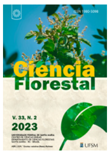
UFSM

Ciência Florestal



ISSN 1980-5098
OPEN ACCESS

Ci. Fl., Santa Maria, v. 33, n. 2, e65886, p. 1-25, Apr./June 2023 • https://doi.org/10.5902/1980509865886
Submitted: 21st/05/2021 • Approved: 9th/02/2023 • Published: 7th/06/2023

Artigos

Morphophysiological characteristics in *Erythroxylum pauferrense* Plowman plants under water stress

Características morfofisiológicas em plantas de *Erythroxylum pauferrense* Plowman sob estresse hídrico

João Everthon da Silva Ribeiro[I], Ester dos Santos Coêlho[I],
Francisco Romário Andrade Figueiredo[I],
Francisco Thiago Coelho Bezerra[II], Thiago Jardelino Dias[II],
Mário Luiz Farias Cavalcanti[II], Manoel Bandeira de Albuquerque[II]

[I]Universidade Federal Rural do Semi-Árido, Mossoró, Rio Grande do Norte, Brazil
[II]Universidade Federal da Paraíba, Areia, Paraíba, Brazil

ABSTRACT

Several abiotic factors, such as water availability, significantly affect the development of forest species found in the understory. This research aimed to evaluate morphophysiological characteristics in plants of *Erythroxylum pauferrense* submitted to different water regimes. The experiment was developed in a greenhouse at the Federal University of Paraíba, Campus II, Areia, Paraíba, Brazil. The experimental design was in randomized blocks, with five water treatments [100%, 80%, 60%, 40% and 20% of the pot capacity (PC)] and four replications. Growth attributes, morphofunctional aspects, gas exchange, chlorophyll fluorescence and chlorophyll indices were evaluated. The data were submitted for analysis of variance, and in cases of significance, polynomial regression analyses were performed. The plants of *Erythroxylum pauferrense* submitted to 100% and 80% water levels of the PC present greater development and morphophysiological performance. In comparison, the evaluated parameters were significantly reduced in the regime of greater water stress (20% of the PC). Thus, the water regime of 80% of PC is the most recommended for producing seedlings of *Erythroxylum pauferrense*, promoting greater growth and significant changes in morphofunctional aspects, gas exchange, chlorophyll fluorescence and chlorophyll indexes.

Keywords: Water availability; Ecophysiology; Erythroxylaceae; Gas exchanges

RESUMO

Vários fatores abióticos, como a disponibilidade de água, afetam significativamente o desenvolvimento das espécies florestais encontradas no sub-bosque. Esta pesquisa teve como objetivo avaliar características morfofisiológicas em plantas de *Erythroxylum pauferrense* submetidas a diferentes regimes hídricos. O experimento foi desenvolvido em casa de vegetação na Universidade Federal da Paraíba, Campus II, Areia, Paraíba, Brasil. O delineamento experimental foi em blocos ao acaso, com cinco tratamentos hídricos [100%, 80%, 60%, 40% e 20% da capacidade do vaso (PC)] e quatro repetições. Foram avaliados atributos de crescimento, aspectos morfofuncionais, trocas gasosas, fluorescência da clorofila e índices de clorofila. Os dados foram submetidos à análise de variância e, nos casos de significância, análises de regressão polinomial. As plantas de *Erythroxylum pauferrense* submetidas a regimes hídricos de 100% e 80% da PC apresentam maior desenvolvimento e desempenho morfofisiológico. Em comparação, os parâmetros avaliados foram significativamente reduzidos no regime de maior estresse hídrico (20% da PC). Assim, o regime hídrico de 80% de PC é o mais recomendado para produção de mudas de *Erythroxylum pauferrense*, promovendo maior crescimento e alterações significativas nos aspectos morfofuncionais, trocas gasosas, fluorescência da clorofila e índices de clorofila.

Palavras-chave: Disponibilidade de água; Ecofisiologia; Erythroxylaceae; Trocas gasosas

1 INTRODUCTION

The growth, development and ecophysiology of understory species vary according to ecosystems' climatic and ecological conditions. Given the seasonal variation in natural environments, the plants found in the understory are directly influenced by abiotic stresses, mainly by high irradiance, high temperature and water deficit. Water deficit is an abiotic factor affecting plant productivity, causing growth and development disorders in different phases or stages (KOCHHAR; GUJRAL, 2020).

Due to water stress, plants respond through various adaptive mechanisms and processes arising from physiological, anatomical and morphological responses due to the need to adapt to the environment (LAMBERS; OLIVEIRA, 2019). Among the effects caused by water deficiency in forest species are reduced growth, associated with a smaller leaf area, low dry matter production, reduction of the aerial part and increased leaf abscission (KUROMORI; SEO; SHINOZAKI, 2018). Such changes are also observed in other morphophysiological aspects, such as reductions in liquid CO_2 assimilation, transpiration, stomatal conductance, water use efficiency, relative water content and potential quantum yield of photosystem II, and increases in internal carbon concentration, thickness and succulence of leaves (D'ODORICO; PORPORATO; RUNYAN, 2019).

Among the understory species, *Erythroxylum pauferrense* Plowman (Erythroxylaceae) is a shrub or tree with a height between 1.5 and 4m (LOIOLA; AGRA; BARACHO; QUEIROZ, 2007). Endemic to the Northeast region of Brazil, the species is restricted to Paraíba, with the type specimen found in the municipality of Areia, in the Agreste Paraibano. According to the Red List of the Flora of Brazil, it is in the category “EN - in danger of extinction” (CNCFLORA, 2020) due to the alteration and reduction of its habitat due to deforestation in order to cause significant declines in individuals in the small existing subpopulations.

E. pauferrense is found in the understory of forest remnants of the Atlantic Forest, specifically in forest fragments of humid forests, called “Brejos de Altitude” (LOIOLA; AGRA; BARACHO; QUEIROZ, 2007). It has ecological importance in the conservation of the genetic resources of these regions, and the fruit serves as food for fauna, especially for birds, acting directly in the dispersal of seeds in troubled regions, such as the Altitude Swamps (RIBEIRO; LEITE; NÓBREGA; ALVES; BRUNO; ALBUQUERQUE, 2019). In addition, its highest rate of seedling emergence occurs in paper and vermiculite substrates at temperatures between 20 and 30°C (RIBEIRO; LEITE; NÓBREGA; ALVES; BRUNO; ALBUQUERQUE, 2019).

Given the importance of *Erythroxylum pauferrense*, studies are fundamental to understanding the morphophysiological behavior of the species in different water conditions, considering that water availability directly influences the photosynthetic process and the production of carbohydrates, which are used in the growth and development of plants. Therefore, this research aimed to evaluate morphophysiological characteristics in plants of *Erythroxylum pauferrense* submitted to different water regimes.

2 MATERIAL AND METHODS

The experiment was conducted in a greenhouse belonging to the Plant Ecology Laboratory, Department of Plant Science and Environmental Sciences, Center for Agricultural Sciences (CCA), Federal University of Paraíba (UFPB), Campus II, Areia, state of Paraíba, Brazil. The experiment site recorded an average temperature of 29.2°C and relative humidity of 52.4%. The measurements were performed with a portable digital thermo-hygrometer (Minipa, model MT-241A).

Erythroxylum pauferrense seeds were collected in different matrices in the Mata do Pau-Ferro State Ecological Park, municipality of Areia, state of Paraíba, Brazil. For the processing of the seeds, the pulp of the fruits was removed, and the seeds were placed in running water for five minutes and planted later. For planting, plastic pots with a capacity of 5 dm^3 were used, containing soil collected at the place of origin of the plants. The soil fertility analysis was: pH: 5.42; P: 55.29 mg dm^{-3}; K^+: 216.41 mg dm^{-3}; Na^+: 0.43 cmol dm^{-3}; H^++Al^{3+}: 4.62 cmol dm^{-3}; Al^{3+}: 0.00 cmol dm^{-3}; Ca^{2+}: 3.50 cmol dm^{-3}; Mg^{2+}: 3.10 cmol dm^{-3}; sum of bases: 7.58 cmol dm^{-3}; cation exchange capacity: 12.20 cmol dm^{-3}; and organic matter: 61.75 g dm^{-3}.

