Floresta e Ambiente 2022; 29(2): e20210047
https://doi.org/10.1590/2179-8087-FLORAM-2021-0047
ISSN 2179-8087 (online)

ORIGINAL ARTICLE – Silviculture

Biochemical and Physiological Modifications in Seedlings of *Schinus terebinthifolius* Raddi. After Hardening with Salicylic Acid

Maria Eunice Lima Rocha[1]
Ubirajara Contro Malavasi[2]
Cândido Ferreira de Oliveira Neto[3]
Jessica Suellen Silva Teixeira[3]
Diana Jhulia Palheta de Sousa[4]
Marlene de Matos Malavasi[2]

[1]Universidade Estadual do Oeste do Paraná, Programa de Pós-Graduação em Agronomia, Marechal Candido Rondon, PR, Brasil.

[2]Universidade Estadual do Oeste do Paraná, Centro de Ciências Agrárias, Marechal Candido Rondon, PR, Brasil.

[3]Universidade Federal Rural da Amazônia, Programa de Pós graduação de Ciências Florestais, Produção Animal na Amazônia e Rede Bionorte, Belém, PA, Brasil.

[4]Universidade Federal de Viçosa, Programa de Pós-graduação em Fitotecnia, Viçosa, MG, Brasil.

Abstract

Exogenous application of stress inductors can facilitate and accelerate some biological responses that promote plant defense. The objective of the experiment was to quantify some compounds linked to nitrogen metabolism as a function of the application of salicylic acid in *Schinus terebinthifolius* seedlings submitted to water deficit. The experiment was constituted of four doses of salicylic acid and three periods of water deficit. Quantifications included levels of nitrate, free ammonium, total soluble amino acids, proteins, proline, glycine-betaine and relative water content. When evaluating the relative water content in seedlings of *Schinus terebinthifolius* at 8 days, it was observed that the dose of 200 mg L^{-1}. Seedlings showed physiological responses when subjected to doses of salicylic acid of 200 and 300 mg L^{-1}. The increase in concentration of proline and glycine are advantageous, because these substances act as osmoregulators and cell protectors against deficit hydrical.

Keywords: Forest species, Free ammonium, Nitrate, Water deficit.

---

1. INTRODUCTION

Among the resources that plants need to develop, water is the most important and the most limiting. On this account, its availability can affect several metabolic processes, resulting in great differences on the type of vegetation that develops along precipitation gradients (Taiz et al., 2017). Drought stress is one of the most studied conditions because it severely limits plant growth, survival, and productivity (Ferreira et al., 2015; Lobo & Oliveira Júnior, 2015; Maseda & Fernández, 2016).

Water deficit (WD) is characterized as an environment factor, which has a negative influence on terrestrial plants. Water deficit on terrestrial plants results in a condition of physiological adversity in which plants does not express its genetic potential, resulting in a decrease of productivity and possible reduction of metabolic capacity, which may take the vegetable to reach the point of permanent wilt and, consequently, death (Cunha et al., 2013; Driever et al., 2013; Taiz et al., 2017).

Metabolist mechanisms are activated in response to stresses in special osmotic adjustment that maintain cell turgidity and, thus, maximizes the use of soil water (Ferrari et al., 2015; Taiz et al., 2017). Molecules of such activation include proline, glycine-betaine, sucrose, polyamine, mannitol, pinitol, among others, and they accumulate in the cell vacuole or cytosol, preserving the integrity of proteins, enzymes, and cell membranes for a short period (Ashraf et al., 2011).

In this sense, some practices can be added to the plant handling in the nursery with the objective of improving seedling performance inducing tolerance and acclimating to field conditions after planting. The use of plant regulators is an option when it comes to modulating the plant morphological, biochemical, and physiological characteristics (D'Avila et al., 2011).

In hardening, the tendency is for those anticipated situations to simulate the condition after seedling expedition, especially stressful situations, and in this way, when exposed to those conditions, seedlings will already have the apparatus linked to defense, so the response will be faster and more efficient, and the post-planting shock will be alleviated (Jacobs & Landis, 2009; Close, 2012). In addition, hardening protocols will facilitate the production of seedlings in quantity and quality, improving post-planting and reducing expenses with replanting.

Salicylic acid (SA) is linked to biological activities, whether regulatory or defensive, and among its functions linked to secondary metabolism is the amplification of signals in stressful conditions. SA will activate enzyme protectors that could promote adaptive responses in plants or more quickly modify their tolerance (Khan et al., 2012; Miura & Tada, 2014; Asgher et al., 2015).

This research aimed to attenuate the effect of water deficit through the application of salicylic acid. In addition, doses of this regulator, between 1 and 50 mM, can alter the biological activities in plants on abiotic stress (Hayat et al., 2010; Kabiri et al., 2012; Hasanuzzaman et al., 2014).

Schinus terebinthifolius Raddi. is classified as tree species native of the Brazilian flora. The species has a wide distribution over the national territory and can be found from the South to the Northeast of Brazil, showing great rusticity. Despite its wide distribution, *aroeira vermelha* as it is popularly known, is more frequent between the states of Rio Grande do Sul and Pernambuco (Gilbert & Favoreto, 2011; Carvalho et al., 2013).

