Universidade Federal Rural do Semi-Árido
Pró-Reitoria de Pesquisa e Pós-Graduação
https://periodicos.ufersa.edu.br/index.php/caatinga
ISSN 1983-2125 (online)

http://dx.doi.org/10.1590/1983-21252024v3712000rc

Scientific Article

Does hydrogel help in the mitigation and recovery of *Eugenia myrcianthes* Nied. under water stress?

O hidrogel auxilia na mitigação e recuperação de *Eugenia myrcianthes* Nied. sob estresse hídrico?

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ABSTRACT - Water deficit or flooding can damage the photosynthetic and enzymatic metabolism in plants such as *Eugenia myrcianthes* Nied. Thus, knowledge of technologies that can mitigate stress damage is necessary for the *ex-situ* cultivation of these species. This study evaluates the potential of hydrogel (H) to mitigate water stress and assist in the recovery of *E. myrcianthes* seedlings under different water conditions. The seedlings were separated into three water regimes: R1 - continuous irrigation (control); R2 - intermittent stress, characterized by two stress cycles: water restriction followed by flooding (stress); and R3 - intermittent stress + hydrogel - Forth® gel (stress + H). Evaluations occurred in six periods: zero time (T0), 1st zero photosynthesis (P0), 1st recovery (REC), 2nd P0, 2nd REC, and END. *E. myrcianthes* seedlings responded to water conditions with reduced CO_2 assimilation rate, transpiration, and stomatal conductance during stressful conditions. We observed decrease in the growth parameters of this species under stress, including leaf area and biomass. Under stress, independent of H, plants increased the activity of the enzymes superoxide dismutase, peroxidase, and the content of the amino acid proline. We observed recovery potential in most of the evaluated traits. *E. myrcianthes* seedlings showed morphophysiological changes due to water deficit and flooding. Still, hydrogel did not contribute to alleviating the effects of water deficit on gas exchange and did not affect flooding. Seedlings showed survival and resumption of metabolism and growth after stressful conditions. This demonstrates their resilience due to physiological plasticity, regardless of the use of hydrogel.

Keywords: Flooding. Pessegueiro-do-mato. Resilience. Water deficit. Water-retaining polymer.

RESUMO - O déficit hídrico ou alagamento podem ocasionar danos nas plantas, como *Eugenia myrcianthes* Nied. Assim, o conhecimento de tecnologias que possam mitigar os danos causados pelo estresse é necessário para o cultivo ex situ dessas espécies. Objetivou-se avaliar o potencial do hidrogel (H) em mitigar o estresse hídrico e de auxiliar na recuperação de mudas de *Eugenia myrcianthes* Nied. sob flutuações hídricas. As mudas foram separadas em três regimes hídricos: R1) irrigação contínua (controle), R2) Estresse intermitente: caracterizado por dois ciclos de estresse, sendo o primeiro a restrição hídrica, e o segundo alagamento (Estresse hídrico) e R3) Estresse intermitente + Hidrogel - Forth® gel (Estresse + H). As avaliações foram realizadas em seis épocas: tempo zero, 1ª fotossíntese zero (F0), 1ª recuperação (REC), 2ª F0, 2ª REC e FINAL. Foram avaliadas características fotoquímicas, morfológicas e antioxidantes. As mudas de *E. myrcianthes* responderam às flutuações hídricas, havendo redução das trocas gasosas. As plantas sob estresse, independente do H, aumentaram a atividade das enzimas superóxido dismutase, peroxidase e do conteúdo do aminoácido prolina. Conclui-se que mudas de *E. myrcianthes* apresentaram mudanças fisiológicas quanto ao déficit hídrico e alagamento, mas o H não contribuiu em aliviar o déficit hídrico sobre as trocas gasosas e não prejudicou no alagamento. As mudas apresentaram sobrevivência, retomada do metabolismo após as condições estressantes demonstrando capacidade de resiliência por plasticidade fisiológica, independente do uso do hidrogel.

Palavras-chave: Alagamento. Pessegueiro-do-mato. Resiliência. Déficit hídrico. Polímero hidroretentor.

Conflict of interest: The authors declare no conflict of interest related to the publication of this manuscript.

Received for publication in: June 6, 2023.
Accepted in: February 16, 2024.

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INTRODUCTION

Climatic seasonality influences soil water availability due to lack and excess, which can damage both the photosynthetic and the enzymatic metabolism of plants in phyto-ecological regions (BOGATI; WALCZAK, 2022). Some species may or may not tolerate these stressful conditions through the activity of antioxidant metabolism enzymes (GARCÍA-CAPARRÓS et al., 2021).

Under low water status due to irregular rainfall, some plants are usually susceptible to cell damage. This hinders the production of energy necessary for their metabolic processes due to stomatal closure and leaf fall from the increase in abscisic acid (REHMAN et al., 2022).

On the other hand, excess water in rainy seasons or areas close to water courses becomes an aggravating factor for species that are not adapted to anoxic/hypoxic environments, that is, environments with a marked decrease in O_2 levels in soil (OLIVEIRA; GUALTIERE, 2017). In this sense, water deficit and/or flooding led to the lowest CO_2 assimilation and carboxylation efficiency (SHARMA et al., 2022).

Knowledge of agents that can mitigate the negative effects of stress on the native tree and fruit plants in the Cerrado is necessary to establish ex-situ cultivation practices. The water-retaining polymer, known as hydrogel, appears to be a promising technology. When in contact with water, the polymeric chains that make up the hydrogel interact with the medium, which causes them to expand up to 400 times their original size (SINGH et al., 2021). This makes water available slowly and locally when the product is placed in the pit for plants under low water availability (BELTRAMIN et al., 2020).

Thereby, hydrogel can be a good alternative for planting seedlings in the recovery of degraded areas (FONSECA et al., 2017), especially under water deficit. However, due to edaphoclimatic variations in the different phytophysiognomies, many areas are subject to flooding at certain times of the year. Thus, it is important to know the responses of seedlings cultivated with hydrogel in areas subject to drought and subsequent flooding.