In planting, five seeds per pot were used for sowing and thinning was performed 30 days after emergence (DAE), selecting the uniform seedlings, 5 cm long and one pair of cotyledons. The plants were grown under 30% shading using a shady screen to minimize the stress caused by high irradiance. The soil was maintained at 100% of the maximum water retention capacity, after free drainage, for subsequent application of water treatments, from the gravimetric method.

For the acclimatization of the plants, 100% of the pot capacity (PC) was maintained for 30 days. After this period, treatments were initiated. The maintenance of the treatments was performed from daily weighing of the vessels and the replacement of the water lost by evaporation until the vessels reached the weight corresponding to each treatment. These measurements were performed with a digital scale with a capacity of 6kg (Balmak UD model).

The experimental design was in randomized blocks, with five water treatments: 100% (control), 80%, 60%, 40% and 20% of the PC, and four replications, with two plants per experimental plot, totaling 40 plants.

Growth analyses began at 60 DAE, with evaluations of plant height (Ph) (cm), stem diameter (Sd) (mm) and the number of leaves at 60, 90, 120, 150 and 180 DAE. The determination of absolute and relative growth rates for plant height (Eq. (1) and (2)) and stem diameter (Eq. (3) and (4)) were obtained according to the methodology proposed by Benincasa (2003):

$$AGR_{plant\ height} = \frac{(Ph2-Ph1)}{(t2-t1)} \tag{1}$$

$$RGR_{plant\ height} = \frac{(lnPh2-lnPh1)}{(t2-t1)} \tag{2}$$

$$AGR_{stem\ diameter} = \frac{(Sd2-Sd1)}{(t2-t1)} \tag{3}$$

$$AGR_{stem\ diameter} = \frac{(lnSd2-lnSd1)}{(t2-t1)} \tag{4}$$

In where: AGR is the absolute growth rate (cm day^{-1}); RGR is the relative growth rate (cm cm^{-1} day^{-1}); Ph1 and Ph2 are the plant heights (g) at times 1 and 2, respectively; Sd1 and Sd2 are the stem diameters (mm) at times 1 and 2, respectively; and ln is the natural logarithm.

At the end of the experiment, at 180 DAE, the plants were removed from the pots and the leaves, stems, and roots were separated. Then, the roots were washed in running water for cleaning and removal of the residues, and thus, the length of the primary root (LR) (cm) was measured from a ruler graduated in millimeters. Subsequently, the root system volume (RSV) was measured from the displacement of the water column in a graduated beaker, inserting the roots with a known volume of water (50 mL). From the observed difference, the volume of the root system was obtained directly through the equivalence of units (1 mL = 1 cm^3).

The leaf area (cm^2) was determined using digital images, in which the leaves of each plant were highlighted and digitized with a flatbed scanner (Canon model P-215II). The images were processed and analyzed by the ImageJ software. Subsequently, from the results of leaf area, the leaf area index (LAI), specific leaf area ($cm^2\ g^{-1}$), leaf area ratio ($cm^2\ g^{-1}$) and specific leaf weight ($g\ cm^{-2}$) were determined according to the methodology of Benincasa (2003).

The leaves, stem, and roots were kept on Kraft paper and placed in an oven with forced air circulation at 65 ºC for 72 hours until reaching a constant mass. Next, the dry mass of leaves (DML), stems (DMS), roots (DMR) and total dry mass (TDM) were measured, and the results were expressed in g $plant^{-1}$.

The relationship between shoot and root dry mass (DWSR) was calculated to evaluate the quality of the seedlings. In addition, the Dickson quality index (DQI) was determined from the total dry mass (TDM), shoot and root dry mass ratio (DWSR) and the ratio of plant height and stem diameter (Ph/Sd), using the equation (DICKSON; LEAF; HOSNER, 1960), Eq. (5):

$$DQI = \frac{TDM}{DWSR + \frac{Ph}{Sd}} \tag{5}$$

The morphofunctional attributes of the leaves were determined from the collection of 10 leaf discs (1 cm^2) per plant. Initially, the discs' fresh mass (FM) was determined with a digital scale (0.0001g). The discs were kept in distilled water in closed Petri dishes for 24 hours until reaching maximum water saturation. Next, the leaf thickness (TH) (mm) was determined using a digital caliper (± 0.001 mm), and the turgid mass (TM) was measured using a digital scale. Subsequently, to determine the dry mass (DM), the discs were stored on Kraft paper and placed to dry in an oven with forced air circulation at 65°C for 72 hours. From these data, we calculated the leaf mass per unit area (LMA) (g m^{-2}), which is the ratio between the dry mass and the disc area (POORTER; NIINEMETS; POORTER; WRIGHT; VILLAR, 2009) and the succulence (SUC) (g m^{-2}) which is calculated from the difference between the turgid mass and dry mass divided by the disc area.

The density (DEN) (mg mm^{-3}) was measured using the Eq. (6):

$$DEN = \frac{LMA}{TH} \tag{6}$$

The relative water content (RWC) was measured according to the Eq. (7):

$$RWC = \frac{FM-DM}{TM-DM}\, x\, 100 \tag{7}$$

The rate of photosynthesis (A) (µmol m^{-2} s^{-1}), stomatal conductance (gs) (mol m^{-2} s^{-1}), transpiration (E) (mmol m^{-2} s^{-1}), the internal concentration of CO_2 (Ci) (µmol mol^{-1}) and leaf temperature (ºC) were measured at 180 DAE. From these variables, we calculated the instantaneous efficiency of water use (WUE: A/E) [(µmol m^{-2} s^{-1})/(mmol m^{-2} s^{-1})], intrinsic efficiency of water use (iWUE: A/gs) [(µmol m^{-2} s^{-1})/(mol m^{-2} s^{-1})] and instantaneous carboxylation efficiency (iCE: A/Ci) [(µmol m^{-2} s^{-1})/(µmol mol^{-1})]. The analyses were performed on healthy, non-detached and completely expanded leaves in the plants' middle third. The measurements were performed on days with total solar luminosity conditions (zero cloudiness) between 11 and 12 hours. The measurements were performed using a portable infrared carbon dioxide (IRGA) analyzer (LI-COR, model LI-6400XT). The protocol for the evaluations with the IRGA was: leaf chamber of 6 cm^2; density of photosynthetically active photons of 1,200 µmol m^{-2} s^{-1}; relative humidity between 50 and 60%; airflow of 300 µmol s^{-1}; and atmospheric CO_2 concentration of 400 µmol mol^{-1}.

The fluorescence of chlorophyll a was quantified with a modulated fluorometer (LI-COR, model LI-6400-40) coupled to the IRGA. At 180 DAE, the following chlorophyll fluorescence parameters were determined: initial fluorescence (F_0), maximum fluorescence (F_m), variable fluorescence (F_v), the maximum quantum yield of PSII (F_v/F_m) and the ratio F_v/F_0. The measurements were performed on healthy leaves in the middle third of the plants, adapted to the dark, using leaf clips for 30 minutes. These measurements were performed in the same period as the gas exchange analyses.

The quantification of the chlorophyll index *a*, *b*, total and ratio *a*/*b* at 180 DAE was performed using the non-destructive method using the portable chlorophyll meter (ClorofiLOG, model CFL 1030) in four leaves of the middle third of the plants and the data were expressed in FCI (Falker Chlorophyll Index).

The data were submitted to analysis of variance (F test) at the level of 5% probability. In cases of significance, polynomial regression analyses were performed. The analyses were performed using the statistical program SAS University.