Schinus terebinthifolius Raddi. is a great option for use, mainly for its pioneering characteristics, rusticity and great phenotypic plasticity. In addition, it can be explored, if it follows the legislation, regarding its timber (furniture, charcoal, and firewood) and non-timber (therapeutic, antifungal, anti-inflammatory properties) potential and can be introduced in places with the objective of restoring degraded areas. Furthermore, the Brazilian Unified Health System and the National Surveillance Agency recently approved commercial use of the species. Thus, knowing the potential of this species and the practices carried out in the nursery phase is a strategy to improve the quality of seedlings produced, as well as knowing the potential for them to have the best destination (DEGÁSPARI et al., 2005; KHALED et al., 2009; COSTA et al., 2010; FREIRES et al., 2011; SILVA et al., 2011; BULLA et al., 2015).

Thus, according to the previous arguments, the objective of the experiment was to quantify some compounds linked to nitrogen metabolism as a function of the application of salicylic acid in *Schinus terebinthifolius* seedlings submitted to water deficit.

2. MATERIAL AND METHODS

The experiment was conducted in a protected shade house covered with a 150-micron-thick anti-UV and low-density polyethylene film, equivalent to 20% shading in the western region of the State of Paraná with coordinates of 24° 33' 24" S and 54° 05' 67" W and an altitude of 420 m. The climatological classification according to Koppen for the region is the Cfa type, subtropical humid mesothermal (Alvares et al., 2013).

We used 400 three months old *Schinus terebinthifolius* seedlings obtained from the Itaipu Binacional nursery, which were propagated in 120 cm^3 plugs filled with Humusfertil® with electrical conductivity of 1.5 ± 0.3 ds m^{-1}, density of 480 kg m^{-3}, hydrogen potential (pH) of 6 ± 0.5, maximum humidity, and water holding capacity in weight:weight basis equal to 60%.

Hardening was realized with salicylic acid with applications over a period of 2 months. The solutions of SA was obtained from the sum of deionized water, salicylic acid and non-ionic surfactant (Agral®) in the proportion 30 mL to 100 L of water, applied weekly for 2 months from September 26th to November 14th 2017, according to Mazzuchelli et al. (2014) applied with a hand sprayer between 6:00 and 8:00 AM in order to avoid unfavorable weather conditions.

After hardening with SA, seedlings were transferred from the plugs to larger containers (3 L pots) to start the acclimatization process, which lasted approximately 20 days in a shade house, with irrigation 3 times a day (8:00 AM, 12:00 PM and 8:00 PM) with weekly weedig. The 7-month-old seedlings transplanted into 3-liter pots filled with a mixture of local soil (Latosol RED Eutrofferric with a very clayey texture) and humus in the proportion of 3:1. Afterwards, the treatments of the water deficit were imposed.

Before the imposition of the water deficit, seedlings presented the following averages for height 27.67; 27.52; 24.74; and 26.33 cm for seedlings that received doses of 0, 100, 200, and 300 mg L^{-1} of salicylic acid; stem diameter of 5.14; 5.07; 4.83; and 4.98 mm and the average number of leaves of 15.83; 08.17; 13.83; and 16.5 , respectively.

The imposition of water deficit consisted of 4 doses of SA (T1- 0 mg L^{-1}; T2- 100 mg L^{-1}; T3-1; 200 mg L^{-1}; T4- 300 mg L^{-1}), 3 periods of water deficit (E1-4; E2-8; and E3-12 days of water deficit), with 5 repetitions, totaling 60 experimental units.

The analyzes were performed at the end of each water deficit period by harvesting seedlings from the containers, oven drying at 65 °C for 48 hours, grounding dry tissues, and their biochemical parameters determined. Relative water content (RWC) was quantified according to Slavick (1979), as well as nitrate and free ammonium (Weatherburn et al., 1967), total soluble amino acids (Peoples et al., 1989), proline (Bates et al., 1973), and glycine-betaine (Grieve & Grattan, 1983). The analyses were performed at the Laboratory for the Study of the Biodiversity on Superior Plants (Laboratório para Estudo da Biodiversidade em Plantas Superiores — EBPS), located at the Universidade Federal Rural da Amazônia (UFRA), Belém, Pará.

For statistical purposes, the normality and homogeneity tests were performed using the common covariance matrix using Genes software followed by analysis of variance and chi-square (Cruz, 1998). When appropriate, treatment means were compared by Tukey test. The graphs were constructed with Excel and the curves were determined according to the averages and their respective standard deviations.

3. RESULTS AND DISCUSSION

After 4 days of water restriction, relative water content values were not influenced by SA treatments but differed from the control treatment. The purpose of the application of plant regulators is to mitigate the effects of water loss by adjusting compatible solutes, attenuating the adverse condition, and allowing plants to continue functioning, even if in a restricted way until the cell turgidity is restored.

At 8 days of water deficit, an increase in relative water content was calculated from seedlings that received 200 mg L^{-1} of SA, indicating that the application of SA is more efficient when the stress condition was more intense and signaled by plant tissues. At 4 days, the plants still had green and turgid leaves, as the species used showed good tolerance to water deficit and, therefore, featured more efficient use of water when the water restriction was increased (Figure 1). In the long run, however, an application of the regulator did not alleviate the effects of the adverse condition, mainly because the maintenance of water inside the cells through the osmotic adjustment is more efficient in the first moments (Bergamaschi & Bergonci, 2017). A the final evaluation period (12 days), a dose of 300 mg L^{-1} resulted (Figure 1) in the lowest average of relative water content (22.87%) and the application of SA did not contribute to alleviate the stressful condition.