Eugenia myrcianthes Nied. (Myrtaceae) is a native fruit and tree species, popularly known in Brazil as “pessegueiro-do-mato”, occurring in riparian forests, seasonal forests, and restinga forests (SILVA et al., 2016). It is a pioneer species that reaches 4 to 6 m in height and generally occurs in moist and well-drained soils (GUARINO et al., 2018). The species presents food potential, being consumed either fresh or processed in the forms of sweets, liqueurs, and jellies (SANTOS; MARCHIORI; SIEGLOCH, 2014). Moreover, its leaves and fruits have antioxidant compounds (INFANTE et al., 2016). Its seedlings can be included in programs to recover degraded areas and integrated production.

Considering the deleterious effects of water stress (due to both scarcity and excess) on photosynthetic metabolism and growth, and based on the natural habitat of *E. myrcianthes*, we raise the following questions: i) does hydrogel attenuate the damage in the reaction centers and gas exchange of this species under water stress? ii) can hydrogel use interfere negatively during the period when plants were exposed to flooding? iii) does the species have physiological plasticity that guarantees survival during stress and recovery after that period?

Thus, this study evaluates the potential of hydrogel to mitigate water stress and assist in the recovery of *Eugenia myrcianthes* Nied. seedlings under water fluctuations.

MATERIALS AND METHODS

Collection site and seedling obtention

Eugenia myrcianthes Nied. fruits were obtained in the remaining Cerrado areas. The species was identified and corresponded to a voucher deposited in the DDMS Herbarium of the Federal University of Grande Dourados under No. 5169. The seeds were removed manually from the fruits and sown in 290 cm^3 tubes filled with commercial substrate (Carolina®).

At 60 days after emergence, when the seedlings had an average height of 15.0 cm, they were transplanted to 7.0 kg plastic pots filled with Dystrophic Red Latosol of clayey texture, corresponding to Oxisols soil USDA classification + coarse sand (1:1 v/v). The pots were kept in a greenhouse, under 30% shading (Sombrite®). Irrigation was performed daily, maintaining 70% of the water retention capacity in the substrate.

Water regimes and experimental design

The seedlings were separated into three water regimes: 1 - continuous irrigation (control), performed daily by gravimetry, maintaining 70% of the water retention capacity (SOUZA et al., 2000); 2 - intermittent water stress (stress), characterized by two stress cycles: water restriction followed by flooding; and 3 - intermittent water stress + hydrogel (stress + H): water cycle carried out according to the previous group; however, the seedlings were transplanted again by adding 20 mL of hydrogel to the pit. This dose was defined based on articles such as Silva et al. (2021), in which the hydrogel was used in a similar way on seedlings of native species.

The water-retaining polymer used was Forth® gel for planting, which is an acrylic copolymer of potassium and acrylamide, with the following characteristics: exchange capacity: 532.26 ($mmol/dm^3$), water retention capacity: 1,526.69%, and physical nature: bran - soil conditioner, class E. The hydrogel was diluted at 4 g L^{-1} and left to rest for 20 minutes until it presented a gel aspect.

Evaluations occurred in six periods, starting at zero time (T0 - 0 day), the period before subjecting plants to different water regimes. Treatments with water stress were conducted in two cycles, the first being water restriction, in which seedlings were grown under plastic cover to protect against rainfall, and irrigation was suspended until the seedlings of one of the water stress treatments showed photosynthetic rate (A) values close to zero (1st P0: 21 days). At this moment, irrigation was resumed until A values were close to those of the control (1st REC: 22 - 37 days).

Seedlings were submitted to the second cycle of water stress (flooding) after the 1st REC. For that, the plants were placed in pools with water depth ± 5.0 cm above the substrate level, being monitored until A decreased to near zero again (2nd P0: 38 –76 days). Subsequently, the seedlings were removed from flooding and left to drain naturally for seven days. Irrigation was then resumed until normalization of A values (2nd REC: 77 - 91 days). The last evaluation (END) occurred 56 days after the 2nd REC, totaling 147 days of the experimental period. Photosynthetic rate (A) was monitored with the IRGA device (LCI Pro-SD ADC Bio Scientific Ltd.) every two days under favorable climatic conditions for evaluation.

Evaluation Morphophysiological characteristics

Assessments of gas exchange, chlorophyll index, and chlorophyll-*a* fluorescence were conducted between 8 am and 10 am using fully expanded leaves located in the middle third of the plants.

Gas exchange: a portable infrared gas analyzer system was used to quantify photosynthesis (A), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E). The analyses were conducted with a portable photosynthesis meter (model LCI Pro-SD, ADC BioScientific Ltd.) between 8 am and 10 am. Subsequently, intrinsic Rubisco carboxylation efficiency (A/C_i) and water use efficiency (iWUE - A/gs) were calculated.

Chlorophyll index: determined using a portable chlorophyll meter - SPAD (Soil Plant Analysis Development).

Chlorophyll-*a* fluorescence: the leaves were adapted to the dark condition for 30 min, using leaf clips, and soon after, the quantic photochemical efficiency of photosystem II – PS II (F_v/F_m) was measured using a portable fluorometer (OS-30p; Opti-Sciences Chlorophyll Fluorometer, Hudson, NY, USA) at 1.500 µmol m^{-2} s^{-1} light intensity. The absorbed energy conversion efficiency (F_v/F_0) was calculated.

Relative water content (RWC): determined according to Turner's methodology (1981).

Initial growth: seedlings were harvested and separated into shoots and roots, and then leaf area (LA) was measured using an area integrator (LI-COR, 3100 C). The fresh material from the shoot and roots were packed in Kraft paper bags, placed in an oven with forced air circulation at 60±5°C for 72 hours, and weighed on a precision scale.

Enzymatic activity: samples of leaves and roots were collected and frozen in liquid N. Then, 1 g of each sample was weighed, macerated in 6 mL of solution containing 0.3 g of polyvinylpyrrolidone (PVP) diluted in 100 mL of potassium phosphate buffer (0.2 M), and centrifuged at 12000 rpm for 20 minutes at 4 °C. The supernatant was used as an enzyme extract to determine the activity of superoxide dismutase (SOD E.C. 1. 15.1.1) and peroxidase (POX E.C 1.11.1.7), according to Broetto (2014). The extraction method used was from potassium phosphate buffer solution. Absorbance readings were taken at wavelengths of 560 and 470 nm, respectively, for SOD and POX, on a spectrophotometer (METASH, model: V-5000 visible spectrophotometer).