3 RESULTS AND DISCUSSION

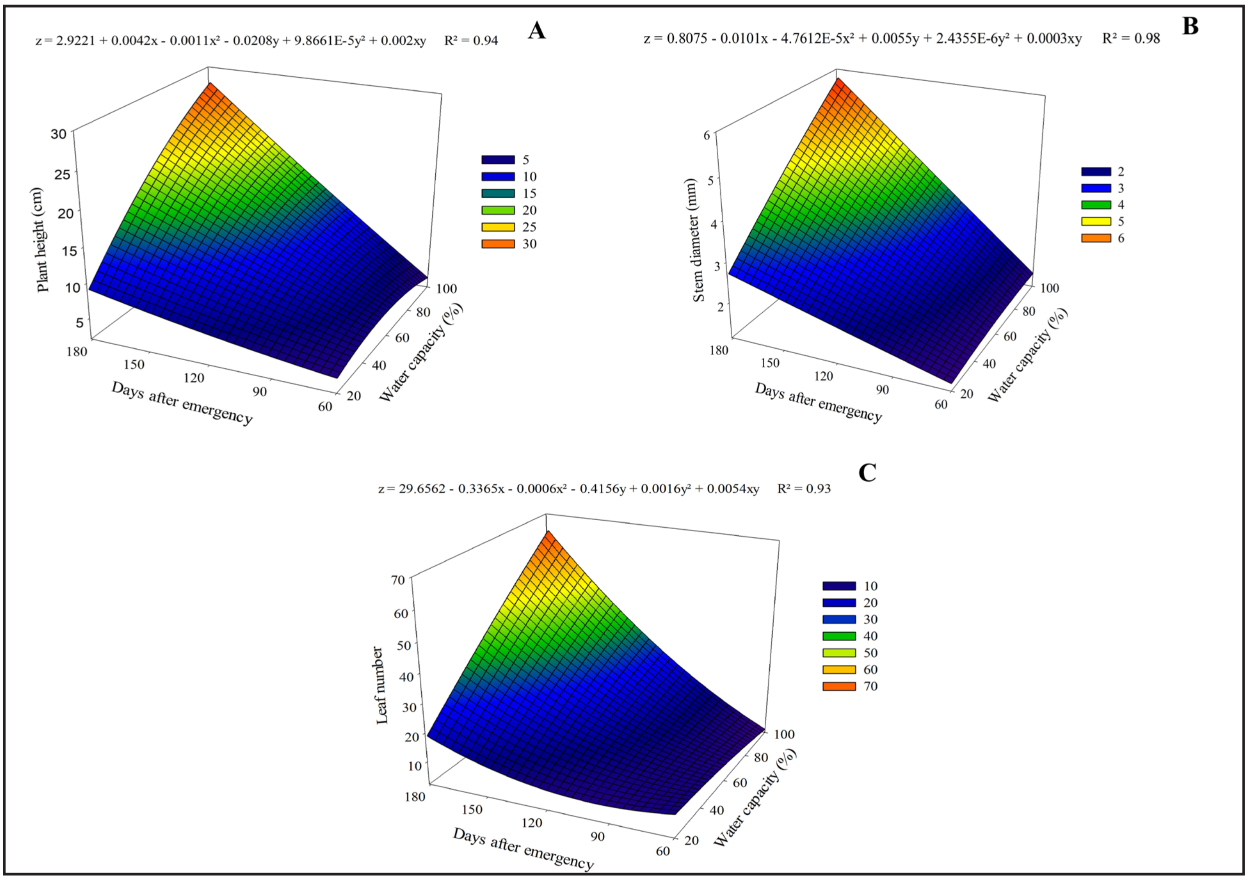
The height of plants was affected by water treatments from 120 DAE until the final period of the experiment (180 DAE), with the highest values recorded in 80% of PC and the lowest in plants submitted to the highest water stress in 20% of PC (Figure 1A). The stem diameter was significantly reduced with the increased water stress, presenting differences in the different water conditions at 150 and 180 DAE (Figure 1B). The highest values were recorded in conditions of greater water availability (100% and 80% of PC), with 5.05 mm and 4.99 mm, respectively (Figure 1B).

The reduction observed in plant height and stem diameter at the highest level of water stress possibly occurred due to a decrease in the turgescence of the cells before the closure of the stomata, negatively affecting the metabolism and providing a decrease in the growth and development of plants (SHABALA, 2012).

The number of leaves was reduced during the entire period of stress, being lower under the conditions of 20% of the PC, with higher values in the water regimes of 80% and 100% of the PC, obtaining 65.37 and 59.68 leaves/plant at 180 DAE, respectively

(Figure 1C). The decrease in the number of leaves is possibly related to one of the defense mechanisms of plants under stress conditions, to cause the acceleration of senescence and increased leaf abscission, limiting the loss of water by transpiration and metabolite processes for tissue maintenance (DUCA, 2015).

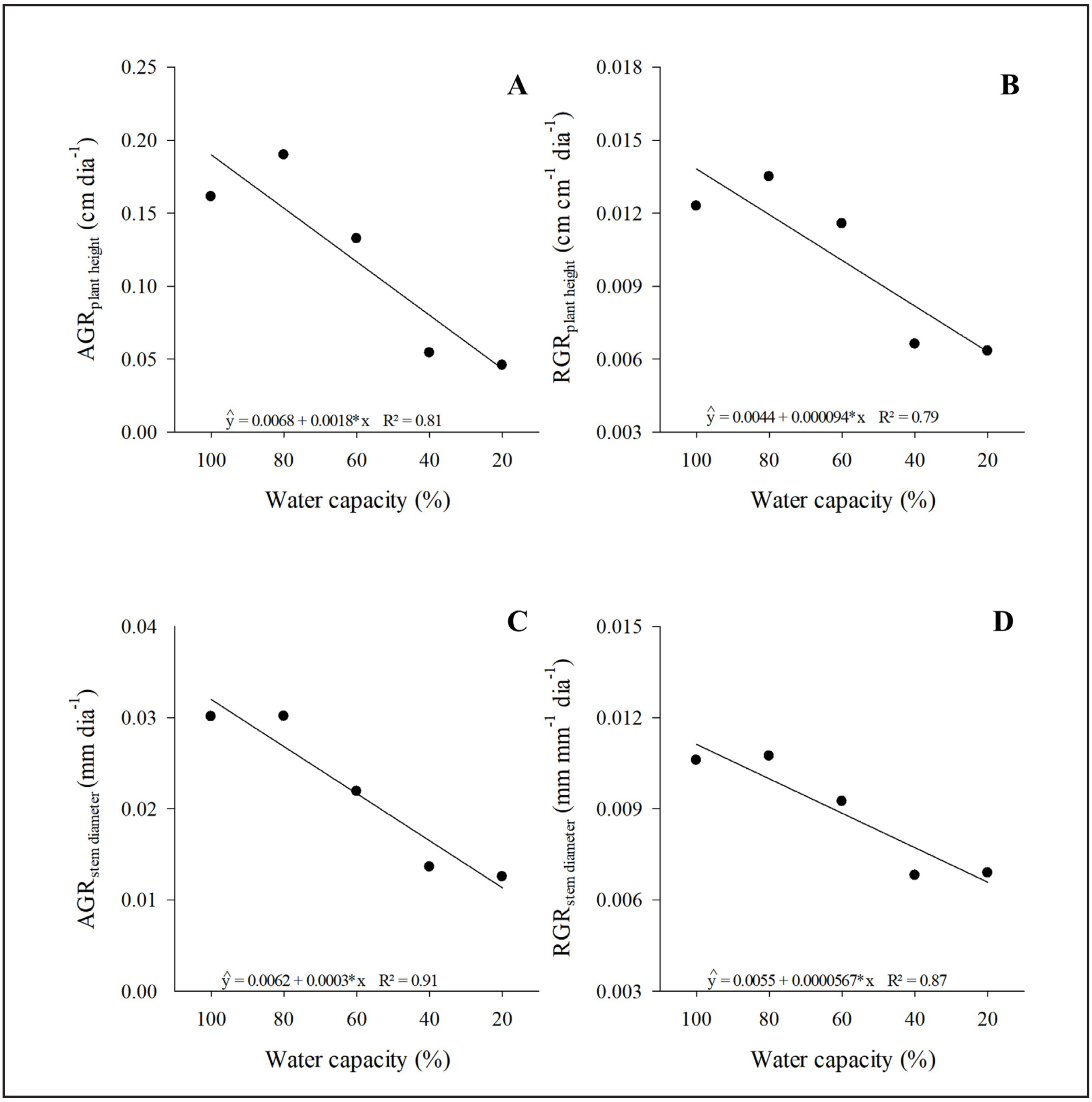
Figure 1 – Plant height (A), stem diameter (B), and the number of leaves (C) in *Erythroxylum pauferrense* plants as a function of water regimes and evaluation time



Source: Authors (2023)

The absolute and relative growth rates for plant height and stem diameter showed significant differences between the water regimes used (Figure 2). The absolute and relative growth rates for plant height were strongly influenced, with high reductions as water stress increased, with decreases of 75.8 and 53.1%, respectively, when comparing the highest and lowest values recorded (Figure 2A-B). The absolute and relative growth rates for stem diameter showed similar behaviors, with the highest values recorded in plants submitted to 80% of PC (0.0301 mm day^{-1} and 0.0107 mm mm^{-1} day^{-1}, respectively), differing 58.1 and 36.3% between the highest and lowest values found in the different water regimes (Figure 2C-D).