In *Schinus terebinthifolius* seedlings, the nitrate content in the leaves was increased with the application of 100 mg L^{-1} at 4 and 8 days of water deficit, while with the application of 200 mg L^{-1} the levels were decreased compared to control treatment (Figure 2). The reduction may result from the conversion of nitrogen in the cells conditioned to the functioning of the nitrate reductase. According to Sharner & Boyer (1976) the objective is to prevent those substances from being lost with the leaf abscission process, since in extreme situations of light or temperature, the transport of substances from the leaves to the roots can occur and thus decrease their concentrations in the above ground system.

The intensification of stress can modify cellular content, causing an imbalance between cellular components and substances, namely sucrose, carbohydrates, proline, glycine, among others. At 12 days (Figure 2) of water deficit, the reduction was not mitigated with the application of SA. Several factors can cause changes in seedling physiology when exposed to water deficit. However, one of the first organs affected will be the leaf. Because in that condition there will be an imbalance in the water potential of the cells, a reduction in stomatal conductance as a consequence of stomatal closure, a reduction in the efficiency of the photosystems and relative water content. In addition, osmotic imbalance of the cells can occur resulting from the increase in compatible solutes (Lobato et al., 2008; Campos et al., 2010).

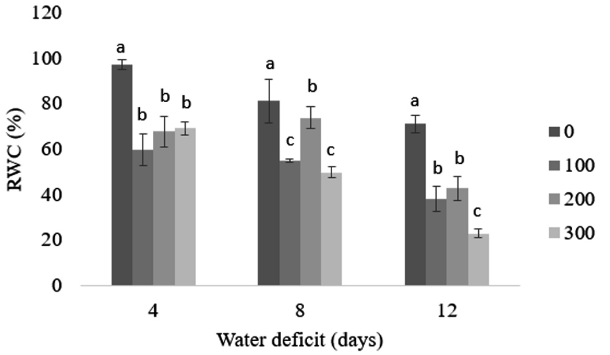

Figure 1. Relative water content (RWC) in *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. Means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1})

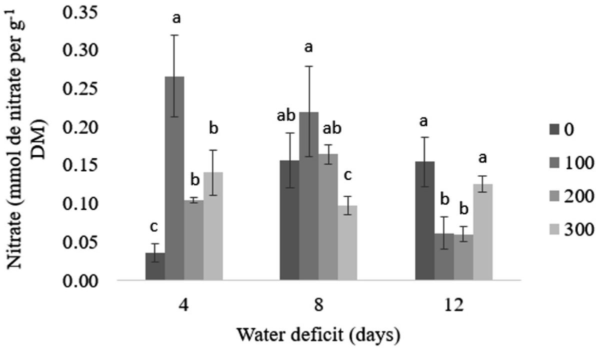

Figure 2. Nitrate content in leaves of *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

Under stressful conditions, the allocation of biomass is redirected from the leaves and stems to the roots improving rooting, water absorption, and nutrient uptake. The strategy is to promote physiological, morphological, biochemical, and cellular changes that will make the plants prepared for future adversities. This is a result of the allocation of photoassimilates, as well as photosynthesis by-products that will stimulate an increase in plant dry biomass, especially in root tissues, as a strategy to increase the surface of contact from the roots with available water in the soil (Correia & Nogueira, 2004; Verma et al., 2012; Sapeta et al., 2013).

Hence, the contents stored in the leaves, mainly sugars and amino acids were translocated to the roots. This can be seen in Figure 3, where root levels were increased, because of the programmed translocation due to the water deficit. At 4 days, the nitrate contents present in the roots increased with the application of 300 mg L^{-1} of SA in comparison to other treatments. The same trend was observed at 12 days, where the levels decreased in the leaves and were increased for the roots, with the doses of 100 and 200 mg L^{-1}. Many studies have suggested that both the accumulation and translocation of solutes are influenced by stressful conditions, whether these are biotic or abiotic. In addition, the application of plant regulators can cause that activity, stimulating tolerance (Suwa et al., 2006; Taiz et al., 2017).

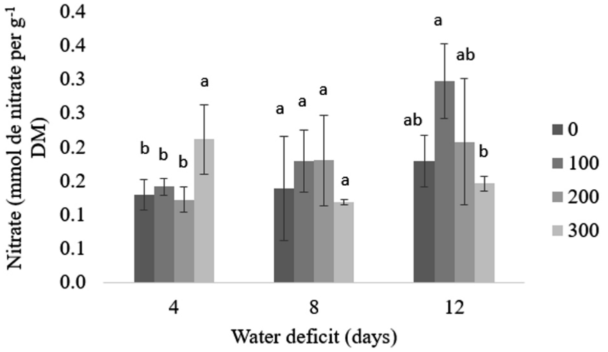

Figure 3. Nitrate content in roots of *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

The concentrations of free ammonium in leaves of *Schinus terebinthifolius* seedlings at 4 and 8 days under water deficit did not show difference ($P > 0.05$) with averages of 5.62 and 5.76 mmol of NH_4^+ kg^{-1} of DM (Dry Matter), respectively. At 12 days, the highest values were obtained with application of 300 mg L^{-1} of SA and the averages obtained were equal to 3.62, 5.89, 5.81, and 5.0 mmol of NH_4^+ kg^{-1} of DM for doses of 0, 100, 200, and 300 mg L^{-1}, respectively. Such trend reinforces the assumption that high doses of SA intensify stress by increasing concentration of solutes (Figure 4).