Proline: quantified with dry material according to the methodology described by Bates, Waldren and Teare (1973).

Statistical design and data analysis

The experimental design was completely randomized, with treatments arranged in a split-plot scheme. The plots consisted of the three water regimes (control, stress, and stress + H), and the subplots corresponded to the six evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC, and END), with three replicates with two seedlings each.

The data were submitted for analysis of variance (ANOVA). When significant by the F-test ($p \leq 0.05$), the means were compared by Tukey's test for water regimes, and the Scott-Knott clustering algorithm was used to group the evaluation times, both at $p \leq 0.05$ ± standard deviation (SD), using the SISVAR software.

RESULTS AND DISCUSSION

E. myrcianthes seedlings responded to water fluctuations, showing reduced gas exchange and growth during stressful conditions. Conversely, under stress conditions, *E. myrcianthes* increase values of superoxide dismutase and peroxidase activity and proline amino acid, regardless use of H, as protection mechanisms. The seedlings presented the potential to recover most traits, demonstrating physiological plasticity.

Seedlings under water deficit reached the first zero photosynthesis (1st P0) at 21 days of water restriction, with *A* value of 0.69 µmol CO_2 m^{-2} s^{-1}, as did seedlings grown with the presence of hydrogel (H) in the same period also showed lower *A* values (1.90 µmol CO_2 m^{-2} s^{-1}) (Figure 1A). At 16 days after the resumption of irrigation (1st REC), these seedlings had *A* values equal to those of the control (Figure 1A). In the 2nd P0, regardless of the use of H, the *A* values of seedlings under water deficit decreased (0.62 and 0.57 µmol m^{-2} s^{-1}, respectively) at 39 days of flooding (Figure 1A).

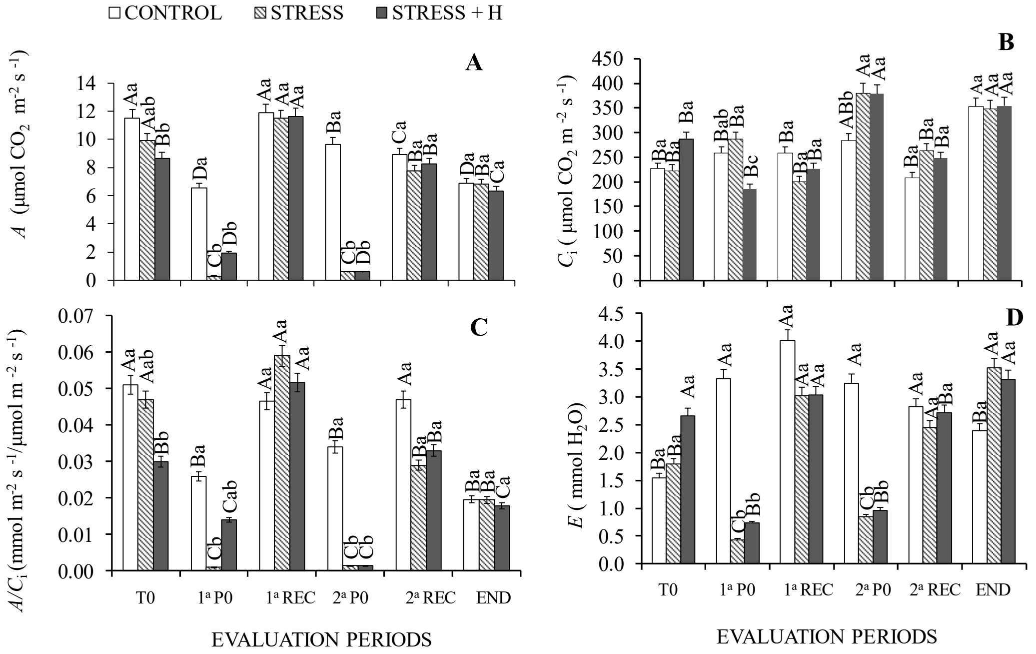

Figure 1. Photosynthesis - *A* (A), intercellular CO_2 concentration - C_i (B), intrinsic Rubisco carboxylation efficiency - A/C_i (C), and transpiration rate - *E* (D) in leaves of *Eugenia myrcianthes* Nied. seedlings under different water conditions (control, stress, and stress + H) and evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC, and END) (n=3). Uppercase letters group the evaluation periods within each water condition (Scott-Knott, $p \leq 0.05$), and lowercase letters compare the water conditions within each evaluation period (Tukey, $p \leq 0.05$). Vertical bars indicate the standard deviation.

The reduction of *A* under low water availability, here characterized by the 1st P0, is due to the importance of water in metabolic processes. This element correlates with the release of protons and electrons in the photochemical step and with stomatal opening and closure, allowing CO_2 absorption and mobilization of photoassimilates by the plant (YANG et al., 2021).

Water deficit and flooding cause stomatic and non-stomatic effects. Stomatic effects reduce *A* by increasing the resilience of CO_2 diffusion as stomata close due to the decrease in leaf water potential and soil water content. In turn, non-stomatic effects involve photochemical and biochemical processes, with a reduction in electron transport affecting the synthesis of ATP and NADPH (CORDOBA-NOVOA et al., 2022). Flooding conditions may decrease Rubisco's capacity for regeneration and activity (OLIVEIRA; GUALTIERE, 2017).

Seedlings grown without H in the 1st P0 showed increase in C_i (258 and 287 µmol CO_2 m^{-2} s^{-1}), while seedlings grown with H corresponded to the lowest (186 µmol CO_2 m^{-2} s^{-1}) and, consequently, this reflected in intrinsic Rubisco carboxylation efficiency (A/C_i) equal to control for seedlings with hydrogel (0.01 mmol m^{-2} s^{-1}/µmol m^{-2} s^{-1}) in the same period (Figure 1C). On the other hand, the 2nd P0 period showed no differences between plants under stress with and without H (Figure 1B).