Figure 2 – Absolute and relative growth rates for plant height and stem diameter in Erythroxylum pauferrense plants under water regimes



Source: Authors (2023)

In where: A - Absolute growth rate for plant height ($\text{AGR}_{\text{plant height}}$); B - relative growth rate for plant height ($\text{RGR}_{\text{plant height}}$); C - absolute growth rate for stem diameter ($\text{AGR}_{\text{stem diameter}}$), D - relative growth rate for stem diameter ($\text{RGR}_{\text{stem diameter}}$) (D); in *Erythroxylum pauferrense* plants under water regimes.

The behavior of absolute and relative growth rates for plant height and stem diameter is similar to the crude values obtained in height and diameter since there were significant decreases in plants submitted to greater water stress. According to Benincasa (2003), the absolute growth rate is an index expressing the average plant growth speed over the observation period. Although this index is essential in physiological studies, it becomes more attractive for some researchers to obtain the relative growth rate to express the increase in plant growth in a given time interval as a function of the initial size (initial period of observation).

Regarding the length of the primary root, it is observed that there was a significant effect of the water treatments, with a reduction of 28.5% between the control treatments (100% of the PC) and the one with the lowest water regime (20% of the PC) (Figure 3A). The volume of the root system decreased significantly in response to water availability, with the highest values recorded under the conditions of 80% and 100% of PC (3.55 and 3.45 mL, respectively) (Figure 3B). From the analyses of the length of the primary root and volume of the root system, it is observed that the plants of *Erythroxylum pauferrense*, within the conditions of the present study, deepened the root system from the main root. However, the high-water stress did not allow us to generate a sufficient volume of roots, so it allowed us to explore a greater volume of soil, obtaining a greater absorption of nutrients. Root growth in plants under conditions of water stress depends on the turgor pressure in the cells being sufficient to allow a greater elongation of the cell wall and, consequently, cell growth (SCALON; MUSSURY; EUZÉBIO; KODAMA; KISSMANN, 2011).

The relationship between shoot and root dry mass (DWSR) was higher under 80% of PC, with a decrease of 55.8% for the treatment of 40% of PC (Figure 3C). The Dickson Quality Index (DQI), which uses the main growth parameters together, showed a similar behavior (6.00 in 80% of the PC, respectively) with an increase of 61.2% when comparing the highest and lowest value observed in the different water conditions (Figure 3D).

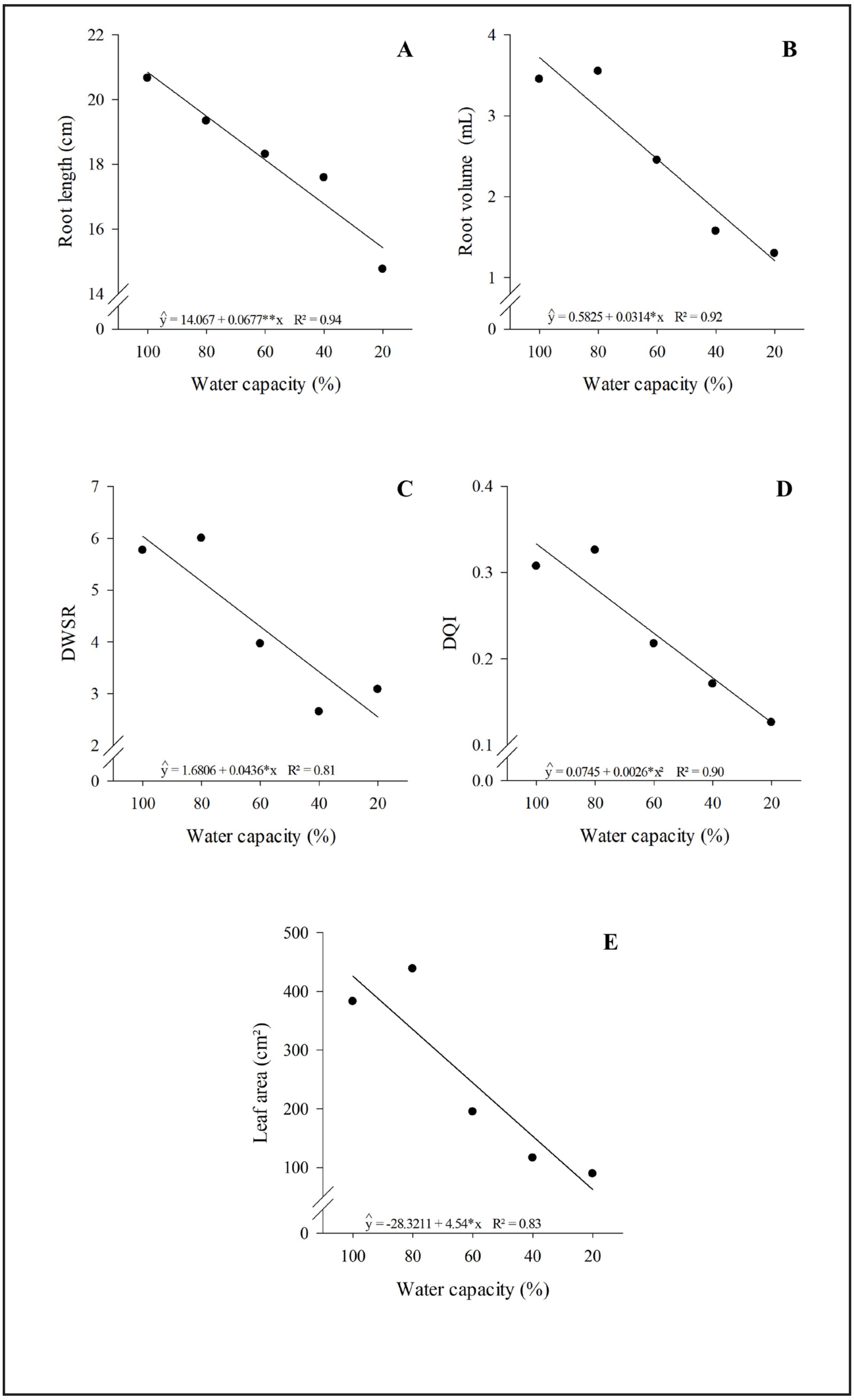
According to Taiz, Zeiger, Møller and Murphy (2017), a lower DWSR under water restriction may indicate that plants develop a lower capacity to exploit water, requiring a greater root system development to supply transpiration activities in the photosynthetic process. Similarly to the DWSR, the DQI is considered one of the most used indicators to evaluate the quality and vigor of seedlings, so it analyzes several growth parameters simultaneously. Thus, the increase observed in the present study may be related to higher production of total dry biomass.

According to Hunt (1990), the plants that register a minimum DQI of 0.20 present good-quality of seedlings. In the present study, plants under the lowest water conditions (20% and 40% of the PC) presented values below 0.20, indicating the lack of quality for producing seedlings of *Erythroxylum pauferrense* under the experimental conditions.

Regarding the specific leaf area, leaf area ratio and specific leaf weight, there were no significant effects of the water treatments, with mean values of 271.42 $cm^2 g^{-1}$, 106.87 $cm^2 g^{-1}$ and 0.0106 $g cm^{-2}$, respectively. The water stress used was sufficient to promote significant changes in leaf area (LA) (Figure 3E). The plants submitted to water treatments of 80% of the PC presented the highest values of LA (438.18 cm^2), while the plants under the water regime of 20% of the PC presented the lowest values (89.19 and 0.18 cm^2, respectively) (Figure 3E).