It is worth noting that high concentrations of ammonium can be toxic and inhibit numerous metabolic activities essential for plant development by altering the photoassimilates production routes and reducing plant growth (Almeida & Vieira, 2010).

The levels of ammonium on the roots after 4 days of water deficit induced a decrease of the averages with the lowest value obtained with the dose of 300 mg L^{-1}. With the worsening of water deficit condition, there was an inversion in the ammonium concentrations on the roots at 12 days and the highest averages were obtained with doses of 200 and 300 mg L^{-1} however not differing from the treatment without application of SA. Therefore, it is inferred that the high concentrations for this evaluation period were stressful, increasing the levels of the evaluated solute (Figure 4). Another explanation is that as the levels were increased, there was signaling of plant defense to cease biological activities and thereby avoid energy expenditure and reduce water consumption.

Similar results were reported by Teixeira et al. (2015) where there was an increase in ammonium concentrations on the leaves (4.3 to 8.6 mmol of NH_4^+ kg^{-1} of DM) and roots (13.7 to 18.5 mmol NH_4^+ kg^{-1} DM) of *Morinda citrifolia L.* subjected to water deficit. This increase can be justified by the reduction in the activity of the glutamine synthetase enzyme, since the lack of energy (ATP) and the decrease in glutamate concentrations negatively impact this conversion from ionic sources (NO_3^- and NH_4^+) to essential amino acids (Sodek, 2019).

In addition to the translocation process, another reason for reductions of both ammonium and nitrate on the leaves is the conversion of these inorganic sources to their organic forms. In this case, amino acids or proteins will be returned to the roots via phloem, or redistributed to fruit seeds and flowers if it is the reproductive period. This conversion can occur in the leaves or roots. Depending on the urgency and demand of the plants, both forms are soluble and mobile in the plant conduction system (Sodek, 2019).

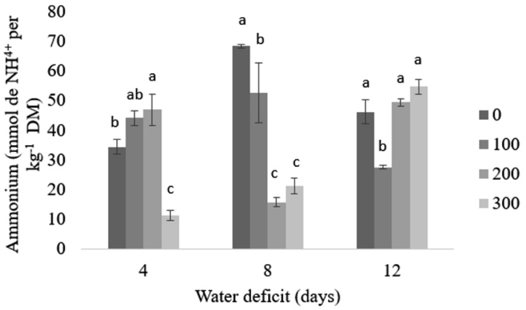

Figure 4. Ammonium content in roots of *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

In leaves and roots of *Schinus terebinthifolius* seedlings there was no difference ($P > 0.05$) between treatments for the concentrations of amino acids and the averages obtained for the respective organs were 9.13 and 8.23 umol of amino acids g^{-1} of DM, respectively.

Proline has the function of osmoregulation, establishment of membranes, and preservation of the structure of proteins, in addition to eliminating free radicals and adjusting the redox potential in cells. This amino acid acts as a reserve of carbon and nitrogen in the detoxification of excess ammonia and the stabilization of membranes (Ashraf & Foolad, 2007). In addition, this compound has an increased content due to abiotic stresses and studies have highlighted that its content can increase up to 100% in conditions of water deficit (Cvikrová et al., 2013; Filippou et al., 2014).

The proline content of *Schinus terebinthifolius* leaves at 4 and 12 days of water deficit showed no difference ($P > 0.05$) with averages of 3.1655 and 2.8102 of Pro g^{-1} of DM. At 8 days, however, the highest concentration of proline was obtained at the maximum dose of SA without however differing from the control treatment (Figure 5).

Accumulation of proline can be a parameter that defines resistance in plants, as it is considered an osmoprotector and ensures greater sensitivity to plants and therefore it is one of the most studied amino acids (Ashraf et al., 2011; Iqbal et al., 2014; Trovato et al., 2008). Therefore, the increase in proline, in addition to signaling, acts to protect against mechanical damage and to prevent overflow of ions as a result of degradation of the cell membranes.

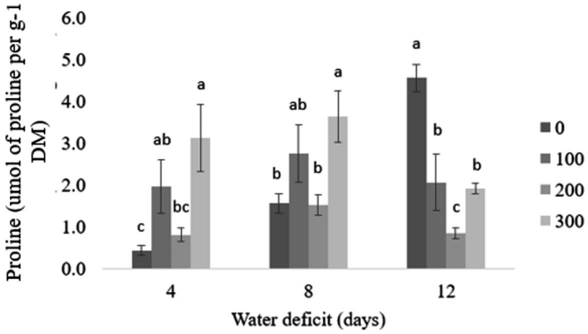

Figure 5. Content of proline in leaves of seedlings of *Schinus terebinthifolius* exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

The concentrations of proline in roots of *Schinus terebinthifolius* seedlings resulted in differences ($P < 0.05$) depending on the number of days of water deficit. At 4 days, the levels increased dramatically from the control treatment (without SA) in contrast with the dose of 300 mg L^{-1} (Figure 6). The levels of proline tend to increase to balance and detoxify the cells, avoiding toxicity caused by the accumulation of ammonium (Figure 6). Ammonium can be influenced by the content of proline, since the high concentration of the latter generates detoxification of the excess ammonium and thus the stabilization of proteins and amino acids present on plants (Kavi Kishor et al., 2005).