Regarding internal CO_2 concentration (C_i), the fact that the seedlings grown with H have lower values in the 1st P0 demonstrates that H possibly helped in this response. Hydrogel contributes to maintaining the soil water status during water restriction, providing aeration. The product acts as a soil conditioner, improving physicochemical properties and reducing the number of irrigations and nutrient loss (KUMAR et al., 2020). This reflects the maintenance of A/C_i, as the stress + H and control treatments were the same for this parameter. In this sense, plants grown under the latter conditions differed from those grown under stress without H in the same period.

Similarly, hydrogel promoted physiological recovery in citrus seedlings under water deficit (FERREIRA et al., 2014). The C_i of flooded seedlings increased regardless of H, thus decreasing A/C_i and *A*.

Under flooding/hypoxia, there is a reduction in the functioning of the photosystem II electron transport chain, resulting in less NADPH production, and then CO_2 is not used by Rubisco, while the energy substrates from the photochemical phase are then dissipated via photorespiration, contributing to increasing C_i in these conditions (BISPO; VIEIRA, 2022; SHARMA et al., 2022). This reduction in the use of CO_2 by the plant reflects lower production of photoassimilates, interfering negatively with its growth.

For transpiration (*E*), we found a reduction in plants under stress, regardless of H, compared to control seedlings, but these same values increased in the 1st and 2nd REC (Figure 1D).

Stomatal conductance (g_s) was influenced by isolated factors, decreasing in seedlings under stress (0.10 mol m^{-2} s^{-1}) (Figure 2A). Regarding evaluation periods, g_s was higher at END and lower at 1st P0, followed by 2nd P0 and 2nd REC. In the 2nd P0 and 2nd REC, g_s values did not differ from each other (Figure 2B).

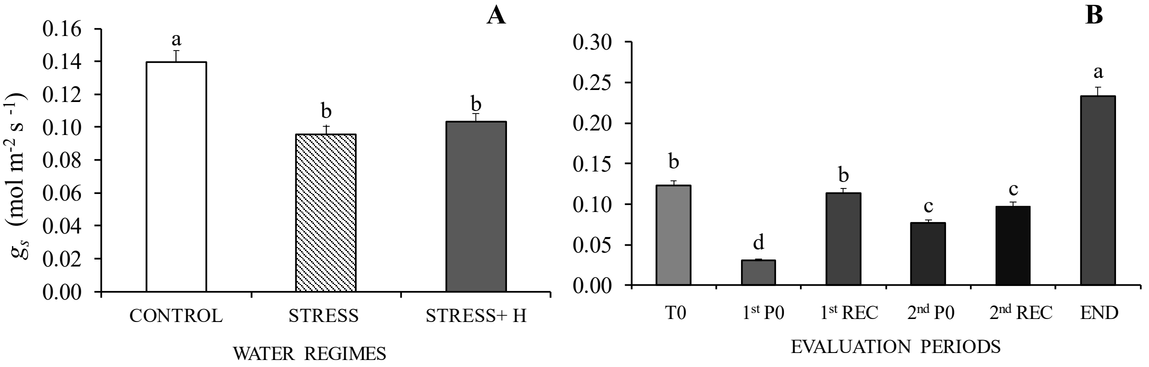

Figure 2. Stomatal conductance (g_s) in leaves of *Eugenia myrcianthes* Nied. seedlings under different water conditions and evaluation periods (*n*=3). Tukey test ($p \leq 0.05$) compares water conditions and Scott-Knott test ($p \leq 0.05$) groups evaluation periods. Vertical bars indicate the standard deviation.

The decrease of g_s and *E* in seedlings under stressful conditions, regardless of the use of H, is due to the reduction in cell turgor, which resulted in stomatal closure (LANGNER et al., 2021), in addition to being a strategy to avoid excessive water loss in these conditions, but which limits the entry of CO_2. The dose of H in the seedling pit was possibly insufficient to promote better physiological results in *E. myrcianthes* seedlings under stressful conditions since there was no significant effect of the presence and/or absence of H.

In the 1st P0, seedlings grown with H showed higher values of i*WUE* than those grown without H. There were no differences between water regimes in the 2nd P0 and END periods (Figure 3A). This indicates CO_2 entry even at low g_s values, which minimized water losses through water use optimization. The chemical composition of H may have possibly contributed to this. Polyacrylamide can absorb water and store it for plants as needed for their development (SINGH et al., 2021), helping to prevent tissue dehydration even under low water availability. The seedlings recovered after periods of stress, reinforcing the physiological plasticity of *E. myrcianthes*.

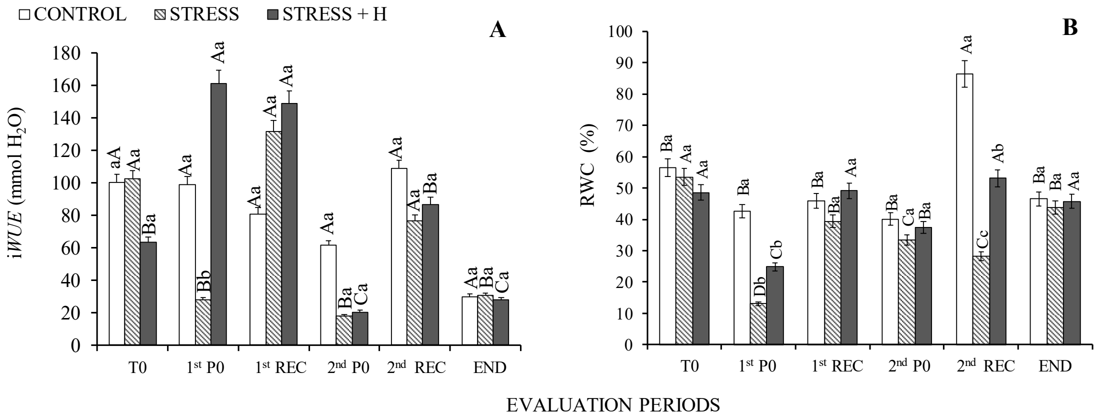

Figure 3. Water use efficiency (i*WUE* - *A/gs*) (A) and relative water content (RWC) (B) in leaves of *Eugenia myrcianthes* Nied. seedlings under different water conditions (control, stress, and stress + H) and evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC, and END) (*n*=3). Uppercase letters group the evaluation periods within each water condition (Scott-Knott test, $p \leq 0.05$), and lowercase letters compare the water conditions within each evaluation period (Tukey test, $p \leq 0.05$). Vertical bars indicate the standard deviation.