Low values of leaf area in the lowest water regime possibly occurred due to a defence mechanism of the plants of *Erythroxylum pauferrense*, thus decreasing the emission of new leaves and causing the loss of leaves, aiding in tolerance against water stress (OJEDA; RUBILAR; MONTES; CANCINO; ESPINOSA, 2018). According to Taiz, Zeiger, Møller and Murphy (2017), this mechanism is one of the first responses of plants to low water conditions that allows the plant to maintain its metabolism.

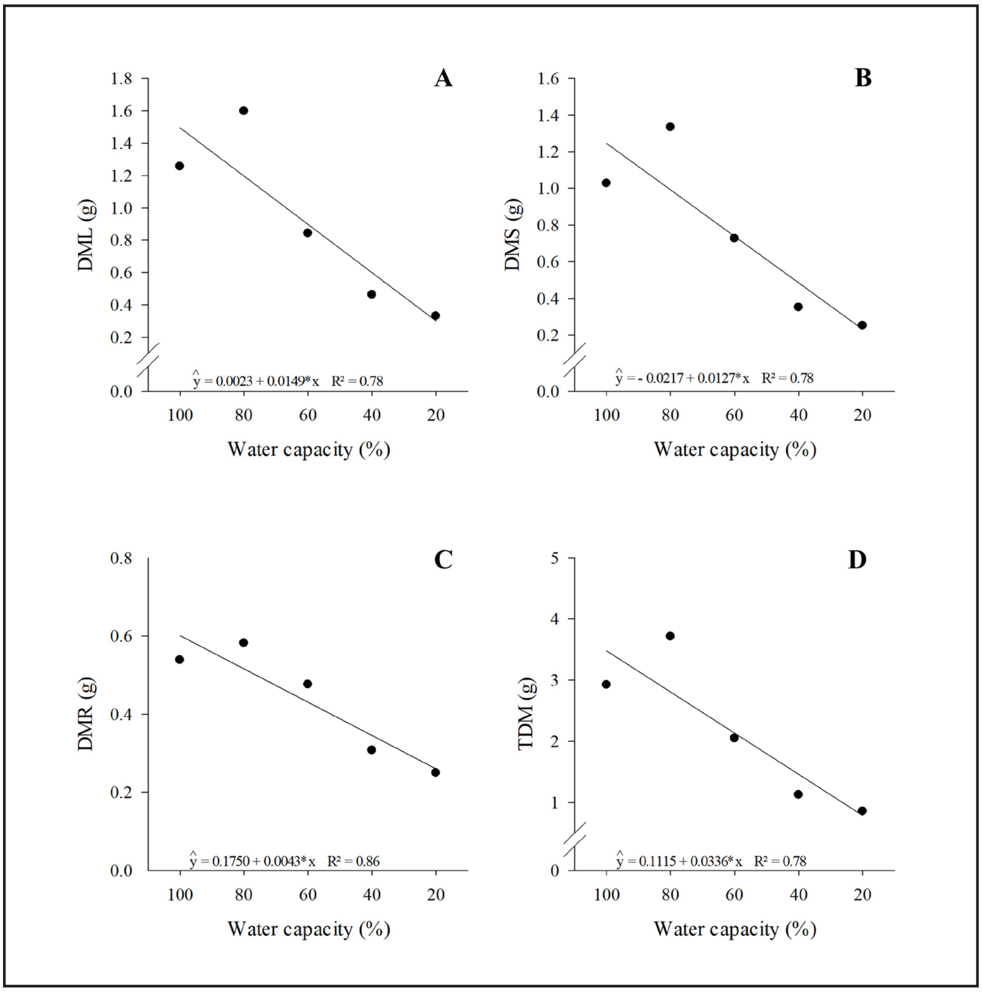
Figure 3 – Root length (A), root volume (B), ratio between dry shoot mass and root dry mass (DWSR) (C), and Dickson's quality index (IQD) (D) in *Erythroxylum pauferrense* plants under water regimes



Source: Authors (2023)

Water stress significantly reduced the dry mass of the different parts (leaves, stems and roots) of *Erythroxylum pauferrense* plants (Figure 4A-C). It was observed that for the dry mass of leaves, the treatment of greater water stress presented reductions of 79.3%, about that of 80% of PC (Figure 4A). Regarding the dry mass of stems, a significant decline of 81.1% was observed between the highest (1.33 g plant^{-1} in 80% of the PC) and the lowest value (0.25 g plant^{-1} in 20% of the PC) (Figure 4B). The dry mass of roots showed a similar trend, with a constant reduction with the increase of water stress, where the more severe treatment (20% of PC) promoted a reduction of 57.1% compared to 80% of PC (Figure 4C). The total dry mass increased by 77.0% compared to the highest and lowest values observed in the water regimes (Figure 4D).

Figure 4 – Dry mass of leaves (DML) (A), stems (DMS) (B) and roots (DMR) (C), and total dry mass (TDM) (D) in *Erythroxylum pauferrense* plants under water regimes



Source: Authors (2023)

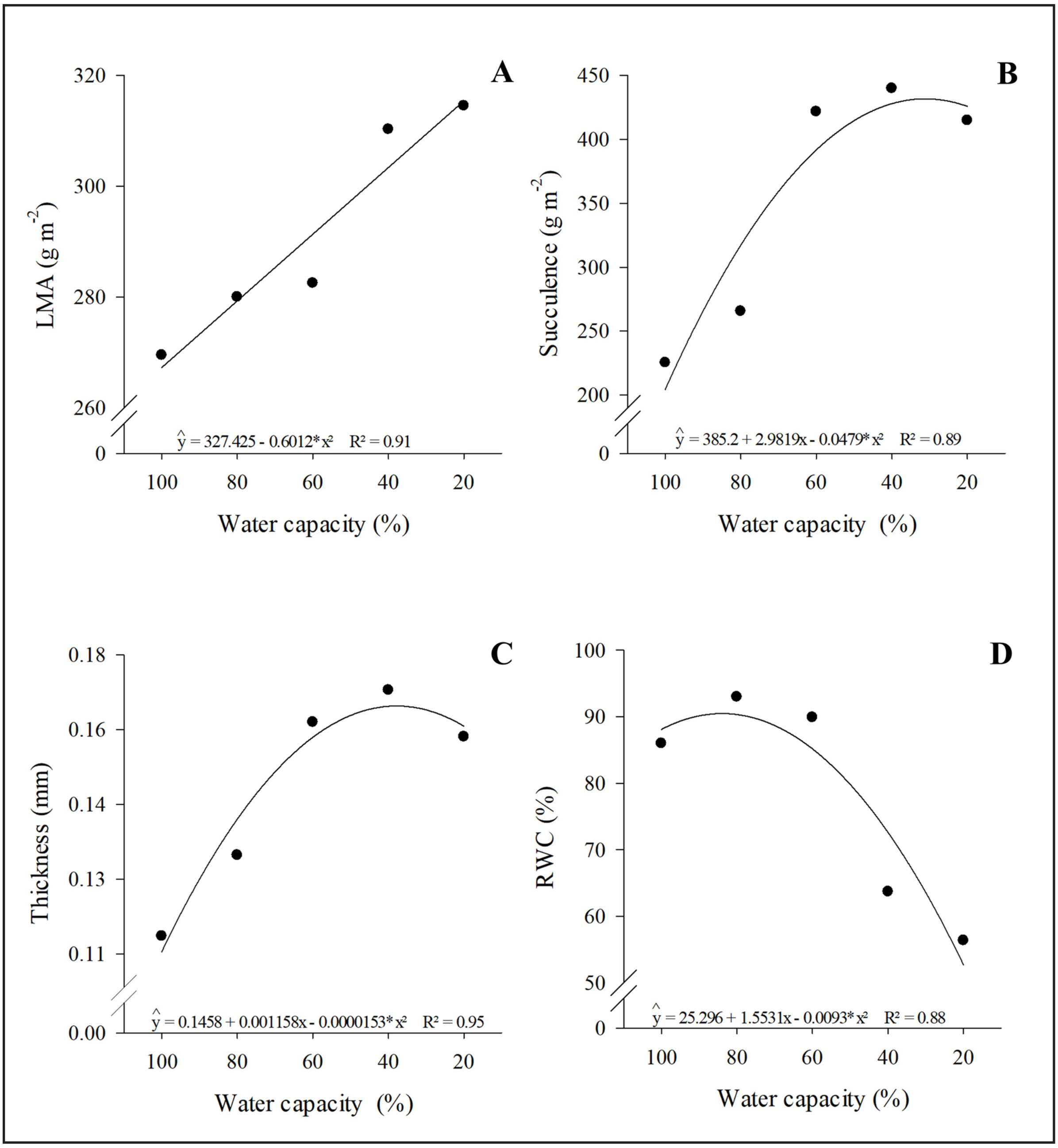
DML and DMS were the most sensitive to water deficit, showing greater reductions with increasing stress intensity. This observed decrease probably occurred due to plants' low assimilation of CO_2, which reduced the production of photoassimilates necessary for the growth and accumulation of dry biomass (ALVES; ALBUQUERQUE; PEREIRA; FELIX; AZEVEDO, 2018).