At 12 days of water deficit, proline levels increased significantly in the control seedlings (Figure 6); that is, in untreated seedlings the main stress condition was the lack of water. On the other hand, after the application of salicylic acid, the concentrations were reduced (Figure 1). These averages coincided with the reduction of the relative water content, reinforcing that the osmotic adjustment is a short-term alternative and consequently with the evolution of the stress period, both the application of treatments and the defense mechanism of the plants becomes more efficient. Therefore, in the same proportion that there was a reduction in water, proline, and the osmotic adjuster. Contrasting results have been described for *Cajanus cajan* L. in cultivars BRS Mandarim and Caqui, where proline levels in leaves and roots increased with decreasing water levels and increasing salinity (Monteiro et al., 2014).

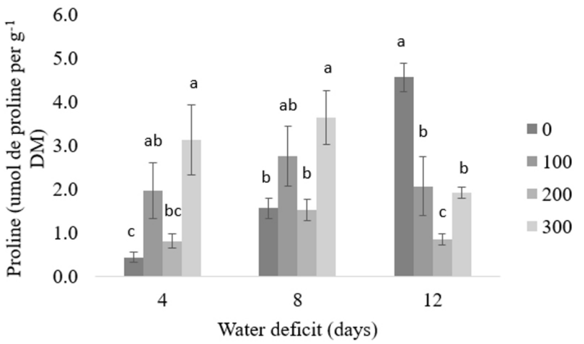

Figure 6. Proline content in roots of *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

Under adverse conditions, there may be an increase in glycine levels in order to balance water and osmotic potential on plant cells. The strategy is to encourage an increase in the number of solutes of the roots, thus decreasing the water potential and facilitating the transport of water in favor of a positive gradient from the soil to the roots (Anjum et al., 2011).

In *Schinus terebinthifolius* seedlings submitted to 8 days of water deficit, the dose of 200 mg L^{-1} of SA showed a higher average equal to 16.62 mg of glycine betaine g^{-1} DM differing from the other treatments (Figure 7).

SA can induce accumulation of glycine betaine at a dose of 0.5 to 2.5 mM on plants subjected to water, salt, and cold stresses. That increase can improve the overall plant development such as primary and secondary growth, seedling biomass, as well as adjustments of photosynthetic metabolism (Wang et al., 2010; Misra & Misra, 2012; Bharwana et al., 2014; Khan et al., 2014) as observed in the present research, where application of SA resulted in accumulation of the amino acid.

For the doses of 200 and 300 mg L^{-1} there was an increase on the levels of glycine betaine up to 8 days of water deficit with values of 16.62 and 13.22 mg of glycine betaine g^{-1} DM, respectively. In this experiment and for this species, the highest dose reduced the glycine betaine content which may be associated with attenuation of the water deficit (Figure 7). The obtained increase was 76.81% of the control treatment compared to the application of 200 mg L^{-1} of SA. However, after 12 days of water deficit, the levels began to decrease, leading to the understanding that, in the long run, SA may lose its potential to attenuate or even intensify the plant responses to water deficit since the osmotic adjustment is a strategy efficient only for short periods. Carlin & Santos (2009) reported an increase of 26.4% in the concentrations of glycine betaine in plants under water restriction.

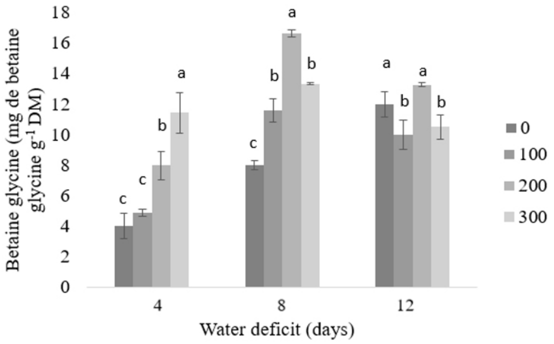

Figure 7. Glycine content in leaves of *Schinus terebinthifolius* seedlings exposed to water deficit and treated with salicylic acid. The means followed by the same letter do not differ statistically from each other by the Tukey test at the level of 1% probability. Caption- The bars represent the doses of salicylic acid (0, 100, 200 and 300 mg L^{-1}).

Glycine-betaine was not affected by the doses of SA and number of days of water deficit on roots of *Schinus terebinthifolius* seedlings. The averages were 21.26, 22.82, and 24.63 mg of glycine betaine g^{-1} of DM after 4, 8 and 12 days of water deficit, respectively.