The RWC of seedlings under stress grown without and with H decreased in the 1st P0, reaching 13 and 24.8%, respectively. The 2nd P0 accounted for no differences between water regimes. In the 2nd REC, although not reaching values equivalent to those of the control (86.38%), seedlings grown with H presented good hydration (53.1%), differing from seedlings grown without H (28.22%) (Figure 3B). The lowest RWC of seedlings grown with and without H in the 1st P0 reinforces our idea that the dose of H applied to the pit in this experiment may have been insufficient to maintain leaf turgor.

Regarding Fv /Fm in the 1st and 2nd P0, seedlings grown with H showed lower values (below 0.70) than those grown under other water regimes. However, their photochemical performance improved again in the 1st REC. In the 2nd REC, seedlings under water stress, regardless of the use of H, showed higher F_v/F_m than control seedlings (Figure 4A). The use of H in seedlings grown under water stress reduced F_v/F_0 in the 1st and 2nd P0. Conversely, there were no differences between water regimes in the END period (Figure 4B).

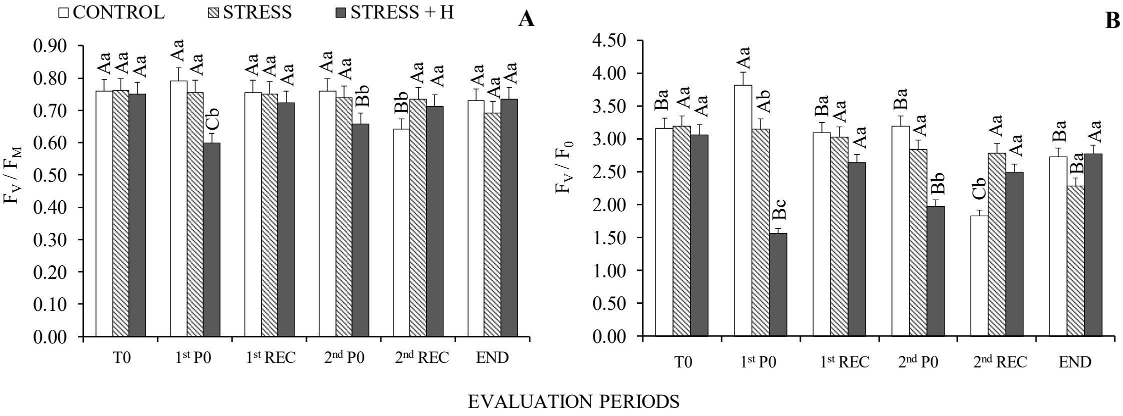

Figure 4. Quantic photochemical efficiency of photosystem II – PS II (F_v/F_m) (A) and Absorbed energy conversion efficiency (F_v/F_0) (B) in leaves of *Eugenia myrcianthes* Nied. seedlings under different water conditions (control, stress, and stress + H) and evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC and END) (*n*=3). Uppercase letters group the evaluation periods within each water condition (Scott-Knott test, $p \leq 0.05$), and lowercase letters compare the water conditions within each evaluation period (Tukey test, $p \leq 0.05$). Vertical bars indicate the standard deviation.

Hydrogel did not contribute to the photochemical activities in photosystem II of *E. myrcianthes* seedlings. Therefore, the ability of H to maintain water status to favor this attribute can vary according to the physiological mechanisms of each species (FERREIRA et al., 2014). We noticed that for *E. myrcianthes,* during the stress phases, the hydrogel impaired the activities in the reaction centers, being an atypical response since we did not observe this behavior in other species.

This behavior may be associated with the fact that although the seedlings had reduced *A* values in the 1st and 2nd P0, the species showed plasticity through mechanisms of maintenance of the photochemical apparatus even under conditions of stress due to deficit and/or flooding since the F_v/F_m and F_v/F_0 values did not differ statistically from the control seedlings, considering the intensity and times of exposure to the stressors. However, regardless of the use of the hydrogel, the reduction of energetic substrates in the photochemical phase impaired the efficiency of carboxylation and carbohydrate production, which negatively affected seedling growth.

Changes in the fluorescence characteristics of chlorophyll-*a* can demonstrate photosynthetic impairment since P0 values vary according to the environment in which the seedling is inserted or cultivated. This trait represents the emission of light by excited chlorophyll-*a* molecules before the energy is dissipated to the reaction center of photosystem II, which may indicate a reduction in energy production optimization for investment in seedling growth and development (FEYZIYEV, 2019). Notwithstanding, the F_v/F_m of seedlings increased in the END period, indicating the ability of these plants to recover photochemical activities.

Although the plant survives this condition, the growth potential in height is altered due to the targeting of energy to other needs (OLIVEIRA; GUALTIERE, 2017). The polymer under study improves soil conditions because its material consists of one or more three-dimensional structured networks formed with macromolecular chains interconnected by covalent bonds. When placed in water, these chains interact with the medium, which causes them to expand while improving substrate aeration and drainage (KUMAR et al., 2020).

Leaf area (LA) varied between the water regimes from the 2nd P0 onwards. Seedlings under stress, regardless of the use of H, had lower leaf area until the END period (Figure 5A). Water stress severely affected the leaf area (LA) of the species, and hydrogel (H) did not improve this attribute. Water stress usually decreases this attribute as the plant needs to minimize transpiration, avoiding further oxidative damage.