The leaf mass per unit area (LMA), juiciness and leaf thickness showed similar behaviors with higher values in plants under greater water stress, with a decline of 14.6%, 48.7% and 34.5% compared to the control treatment (100% of PC) (Figure 5A-C). The density was insignificant in the different water treatments, presenting a mean value of 0.2004 mg mm^{-3}.

LMA plays a central role in the ecological strategies of plants subjected to stress conditions, being an indicator of the performance of species over a given period in rich or resource-poor environments. High values of this index can be interpreted as an adaptation of plants to low water availability, associated with a period of longer leaf life, corroborating the present study. According to Ogburn and Edwards (2012), plants with narrower leaves are less succulent; thus, increased juiciness is associated with a greater thickness of leaves. Higher values of succulence and thickness of the leaves are related to a greater water storage capacity, which provides water reserves during greater water stress. The thickness of the leaves has a fundamental importance in the development and photosynthetic process of plants, being associated with strategies of acquisition and use of resources of the species (TOUNEKTI; AL-TURKI; MAHDHI; KHEMIRA, 2017). Generally, species with thicker leaves are found in resource-poor environments and low water availability (TOUNEKTI; AL-TURKI; MAHDHI; KHEMIRA, 2017), corroborating the present study.

The relative water content (RWC) showed a significant reduction as water restriction increased, with a decrease of 39.4% compared to the 80% water treatment of PC (Figure 5D). The relative water content in the leaves of *Erythroxylum pauferrense* can be considered as an indication of the conditions of plants in different water conditions to determine the ideal regime that favors the physiological performance of plants. The reduction of RWC significantly alters the physiological responses of plants in order to affect the integrity of the photosynthetic apparatus, which can cause disturbances in the growth of vegetables (ZEGAOUI; PLANCHAIS; CABASSA; DJEBBAR; BELBACHIR; CAROL, 2017).

Figure 5 – Leaf mass per unit area (LMA) (A), succulence (B), leaf thickness (C), and relative water content (RWC) in *Erythroxylum pauferrense* plants under water regimes



Source: Authors (2023)

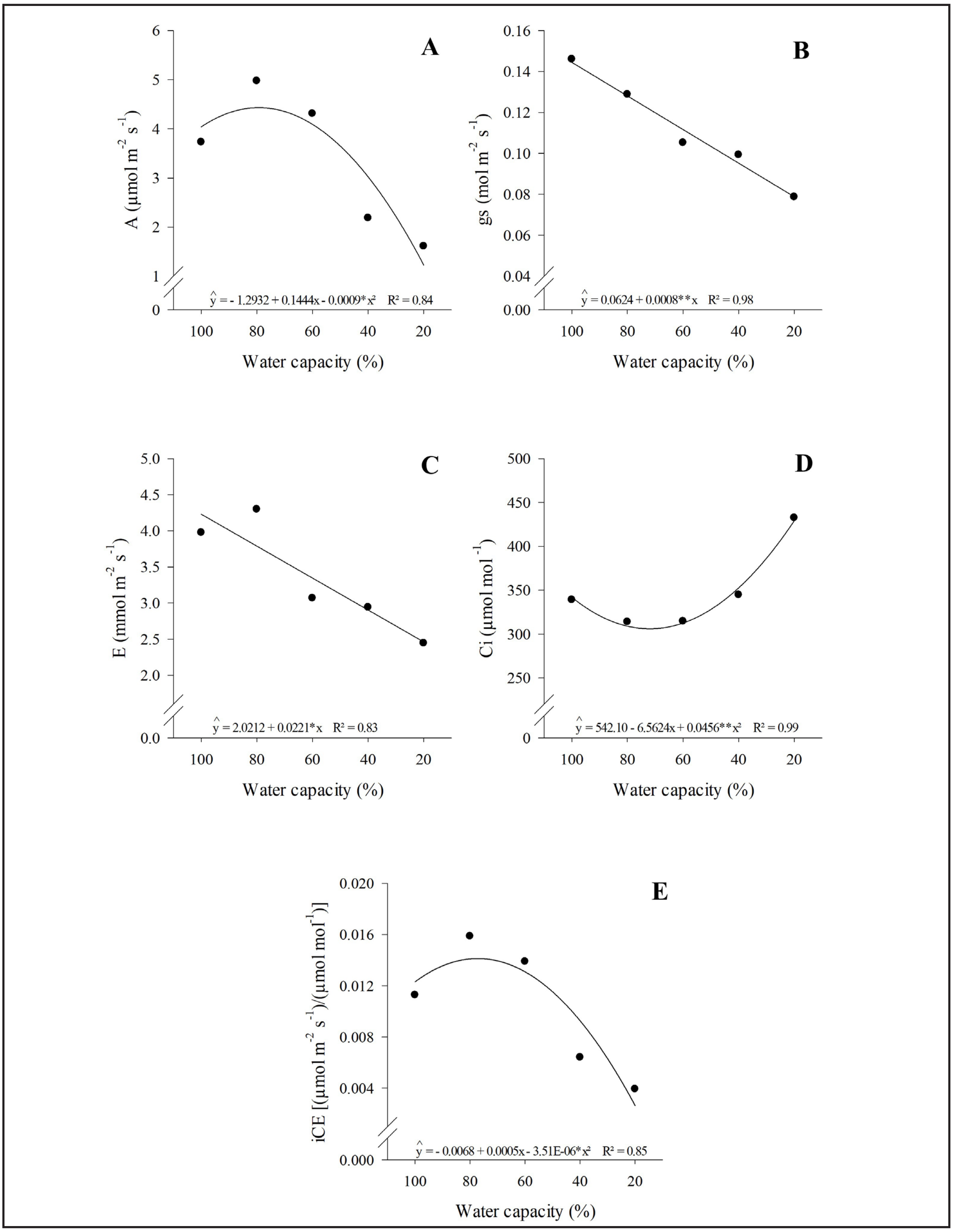
The different water regimes significantly influenced the gas exchange of *Erythroxylum pauferrense* plants (Figure 6A-E), except for leaf temperature, instantaneous water use efficiency and intrinsic water use efficiency, whose mean values were 34.9°C, 0.9817 [(μmol m^{-2} s^{-1})/(mmol m^{-2} s^{-1})] and 30.58 [(μmol m^{-2} s^{-1})/(mol m^{-2} s^{-1})], respectively. It is observed that the rate of photosynthesis (A) showed high decreases as stress increased, differing 67.5% between the highest (4.97 μmol m^{-2} s^{-1}: 80% of the PC) and the lowest (1.61 μmol m^{-2} s^{-1}: 20% of the PC) value found (Figure 6A). Stomatal conductance and transpiration showed similar behaviors, with the highest rates recorded in 100% and 80% of the PC (0.1461 mol m^{-2} s^{-1} and 4.29 mmol m^{-2} s^{-1}, respectively) and the lowest in the treatment of 20% of the PC (Figure 6B-C).

The lower values of A, gs and E found in the treatment with the lowest amount of water available in the soil can be explained by the more rigorous and necessary stomatal control in plants under stress conditions, limiting the stomatal conductance with a smaller opening of the stomata and reducing transpiration from mechanisms that avoid excessive water loss (LAMBERS; OLIVEIRA, 2019).