The SA interacts with other hormones and substances dispersed across cells, harmoniously or not, depending on the conditions to which plants are exposed, in addition to intrinsic factors (Joseph et al., 2010). Therefore, its action will be variable as changes, even minimal ones, occur. Therefore, the use of salicylic acid is a viable option for the studied specie, as it can help, depending on the conditions, improving tolerance to biotic stresses. It is interesting that new studies, both with the species and with the regulator are developed, reducing the gaps, especially in relation to which dose would be more appropriate and whether that would be included in the concentrations used in current research. Additionally, other physiological, enzymatic and biochemical variables that would serve as standards for the development of seedling quality recommendations.

4. CONCLUSION

Nitrogen metabolism of *Schinus terebinthifolius* seedlings was altered according to the number of days of water deficit and the application of salicylic acid. Relative water content value was the biggest influencer of the variation for the compatible solutes, based on the fact that, due to the water imbalance, the plant osmotically adjusts the cellular content.

When evaluating the relative water content in seedlings of *Schinus terebinthifolius* at 8 days, it was observed that the dose of 200 mg L^{-1} helped in the attenuation of water stress, reflecting in increments in the water content. Despite this, the mean obtained in this treatment was still lower than the control treatment (without application of SA). Thus, it is interesting to note that each condition, whether internal or external the seedlings will affect their responses and development and therefore, at 4 days, as it is a rustic species, there was no significant response (doses without significant difference) and at 12 days the plants were subjected to an extremely stressful condition, both due to the fault water condition and to the application of the plant regulator, resulting in low values for the relative water content.

Schinus terebinthifolius seedlings showed physiological responses when subjected to doses of salicylic acid of 200 and 300 mg L^{-1}, since they promoted variation in compatible solutes, among which, nitrate, ammonium, proline, glycine-betaine. Furthermore, the species seedlings showed no variation of concentration for some quantified solutes.

SUBMISSION STATUS

Received: 24 May. 2021
Accepted: 12 Apr. 2022
Associate editor: Natane Miranda

CORRESPONDENCE TO

Maria Eunice Lima Rocha
Universidade Estadual do Oeste do Paraná, *Campus* Marechal Cândido Rondon.
Rua Pernambuco, 1963, 85.960-000, Marechal Cândido Rondon, PR, Brasil.
e-mail: eunice_agronomia@yahoo.com.br

AUTHORS' CONTRIBUTIONS

Maria Eunice Lima Rocha: Conceptualization (Equal); Data curation (Equal); Formal analysis (Equal); Funding acquisition (Equal); Investigation (Equal); Methodology (Equal); Project administration (Equal); Resources (Equal); Software (Equal); Validation (Equal); Visualization (Equal); Writing – original draft (Equal); Writing – review & editing (Equal).

Ubirajara Contro Malavasi: Methodology (Supporting); Supervision (Supporting); Writing – review & editing (Supporting).

Cândido Ferreira de Oliveira Neto: Methodology (Supporting); Supervision (Supporting).

Jessica Suellen Silva Teixeira: Methodology (Supporting).

Diana Jhulia Palheta de Sousa: Methodology (Supporting).

Marlene de Matos Malavasi: Methodology (Supporting); Supervision (Supporting).

REFERENCES

Almeida AQ, Vieira EL. Gibberellin action on growth, development and production of tobacco. Scientia Agraria Paranaensis, 2010; 9(1): 45-57.

Alvares CA.; Stape JL, Sentelhas PC, Gonçalves JLdeM, Sparovek GK. Köppen's climate classification map for Brazil. Meteorologische Zeitschrift, 2013; 22(6): 711–728.

Anjum AS, Xie XY, Wang LC, Saleem MF, Man, C, Lei W. Morphological, physiological and biochemical responses of plants to drought stress. African Journal of Agricultural Research, 2011; 6(9): 2026-2032.

Asgher M, Khan MIR, Anjum NA, Khan NA. Minimizing toxicity of cadmium in plants–role of plant growth regulators. Protoplasma, 2015; 252 (2): 399–413.

Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environmental and Experimental Botany, 2007; 59(2): 206–216.

Ashraf M, Akram NA, Arteca RN, Foolad MR. The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. Critical Reviews in Plant Sciences, 2011; 29(3):162-190.

Bates, LS, Waldren, RP, Teare ID. Rapid determination of free proline for water-stress studies. Short communication. Plant and Soil, 1973; 39(1): 205-207.

Bergamaschi, H, Bergonci, JI. Relações água-Planta. In: Bergonci, JI. As plantas e o clima: Princípios e aplicações. Guaíba: Agrolivros; 2017.

Bharwana SA, Ali S, Farooq MA, Ali B, Iqbal N, Abbas F et al. Hydrogen sulfide ameliorates lead-induced morphological, photosynthetic, oxidative damages and biochemical changes in cotton. Environmental Science and Pollution Research, 2014; 21(1):717–731.

Bulla MK, Hernandes L, Baesso ML, Nogueira AC, Bento AC, Bortoluzzi BB, Serra LZ, Cortez DA. Evaluation of Photoprotective Potential and Percutaneous Penetration by Photoacoustic Spectroscopy of the *Schinus terebinthifolius* Raddi Extract. Photochemistry and Photobiology, 2015; 91(3): 558–566.

Campos MF, Backes C, Roters JMC, Ono EO, Rodrigues JD. Influência de retardantes de crescimento no desenvolvimento de plantas de gladíolo (*Gladiolus communis* L. spp., Iridaceae). Biotemas, 2010; 23(3): 31-36.