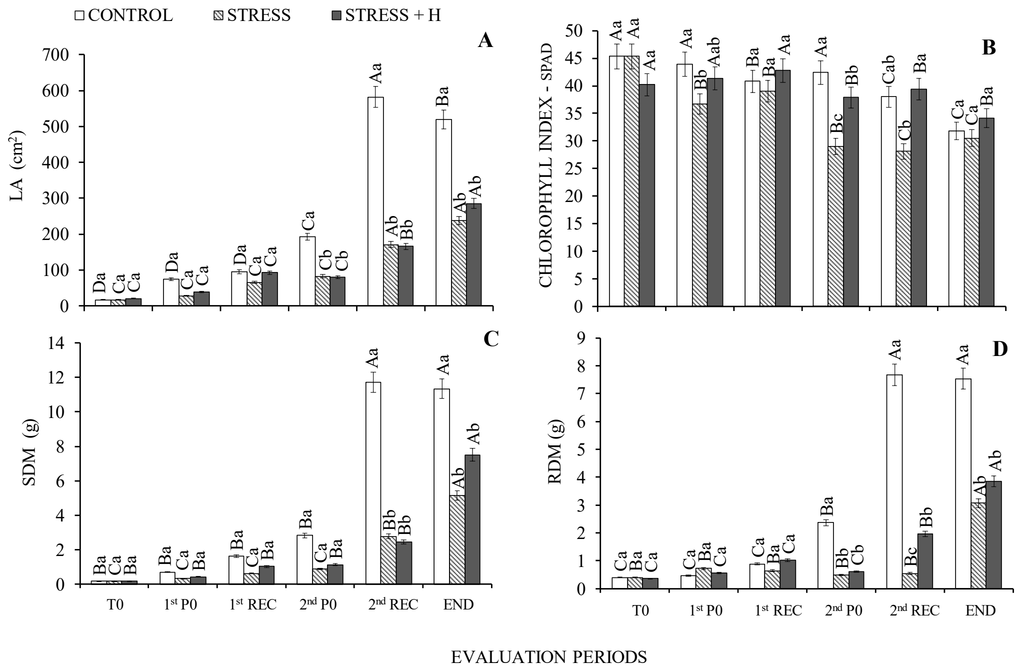

Figure 5. Leaf area (LA) (A), chlorophyll index (SPAD) (B), shoot dry mass (C), and root dry mass (D) in seedlings of *Eugenia myrcianthes* Nied. seedlings under different water conditions (control, stress, and stress + H) and evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC, and END) (n=3). Uppercase letters group the evaluation periods within each water condition (Scott-Knott test, $p \leq 0.05$), and lowercase letters compare the water conditions within each evaluation period (Tukey test, $p \leq 0.05$). Vertical bars indicate the standard deviation.

However, growth responses to the use of H vary between species. *S. terebinthifolia* seedlings grown under water deficit + H had a larger leaf area in the recovery phase (BELTRAMIN et al., 2020).

The chlorophyll index (SPAD) of seedlings under stress was lower in the 1st P0, but control seedlings did not differ from seedlings grown with H in that period. In the 2nd P0, seedlings of the stress + H treatment had higher values (37.9) than those grown without H (Figure 5B). In the two recovery phases, seedlings grown with H had values that did not differ from those of the control. Hydrogel showed a mitigating effect on SPAD during water deficit and flooding periods. Thereby, treatment and control seedlings presented similar values for this parameter. Severe water stress can reduce chlorophyll synthesis and cause chlorophyll degradation by oxidative damage due to carbohydrate accumulation and reactive oxygen species production (BARBOSA et al., 2014).

Hydrogel assists in maintaining soil moisture by minimizing the effects of root dehydration in situations of water deficit and may influence the maintenance of plant turgor under stressful conditions (SINGH et al., 2021). The presence of H in the roots did not have a negative effect during flooding, demonstrating that using the polymer was not harmful in this condition.

In general, the shoot and root dry mass values of plants under stress (1st and 2nd P0) were lower, regardless of the use of H (Figures 5C and 5D). These reductions are due to lower *A* values in these periods of stress, resulting in lower production of photoassimilates. According to Medeiros et al. (2023) increasing water use efficiency and reducing biomass is a strategy to reduce the effects of water stress. The flooding condition, as it contains low availability of oxygen in the soil, affects the development and growth of the plant; thus, the stoppage of growth may have contributed to the tolerance of the stressful situation and survival of the seedlings (ASLAM; ASLAM, 2023).

Superoxide dismutase (SOD) activity in leaves was higher in seedlings under stress, regardless of the use of H, in the 1st P0. The 2nd P0 accounted for a reduction in SOD only in seedlings grown with H. SOD activity in roots increased only in seedlings grown without H in the 1st P0 (Figure 6). Abiotic stresses alter cellular physiology and biochemistry, in which plants develop several mechanisms to deal with oxidative stress, including the production of antioxidants and activation of stress response pathways (MISHRA et al., 2023), which may have contributed to the survival and recovery of *E. myrcianthes* seedlings.

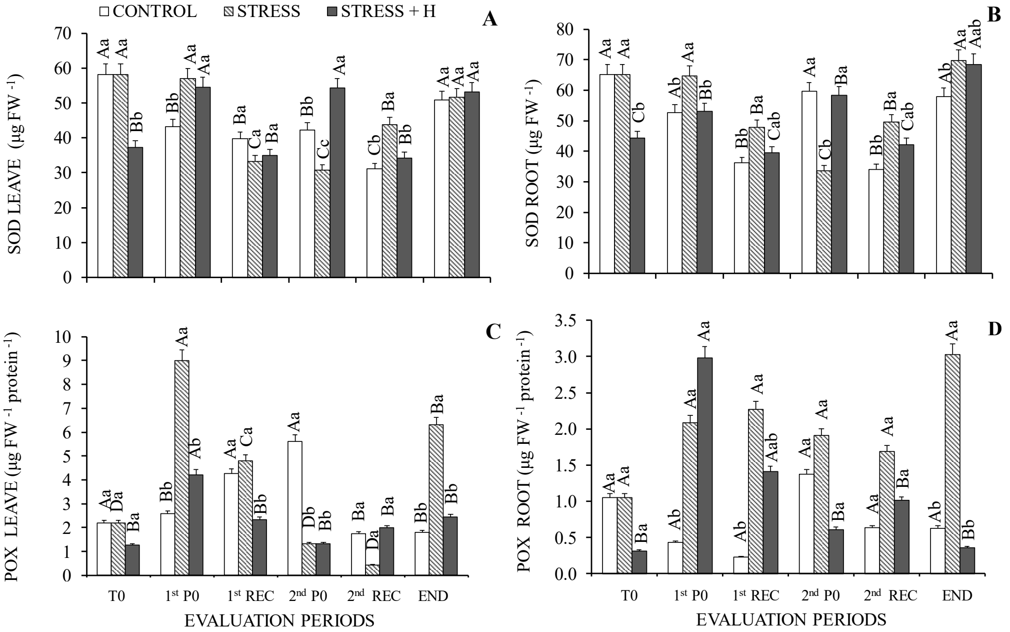

Figure 6. Activity of superoxide dismutase in leaves (A), superoxide dismutase in roots (B), peroxidase in leaves (C), and peroxidase in roots (D) of *Eugenia myrcianthes* Nied. seedlings under different water conditions (control, stress, and stress + H) and evaluation periods (T0, 1st P0, 1st REC, 2nd P0, 2nd REC, and END) (*n*=3). Uppercase letters group the evaluation periods within each water condition (Scott-Knott test, $p \leq 0.05$), and lowercase letters compare the water conditions within each evaluation period (Tukey test, $p \leq 0.05$). Vertical bars indicate the standard deviation.