On the other hand, the internal concentration of CO_2 decreased with the increase in water availability, registering a higher value in the 20% PC regimen with an increase of 27.4% compared to the 80% PC treatment (Figure 6D). Generally, reductions in A and gs are associated with a lower internal concentration of CO_2 if there is no damage to the photosynthetic apparatus caused by stress. Thus, the reduction of Ci as stress decreased may be caused by biochemical factors, that is, by a non-stomatal factor, such as lack of ATP and NADPH, resulting in lower efficiency of photosystem II.

The lower water regime (20% of the PC) negatively influenced the instantaneous efficiency of carboxylation (iCE) in plants *of Erythroxylum pauferrense*, presenting a reduction of 19.6% about the condition of 80% of the PC (Figure 6E). This behavior of the iCE may have occurred due to the stomatal closure associated with water deficiency of the plants, decreasing the photosynthetic rate and consequently the efficiency of carboxylation, thus limiting the assimilation of CO_2 and lower activity of Rubisco (MEJÍA-DE TAFUR; RIAÑO-HERRERA; URREGO-MESA; IBARRA-ESPINOSA; ZAPATA-DUQUE, 2017).

Figure 6 – Photosynthesis rate (A) (A), stomatal conductance (gs) (B), transpiration (E) (C), internal CO_2 concentration (Ci) (D) and instantaneous carboxylation efficiency (iCE) (E) in *Erythroxylum pauferrense* plants under water regimes



Source: Authors (2023)

It was found that there were significant effects on chlorophyll fluorescence of *Erythroxylum pauferrense* plants submitted to water stress (Figure 7A-E). Initial fluorescence showed a significant increase as water availability was reduced, with the highest value (0.0159) observed in the treatment of 20% of PC (Figure 7A). This increase observed in F_0 in the conditions of greater water stress possibly occurred due to the reduction in the capacity of excitation energy transfer to the photosystem II, indicating possible destruction of the reaction center of the PSII (KALAJI; SCHANSKER; BRESTIC; BUSSOTTI; CALATAYUD; FERRONI; GOLTSEV; GUIDI; JAJOO; LI, 2017). As observed in the present study, severe stress conditions can alter the structure of photosynthetic pigments in the PSII, raising the values of F_0 (FEYZIYEV, 2020).

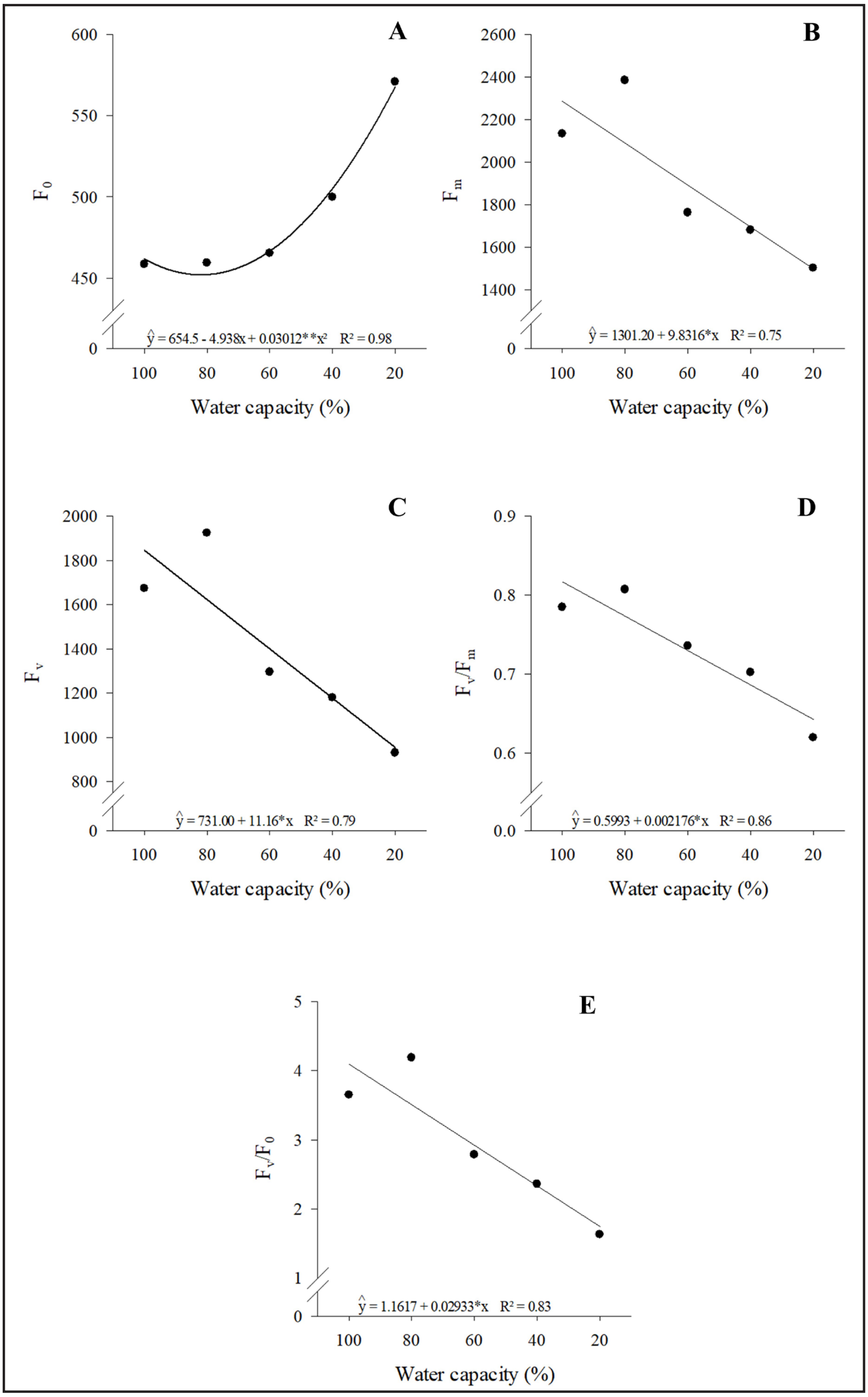
Maximum fluorescence (F_m), variable fluorescence (F_v), the maximum quantum yield of PSII (F_v/F_m) and F_v/F_0 ratio increased in *Erythroxylum pauferrense* plants submitted to 80% of PC, with increases of 37.0%, 51.6%, 23.2% and 61.0%, respectively, compared to the lower water regime (Figure 7B-E).

The decrease in Fm values in *Erythroxylum pauferrense* plants is possibly associated with deficiencies in quinone photoreduction since the reaction centers of the PSII did not reach their maximum capacity, altering the photochemical activities of the leaves, thus promoting a lower capacity of CO_2 fixation in the biochemical process of photosynthesis. The higher values of F_v in plants under greater water availability may be associated with a greater adaptation of the photosynthesizing tissue to the dark, providing greater capacity in the energy transfer of electrons for forming the NADPH and ATP reducer. Thus, in the present study, it was found that this capacity was lost throughout the low water conditions.

The maximum quantum yield (F_v/F_m) represents the energy conversion efficiency of the light absorbed in the reaction center of photosystem II. High values of this variable indicate a greater photochemical activity. According to Soares, Fernandes, Lima, Brito, Nascimento and Arriel (2018), plants that obtain values between 0.75 and 0.85 with the photosynthetic apparatus intact (without damage), evidencing according to the present study that the plants of *Erythroxylum pauferrense* submitted to the highest water levels presented quantum yield good values.

The ratio F_v/F_0 represents the relationship between the flow of absorbed energy and dissipated energy, being one of the main parameters to be influenced by stresses, possibly indicating the maximum efficiency of the PSII or the potential photosynthetic activity, with good values between 4 and 6, observed in the present study in the treatment of 80% of PC.

