photoinhibition (Habibi 2012).

The increased F_0 in plants grown under water deficit without SA (S–SA) reinforces the stress under this cultivation condition, indicating that it causes changes in F_v/F_0 and F_0/F_m. The F_v/F_0 ratio indicates maximum efficiency of the photochemical

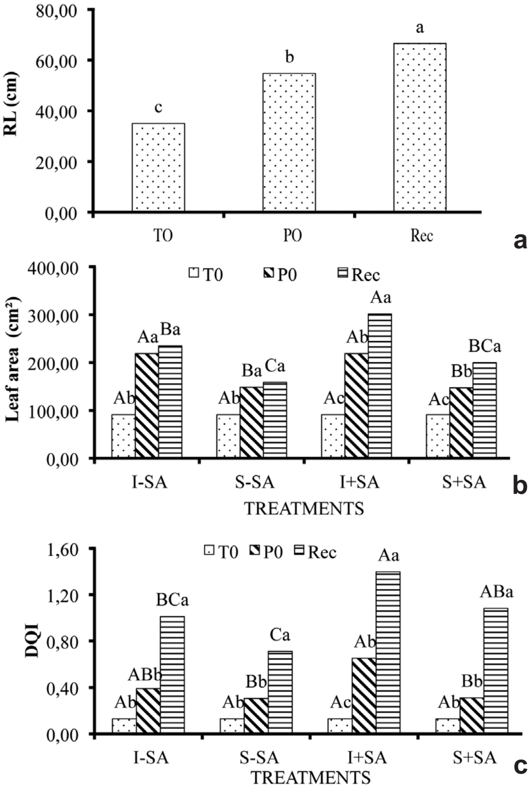

Figure 4 – a-c. *Eugenia myrcianthes* seedlings under different water regimes and salicylic acid applications: irrigation with (I+SA) or without SA (I–SA) and water stress with (S+SA) or without SA (S–SA), in the different evaluation periods: zero time (T0), zero photosynthesis (P0), and recovery (REC) – a. root length - LR; b. leaf area - LA; c. Dickson quality index - DQI. Means followed by the same uppercase letters (evaluation period within different water regimes and salicylic acid application) and lowercase letters (water regime within different evaluation periods) do not differ from each other by the Tukey test ($p < 0.05$).

process in PSII and/or the potential photosynthetic activity, with average values from 4 to 6 when the plant is under favorable cultivation conditions (Zanandrea *et al*. 2006). In our study, we observed that S–SA seedlings in P0 showed levels below that indicated for the ratio (1.56), with stress and damage in the photosynthetic apparatus. The F_v/F_0 increased to satisfactory values after rehydration in REC (5.75%). However, S+SA seedlings showed a value of 3.35 in P0. This value increased to 4.05 after rehydration, showing the beneficial effect of exogenous application of SA to seedlings under water deficit. Moreover, I+SA seedlings did not present values below 4 during all evaluations, reinforcing the favorable cultivation condition.

The increase in F_0/F_m can also be considered an indication of stress, with normal values between 0.14 and 0.20 (Rohácek 2002). The F_0/F_m ratio increased only in P0 in S–SA seedlings, with a value 95% higher than that of 0.20, the maximum limit indicated in the literature. However, these values decreased after rehydration, reaching 0.21 in REC. We observed that the increase was not so expressive in seedlings with SA. The positive effects attributed to salicylic acid on photosynthetic metabolism are related to the activation of plant antioxidant enzymes, favoring the stability of membranes by decreasing the level of lipid peroxidation, which contributes to an increase in *A* and accumulation of biomass in plants (Agarwal *et al*. 2005).