The peroxidase (POX) activity in leaves was higher (8.99 g FW^{-1} protein^{-1}) in the 1st P0 and END periods (6.31 FW^{-1} protein^{-1}) in seedlings under stress grown without H (Figure 6C). The peroxidase activity in roots was higher in the 1st P0 and 1st REC in seedlings under stress. The 2nd P0, 2nd REC, and END periods accounted for the highest values of this parameter in seedlings grown under stressful conditions (Figure 6D). In both organs, the POX activity remained high at the end of the evaluations in seedlings under stressful conditions and grown without using H.

The exposure of plants to several types of stress can cause the overproduction of reactive oxygen species (ROS), leading to an imbalance in cellular metabolism. The activity of SOD and POX is an important parameter to quantify plant responses to environmental stresses, correlating with the adaptation of the species to water stress and its ability to maintain sufficient levels of antioxidants in its tissues (GARCÍA-CAPARRÓS et al., 2021).

The SOD activities in leaves and roots were higher in seedlings under water deficit, without or with H. The increase in antioxidant enzymes acts in the elimination of ROS. Changes in the activities and quantities of these enzymes indicate an alteration in the redox state in plants under stressful conditions.

Stressed seedlings showed higher enzymatic activity during the 2nd REC. This was probably due to the previous stress that the species suffered from water deficit, recovery, and subsequent flooding. The occurrence of fluctuations in the values (even in control seedlings) demonstrates that the alteration of enzymatic activity may correlate with factors other than water regimes, including seasonal changes in temperature, relative humidity, and dry and rainy seasons (SONG-PING et al., 2020).

Seedlings that underwent intermittent stress without H in the soil had higher POX values in the END evaluation. It shows that the seedlings of this treatment may have undergone greater metabolic interference caused by water fluctuations, increasing ROS production. From its ability to maintain soil moisture, H may have helped the plant to maintain balance and minimize ROS production. Intermittent water deficit and flooding promoted oxidative stress and changes in the enzymes SOD and POX of *E. myrcianthes* seedlings, which may correlate with the protective mechanism.

Proline amino acid content in leaves was influenced by the water conditions, with the highest values occurring in seedlings grown under stress (1.70 µg mL^{-1}) (Figure 7A), in the 1st REC and 1st P0 (2.86 and 1.96 µg mL^{-1}, respectively) (Figure 7B).

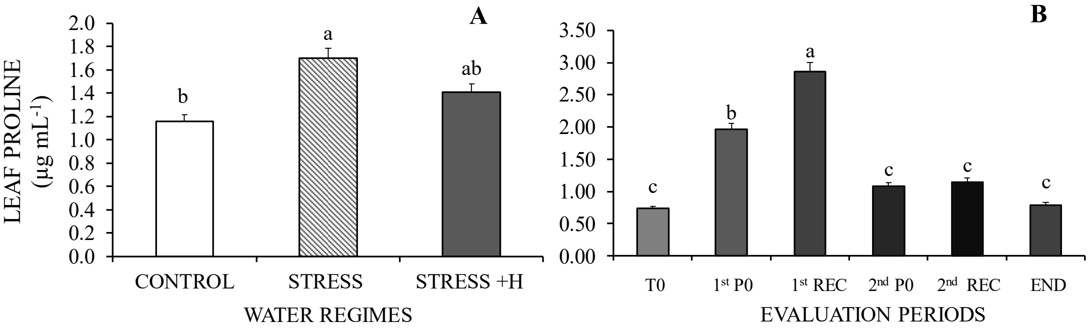

Figure 7. Proline content in leaves of *Eugenia myrcianthes* Nied. seedlings under different water conditions and evaluation periods (*n*=3). Tukey test ($p \leq 0.05$) compares water conditions and Scott-Knott test ($p \leq 0.05$) groups evaluation periods. Vertical bars indicate the standard deviation.

The higher proline content in seedlings grown under stress is because this amino acid relieves cell damage and reduces the negative effects of ROS under water deficit as an osmoregulation agent (WAHAB et al., 2022). Higher values of this parameter in the 1st P0 and 1st REC are due to the negative effect of the deficit and possibly to the recovery period being short for the plant to return to the antioxidant metabolism in a stabilized manner, even with normalized photosynthetic metabolism. Conversely, we observed that proline content in the 2nd P0 was lower, suggesting memory responsive to first stress (water deficit), which may have contributed to possible tolerance induction.

This study shows that the hydrogel did not alleviate the stressful effect of low availability or excess water for *E. myrcianthes* seedlings, rejecting our initial hypothesis. Furthermore, the hydrogel did not accentuate the effect of flooding since we were unsure whether the excess water (2nd P0) associated with the gel around the roots from the water-retaining polymer could further harm the plant's metabolism.

Novel studies with the species, testing the amount of hydrogel per pit and longer exposure times to the stressful conditions tested here, are relevant for future developments. The amount used in this study has been shown to favor the capacity for greater soil water storage but has not been reflected in many of the evaluated traits, especially regarding seedling growth. Furthermore, it is suggested that determinations be made of the activity of other antioxidant enzymes and non-enzymatic compounds to contribute information to understanding the mechanisms of the species and the hydrogel under these stressful conditions.

CONCLUSION

Eugenia myrcianthes Nied. seedlings decreased morpho-physiological attributes under water deficit and flooding, and the hydrogel did not mitigate the damage to the reaction centers and gas exchange of this species.