Carlin SD, Santos, DMMDos. Indicadores fisiológicos da interação entre déficit hídrico e acidez do solo em cana-de-açúcar. Pesquisa agropecuária brasileira, 2009; 44 (9): 1106-1113.

Carvalho MG, Melo AGN, Aragão CFS.; Raffin FN, Moura TFAL. *Schinus terebinthifolius* Raddi: composição química, propriedades biológicas e toxicidade. Revista brasileira de plantas medicinais, 2013; 15(1):158-169.

Close DCA. review of ecophysiologically-based seedling specifications for temperate Australian eucalypt plantations. New Forests, 2012; 43(6): 739-753.

Correia KG, Nogueira RJMC. Avaliação do crescimento do amendoim (*Arachis hypogaea* L.) submetido a déficit hídrico. Revista de Biologia e Ciências da Terra, 2004; 4(2): 1-7.

Costa EMMB, Barbosa AS, Arruda TAde, Oliveira PTde, Dametto FR, Carvalho RAde, Melo MdasD. Estudo *in vitro* da ação antimicrobiana de extratos de plantas contra *Enterococcus faecalis.* Jornal Brasileiro de Patologia e Medicina Laboratorial, 2010; 46 (3):175-180.

Cruz CD. Programa GENES - Aplicativo Computacional em Estatística Aplicada à Genética (GENES - Software for Experimental Statistics in Genetics). Genetics and Molecular Biology, 1998; 21(1): 1-5.

Cunha RLM, Filho BGS, Costa RCL, Viégas IJM. Physiological assessment in young Brazilian and African mahogany plants during the dry and rainy seasons in northeastern Para state, Brazil. Revista Ciências Agrárias, 2013; 56(3): 255-260.

Cvikrová M, Gemperlová L, Martincová O, Vanková R. Effect of drought and combined drought and heat stress on polyamine metabolism in proline over producing tobacco plants. Plant Physiology and Biochemistry, 2013; 73(1): 7-15.

D'Avila FS, Paiva HN, Leite HG, Barros NF, Leite FP. Efeito do potássio na fase de rustificação de mudas clonais de eucalipto. Revista Árvore, 2011; 35(1): 13-19.

Degáspari CH, Waszczynskyj N, Prado MRM. Antimicrobial activity of *Schinus terebenthifolius* Raddi. Ciência e Agrotecnologia, 2005; 29(3): 617-22.

Driever SM, Krormdijk J. Will. C3 crops enhanced with the C4 CO2- concentrating mechanism live up to their full potential (yield). Journal of Experimental Botany, 2013; 64(13): 3925-3935.

Ferrari E, Paz A, Silva AC. Déficit hídrico no metabolismo da soja em semeaduras antecipadas no mato grosso. Pesquisas Agrárias e Ambientais, 2015; 3(1): 67-77.

Ferreira WN, Lacerda CF, Costa RCda, Medeiros Filho S. Effect of water stress on seedling growth in two species with different abundances: the importance of Stress Resistance Syndrome in seasonally dry tropical forest. Acta Botanica Brasilica, 2015; 29(3): 375-382.

Filippou P, Bouchagier P, Skotti E, Fotopoulos V. Proline and reactive oxygen/nitrogen species metabolism is involved in the tolerant response of the invasive plant species *Ailanthus altissima* to drought and salinity. Environmental and Experimental Botany, 2014; 97(1): 1-10.

Freires IA, Alves LA, Jovito VdeC, Castro RDde. Atividade antifúngica de *Schinus terebinthifolius* (Aroeira) sobre cepas do gênero *Candida*. Revista Odontológica do Brasil-Central, 2011; 20(52): 41-45.

Gilbert B, Favoreto R. *Schinus terebinthifolius* Raddi. Revista Fitossanitária, 2011; 6(1): 43-56.

Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant and Soil, 1983;70(1): 303-307.

Hayat Q, Hayat S, Irfan M, Ahmad A. Effect of exogenous salicylic acid under changing environment: a review. Environmental and Experimental Botany, 2010; 68(1): 14-25.

Hasanuzzaman M, Alam MM, Nahar K, Mahmud JA, Ahamed U, Fujita M. Exogenous salicylic acid alleviates salt stress-induced oxidative damage in *Brassica napus* by enhancing the antioxidant defence and glyoxalase systems. Australian Journal of Crop Science, 2014; 8(4): 631-639.

Iqbal N, Umar S, Khan NA, Khan MR. A new perspective of phytohormones in salinity tolerance: Regulation of proline metabolism. Environmental and Experimental Botany, 2014; 100 (1): 34-42.

Jacobs DF, Landis TD. Hardening. In: Dumroese RK, Luna T, Landis TD. (Eds.). Nursery manual for native plants: Guide for tribal nurseries. Washington: United States Department of Agriculture, Forest Service, 2009.

Joseph B, Jini D, Sujatha S. Insight into the role of exogenous salicylic acid on plants grown under salt environment. Asian Journal of Crop Science, 2020, 2(4); 226-235.

Kabiri R., Farahdakhsh H, Nasibi F. Effect of drought stress and its interaction with salicylic acid on black cumin (*Nigella sativa*) germination and seedling growth. World Applied Sciences Journal, 2012; 18(4); 520-527.