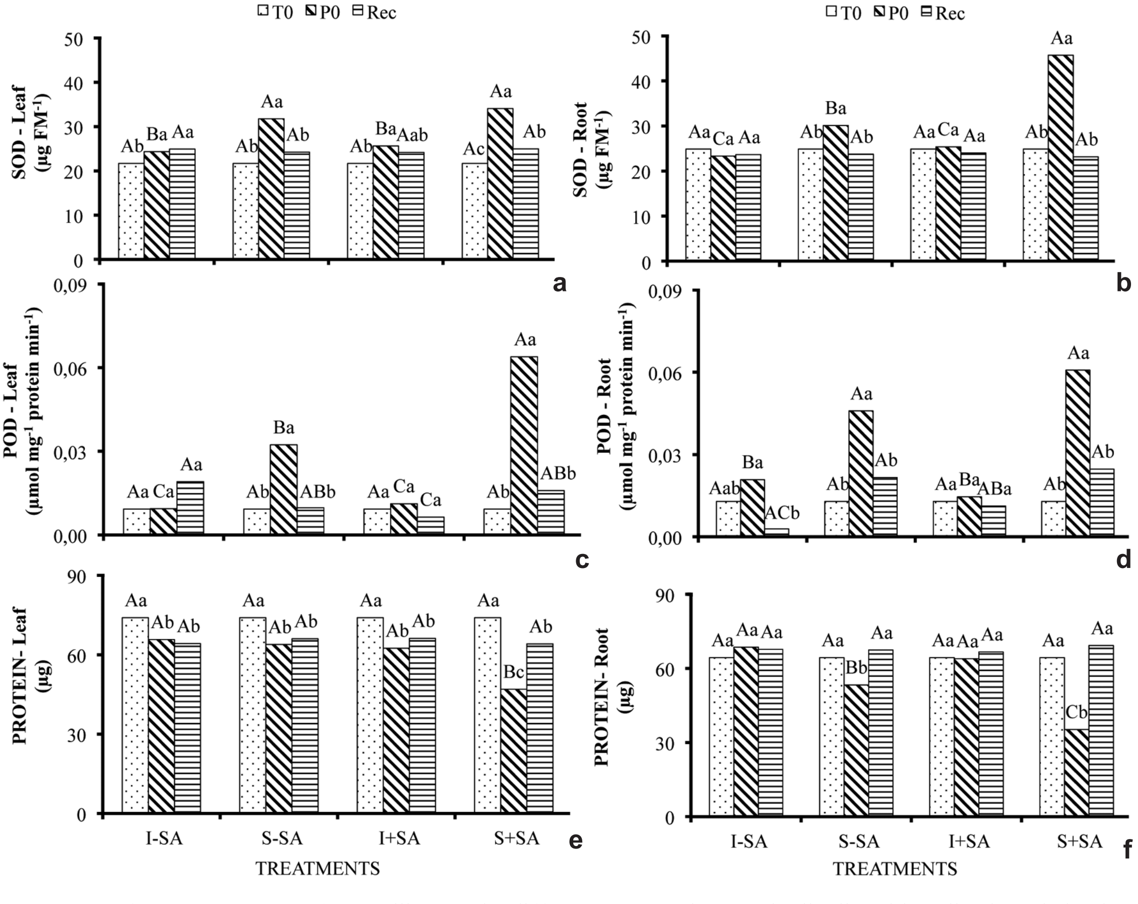

Figure 5 – a-f. *Eugenia myrcianthes* seedlings under different water regimes and salicylic acid applications: irrigation with (I+SA) or without SA (I–SA) and water stress with (S+SA) or without SA (S–SA), in the different evaluation periods: zero time (T0), zero photosynthesis (P0), and recovery (REC) – a-b. enzymatic activity of superoxide dismutase - SOD – a. in leaves; b. in roots; c-d. peroxidase - POD – c. in leaves; d. in roots; e-f. protein – e. in leaves; f. in roots. Means followed by the same uppercase letters (evaluation period within different water regimes and salicylic acid application) and lowercase letters (water regime within different evaluation periods) do not differ from each other by the Tukey test ($p > 0.05$).

Water restriction hindered the increase in leaf area in seedlings under water deficit. However, LA values in plants with SA and after rehydration were higher than those of stressed plants without SA, resulting in a higher DQI of S+SA seedlings.

The biomass gain evaluated by DQI was observed in all seedlings from P0, mainly for SA-treated seedlings, demonstrating the potential of SA for recovery and production of seedlings under stress. In addition, the DQI for S+SA seedlings was also 6.48% higher than that of I–SA seedlings. Other species showed an increase in dry mass under water deficiency and SA application, such as *Eucalyptus globulus* Labill. (Mazzuchelli *et al*. 2014) and *Helianthus annuus* L. (Ebtessam *et al*. 2015). Leaf application of 200 mg SA L^{-1} contributed to increased quality of *S. terebinthifolia* seedlings under low soil water availability (Saracho *et al*. 2021).

The increase in SOD activity in P0 both in the leaves and in the roots of *E. myrcianthes* seedlings under water deficit was already expected, as it has been considered the first antioxidant defense line of plants (Gill *et al*. 2015), which alter their metabolism under these stress conditions and increase the production and accumulation of reactive oxygen species (ROS) (Barbosa *et al*. 2014; Abdelgawad *et al*. 2015) such as superoxide radicals that undergo dismutation by SOD into H_2O_2 and O_2 (Foyer & Noctor 2000).

Thus, although this research did not quantify ROS in *E. myrcianthes*, the increase in SOD when releasing H_2O_2 leads to an increase in POD (Ahmad *et al*. 2010), as observed in the leaves of S+SA seedlings, showing a protective mechanism to the photosynthetic apparatus, reducing ROS production, and ensuring the carboxylation of Rubisco to maintain stable metabolic responses and photoassimilate production.

Also, SA application potentiated the activity of these enzymes in the antioxidant metabolism, as this phytohormone acts asregulates the activity of cellular detoxification enzymes (Kang *et al*. 2014; Brito *et al*. 2019), such as the antioxidant enzymes POD and SOD observed in this research with *E. myrcianthes*, mitigating the collapses to the functioning of the photosynthetic apparatus.

Similar results were observed in other species, such as *V. unguiculata* (Dutra *et al*. 2017) and *Sesamum indicum* L. (Silva *et al*. 2017), which showed increased enzyme activity when receiving SA and subjected to water stress, suggesting induction of resistance of the plants under study. Dianat *et al*. (2016) found that 300 mg SA L^{-1} favored higher physiological performance, growth rate and activity of SOD and POD in *Lippia citriodora* L. under water déficit, a dose close to that used in our study with *E. myrcianthes*.

The reduction of protein concentrations in plant tissues due to the increased activity of antioxidant enzymes interferes with the biochemical metabolism of the plant and acts as a defense against water deficiency (Souza *et al*. 2014). Therefore, the direct effect of SA could be observed on the metabolism of plants under water deficit. During P0, S+SA plants showed the lowest protein levels regardless of the organ, reflecting especially on the antioxidant metabolism and possibly on the induction of resistance.

Bases on our results, we found that exogenous application of SA is a promising practice for production of seedlings of higher quality under adverse conditions, in addition to favoring the potential physiological recovery. In future perspectives, studies on the application of phytohormone in the other nativ species should be arried out in order to establish doses and times that can contribute to ex cultivation and biodiversity conservation.

Salicylic acid application mitigated the negative effects of water deficit on the photosynthetic apparatus in *E. myrcianthes* seedlings, stabilized and favored photosynthetic metabolism and growth of seedlings after re-irrigation.

An increase in the activity of antioxidant metabolism enzymes was observed under water deficit, and SA application enhanced this protective effect, suggesting the promotion of plant resistance induction.

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