The seedlings showed survival and resumption of metabolism and growth after stressful conditions. This demonstrates their resilience due to physiological plasticity, regardless of the use of hydrogel.

ACKNOWLEDGMENTS

To the CAPES and CNPq for granting the scholarships and the FUNDECT for financial support.

REFERENCES

ASLAM, S.; ASLAM, S. Impact of flooding on agricultural crops an overview. **Environmental Processes and Management**, 120: 255-263, 2023.

BARBOSA, M. R. et al. Geração e desintoxicação enzimática de espécies reativas de oxigênio em plantas. **Ciência Rural**, 44: 453-460, 2014.

BATES, L. S.; WALDREN, R. P; TEARE, I. D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, 39: 205-207, 1973.

BELTRAMIN, F. A. et al. Water-retaining polymer mitigates the water deficit in *Schinus terebinthifolia*: photosynthetic metabolism and initial growth. **Engenharia Agrícola**, 40: 684 -691, 2020.

BISPO, T. M.; VIEIRA, A. V. Assimilatory deficit and energy regulation in young *Handroanthus chrysotrichus* plants under flooding stress. **Journal of Plant Research**, 135: 323-336, 2022.

BOGATI, K.; WALCZAK, M. The impact of drought stress on soil microbial community, enzyme activies and plants. **Agronomy**, 12: 189, 2022.

BROETTO, F. **Métodos de trabalho em bioquímica vegetal e tecnologia de enzimas**. [Recurso Eletrônico] / Coordenador: Fernando Broetto – Botucatu: IBB, Cultura. São Paulo, SP: Acadêmica, UNESP, 2014. 92 p.

CORDOBA-NOVOA, H. A. et al. Shading reduces water deficit in strawberry (*Fragaria* x *Ananassa*) plants during vegetative growth. **International Journal of fruit Science**, 22: 725-740, 2022.

FERREIRA, E. A. et al. Eficiência do hidrogel e respostas fisiológicas de mudas de cultivares apirênicas de citros sob déficit hídrico. **Pesquisa Agropecuária Tropical**, 44: 158-165, 2014.

FEYZIYEV, Y. M. Chlorophyll fluorescence and “Maximum

Quantum Efficiency” of photosystem II in plant sciences. **Life Sciences and Biomedicine**, 1: 18-28, 2019.

FONSECA, L. et al. Viabilidade do hidrogel na recuperação de cerrado sensu stricto com espécies nativas. **Floresta e Ambiente**, 24: e20160227, 2017.

GARCÍA-CAPARRÓS, P. et al. Oxidative stress and antioxidante metabolism under adverse environmental conditions: a review. **The Botanical Review**, 87: 421-466, 2021.

GUARINO, E. S. G. et al. **Espécies de plantas prioritárias para projetos de restauração ecológica em diferentes formações vegetais no bioma pampa: primeira aproximação**. Pelotas, RS: EMBRAPA CLIMA TEMPERADO, 2018. 79 p. (Documentos, 457).

INFANTE, J. et al. Antioxidant and anti-inflammatory activities of unexplored brazilian native fruits. **Plos One**, 11: 1-13, 2016.

KUMAR, R. et al. Hydrogel and its effect on soil moisture status and plant growth: A review. **Journal of Pharmacognosy and Phytochemistry**, 9: 1746-1753, 2020.

LANGNER, J. A. et al. Water-deficit tolerance of landrace and improved corn genotypes. **Pesquisa Agropecuária Brasileira**, 56: e02627, 2021.

MEDEIROS, R. L. S. et al. How does water deficit induce changes in primary and secondary metabolism in *Corymbia citriodora* seedlings? **Scientia Forestalis**, 51: e3911, 2023.

MISHRA, N. et al. Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. **Frontiers in Plant Science**, 14: 1110622, 2023.

OLIVEIRA, A. K. M.; GUALTIERE, S. C. J. Trocas gasosas e grau de tolerância ao estresse hídrico induzido em plantas jovens de *Tabebuia aurea* (paratudo) submetidas a alagamento. **Ciência Florestal**, 27: 181-191, 2017.

REHMAN, R. S. et al. Abscisic acid mediated abiotic stress tolerance in plants. **Asian Journal of Research in Crop Science**, 7: 1-17, 2022.

SANTOS, S. R.; MARCHIORI, J. N. C.; SIEGLOCH, A. M. Diversidade estrutural em *Eugenia L.* (Myrtaceae). **Ciência Florestal**, 24: 785-792, 2014.

SHARMA, S. et al. Ultrastructure, adaptability, and alleviation mechanisms of photosynthetic apparatus in plants under waterlogging: a review. **Photosynthetica**, 60: 430-444, 2022.

SILVA, A. B. et al. Levantamento da biodiversidade de arbóreas em fragmento florestal na região sul de Minas Gerais. **Revista Agrogeoambiental**, 8: 47-60, 2016.

SILVA, M. S. et al. Do hydro-retainer polymers attenuate damage from water fluctuations in leaf metabolism and the quality of *Cedrela odorata* seedlings? **International Journal of Agriculture & Biology**, 26: 209-216, 2021.

SINGH, N. et al. 3-dimensional cross limked hydrophilic polymeric network “hydrogels”: na agriculture boom. **Agricultural Water Management**, 253: 106939, 2021.

SONG-PING, L. et al. Effects of seasonal variation on soil microbial community structure and enzyme activity in a masson pine forest in Southwest China. **Journal of Montain Science**, 17: 1398-1409, 2020.

SOUZA, C. C. et al. Avaliação de métodos de determinação de água disponível e manejo da irrigação em terra roxa sob cultivo de algodoeiro herbáceo. **Revista Brasileira de Engenharia Agrícola e Ambiental**, 4: 338-342, 2000.

TURNER, N. C. Techniques and experimental approaches for the measurement of plant water status. **Plant and Soil**, 58: 339-366, 1981.

WAHAB, A. et al. Plants physio-biochemical and phyto-hormonal responses to alleviate the adverse effects of drought stress: a comprehensive review. **Plants**, 11: 1620, 2022.

YANG, X. et al. Response mechanism of plants to drought stress. **Horticulturae**, 7: 1-36, 2021.