Kavi Kishor PB, Sangam S, Amrutha RN, Sri Laxmi P, Naidu KR, Rao KRSS et al. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Current Science, 2005; 88 (3): 424-438.

Khaled FEM, Ahmed HEG, Hamdy AS, Shibamoto T. Chemical Compositions and Antioxidant/Antimicrobial Activities of Various Samples Prepared from *Schinus terebinthifolius* Leaves Cultivated in Egypt. Journal of Agricultural and Food Chemistry, 2009; 57(12): 5265-5270.

Khan MIR, Iqbal N, Masood A, Khan NA. Variation in salt tolerance of wheat cultivars: role of glycinebetaine and ethylene**.** Pedosphere, 2012; 22(6): 746–754.

Khan MIR.; Asgher M, Khan NA. Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). Plant Signaling & Behavior, 2014; 80(1): 67–74.

Lobato AKS, Oliveira Neto CF, Costa RCL, Santos Filho BG, Cruz FJR, Laughinghouse HD. Biochemical and physiological behavior of *Vigna unguiculata* (L.) walp. under water stress during the vegetative phase. Asian Journal of Plant Sciences, 2008; 7(1); 44-49.

Lobo RC, Oliveira Júnior Fde. Efeitos do estresse hídrico nas características morfológicas de plântulas de *Eucalyptus grandis* W. (Hill ex. Maiden). Revista da União Latino-americana de Tecnologia, 2015; 3(1): 09-25.

Maseda, PH, Fernández, RJ. Growth potential limits drought morphological plasticity in seedlings from six Eucalyptus provenances. Tree Physiology, 2016; 36(1): 243–251.

Mazzuchelli EHL, Souza GM, Pacheco AC. Rustificação de mudas de eucalipto via aplicação de ácido salicílico. Pesquisa Agropecuária Tropical, 2014; 44(4); 443-450.

Misra N, Misra R. Salicylic acid changes plant growth parameters and proline metabolism in *Rauwolfia serpentina* leaves grown under salinity stress. American-Eurasian Journal Of Agricultural & Environmental Sciences, 2012; 12(2): 1601-1609.

Miura K, Tada Y. Regulation of water, salinity, and cold stress responses by salicylic acid. Frontiers Plant Science, 2014; 5(4): 1-19.

Monteiro JG, Cruz FJR, Nardin MB, Santos DMMdos. Crescimento e conteúdo de prolina em plântulas de guandu submetidas a estresse osmótico e à putrescina exógena. Pesquisa agropecuária brasileira, 2014; 49(1): 18-25.

Peoples MB, Faizah AW, Reakasem BE, Herridge DF. Methods for evaluating nitrogen fixation by nodulated legumes in the field. Australian Centre for International Agricultural Research Canberra, 1989: 1:89.

Sapeta H, Costa JM, Lourenço T, Marocod J, Lindee PVD, Oliveira MM. Drought stress response in Jatrophacurcas: Growth and physiology. Environmental and Experimental Botany, 2013, 85(1): 76-84.

Sharner DL, Boyer JS. Nitrate reductase activity in maize (*Zea mays* L.) leaves. Plant Physiology, 1976: 58(4): 499-504.

Silva MAda, Pessotti BMdeS, Zanini SF, Colnago GL, Nunes LdeC, Rodrigues MRA, Ferreira L. Óleo essencial de aroeira-vermelha como aditivo na ração de frangos de corte. Ciência Rural, 2011; 41(4): 676-681.

Slavick B. Methods of studying plant water relations. Nova York: Springer Verlang, 1979.

Sodek L. Metabolismo do nitrogênio. In: Kerbauy GB. Fisiologia vegetal. Rio de Janeiro: Guanabara Koogan, 2019.

Suwa R, Nguyen NT, Saneoka H., Moghaieb R, Fujita K. Effect of salinity stress on photosynthesis and vegetative sink in tobacco plants. Soil Science & Plant Nutrition, 2006; 52(2): 243-250.

Taiz L, Zeiger E, Møller IM, Murphy A. Estresse abiótico. In: Blumwald, E.; Mittler, R. *Fisiologia Vegetal*. Porto Alegre, Artmed, 2017.

Teixeira, D. T. de. F.; Nogueira, G. A. dos. S.; Maltarolo, B. M.; Ataíde, W. L. da. S.; Oliveira Neto, C. F. de. Alterações no metabolismo do nitrogênio em plantas de noni sob duas condições hídricas. Enciclopédia biosfera: Centro Científico Conhecer, 2015; 11 (22): 89-106.

Trovato M, Mattioli R, Costantino P. Multiple roles of proline in plant stress tolerance and development. Rendiconti Lincei, 2008; 19(4): 325-346.

Verma KK, Vatsa S, Gupta RK, Ranjan S, Verma CL; Singh M. Influence of water application on photosynthesis, growth and biomass characteristics in Jatropha curcas. Current Botany, 2012; 3(4): 26-30.

Wang LJ, Fan L, Loescher W, Duan W, Liu GJ, Cheng JS. Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. BMC Plant Biology, 2010; 10(1): 34–40.

Weatherburn MW. Phenol hipochlorite reaction for determination of ammonia. Analytical Chemistry, 1967; 39(8): 971-974.