Figure 7 – Initial fluorescence (F_0) (A), maximum fluorescence (F_m) (B), variable fluorescence (F_v) (C), maximum quantum yield of PSII (F_v/F_m) (D) and F_v/F_0 ratio (E) in *Erythroxylum pauferrense* plants under water regimes

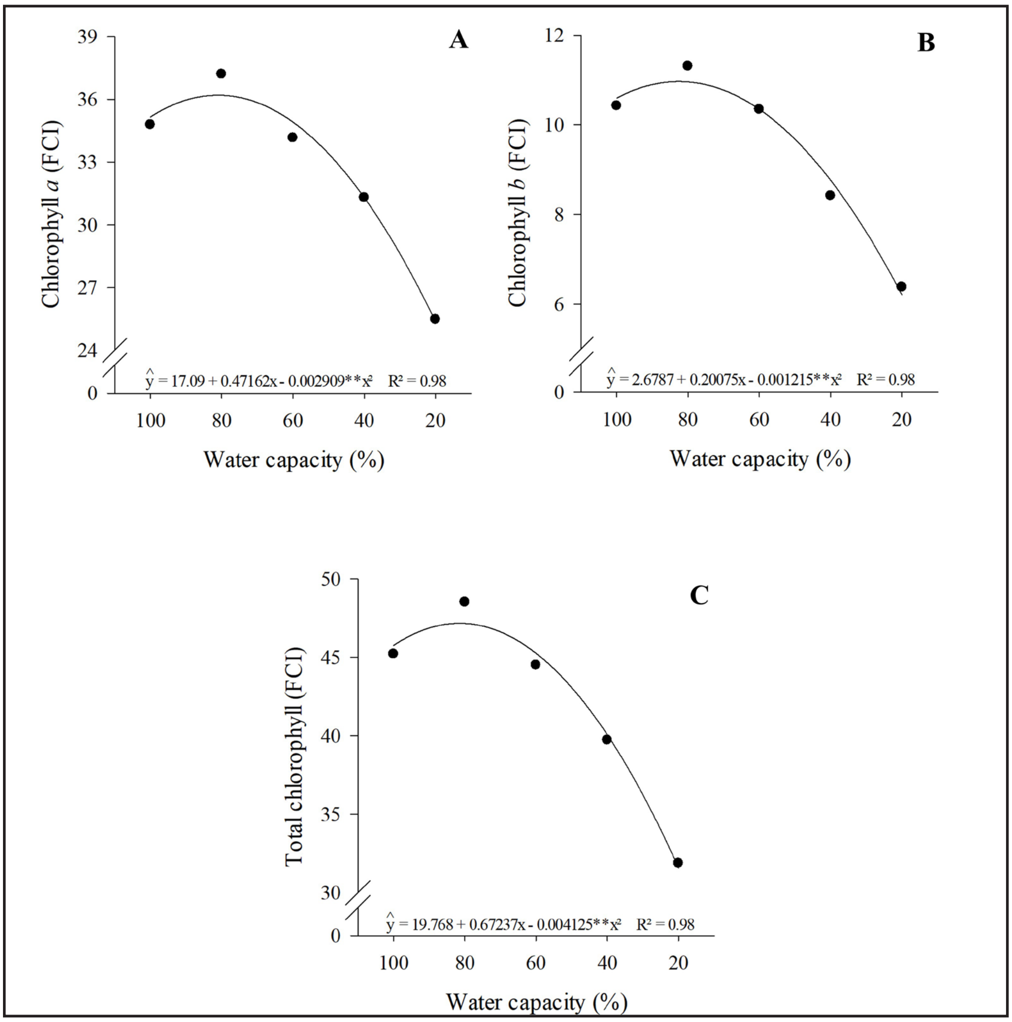


Source: Authors (2023)

The chlorophyll levels in plants *of Erythroxylum pauferrense* were strongly influenced by the different water treatments (Figure 8A-C), except for the ratio between chlorophyll *a* and *b* (*a* /*b*). The chlorophyll *a*, *b* and total indices decreased with the increase in water stress, with decreases of 31.5%, 43.5% and 34.3%, respectively, compared to the 80% water regime of PC (Figure 8A-C).

The reduction of chlorophyll levels (*a*, *b* and total) in plants may have occurred due to the greater sensitivity of these pigments to water stress and consequently caused photoinhibition, decreasing the activity in the photosynthetic apparatus of plants, in addition to influencing cellular processes such as cell expansion and division (KARGAR; SURESH; LEGRAND; JUTRAS; CLARK; PRASHER, 2017).

Figure 8 – Chlorophyll *a* (A), *b* (B) and *a+b* (C) contents in *Erythroxylum pauferrense* plants under water regimes



Source: Authors (2023)

4 CONCLUSIONS

The water regime of 80% of PC is the most recommended for producing seedlings *of Erythroxylum pauferrense*, promoting greater growth and significant changes in morphofunctional aspects, gas exchange, chlorophyll fluorescence and chlorophyll indexes.

Despite tolerating the greatest water stress, the plants submitted to 20% of the PC showed drastic reductions in the rate of photosynthesis, which consequently negatively affected the other morphophysiological variables.

ACKNOWLEDGEMENTS

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

REFERENCES

ALVES, M. M.; ALBUQUERQUE, M. B.; PEREIRA, W. E.; FELIX, V. J. L.; AZEVEDO, J. P. L. Morphophysiological evaluation of *Bauhinia divaricata* L. (Fabaceae) plants at different water regimes. **Bioscience Journal**, Uberlândia, v. 34, n. 1, p. 120-130, dec. 2018.

BENINCASA, M. M. P. **Análise de crescimento de plantas, noções básicas**. Jaboticabal: FUNEP, 2003.

DICKSON, A.; LEAF, A. L.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **Forest Chronicle***, Mattawa,* v. 36, n. 1, p. 10-13, mar. 1960.

D'ODORICO, P.; PORPORATO, A.; RUNYAN, C. W. **Dryland Ecohydrology**. Cham: Springer, 2019. 602 p.

DUCA, M. **Plant Physiology**. New York: Springer, 2015. 315 p.

FEYZIYEV, Y. M. Chlorophyll fluorescence and "maximum quantum efficiency" of photosystem II in plant sciences. **Life Sciences Biomedicine**, v. 1, n. 74, p. 18-28, jan. 2020.

HUNT, G. A. Effect of styroblock design and cooper treatment on morphology of conifer seedlings. In: ROSE, R.; CAMPBELL, S. J.; LANDIS, T. D. **Target seedling symposium, meeting of the western forest nursery associations, general technichal report RM-200**. Roseburg: Proceedings... Collins: United States Departament of Agriculture, 1990. p. 218-222.

KALAJI, H. M.; SCHANSKER, G.; BRESTIC, M.; BUSSOTTI, F.; CALATAYUD, A.; FERRONI, L.; GOLTSEV, V.; GUIDI, L.; JAJOO, A.; LI, P. Frequently asked questions about chlorophyll fluorescence, the sequel. ***Photosynthesis*** Research, New York, v. 132, n. 1, p. 13-66, nov. 2017.

KARGAR, M.; SURESH, R.; LEGRAND, M.; JUTRAS, P.; CLARK, O. G.; PRASHER, S. O. Reduction in water stress for tree saplings using hydrogels in soil. **Journal of Geoscience and Environment Protection**, [s.l.], v. 5, n. 1, p. 27-39, jan. 2017.

KOCHHAR, S. L.; GUJRAL S. K. **Plant Physiology**: Theory and Applications. Cambridge: University Press, 2020. 800p.

KUROMORI, T.; SEO, M.; SHINOZAKI, K. ABA transport and plant water stress responses. **Trends in Plant Science**, Amsterdã, v. 23, n. 6, p. 513-522, mai. 2018.

LAMBERS, H.; OLIVEIRA, R. S. **Plant Physiological Ecology**. Cham: Springer, 2019. 736 p.

LOIOLA, M. I. B.; AGRA, M. F.; BARACHO, G. S.; QUEIROZ, R. T. Flora da Paraíba, Brasil: Erythroxylaceae Kunth. **Acta Botanica Brasilica**, Brasília, v. 21, n. 2, p. 473-487, abr. 2007.

MEJÍA-DE TAFUR, M. S.; RIAÑO-HERRERA, N. M.; URREGO-MESA, J. B.; IBARRA-ESPINOSA, D. M.; ZAPATA-DUQUE, C. M. Effect of soil water availability on gas exchange in young trees of *Eucalyptus grandis* W. Hill ex Maiden. **Acta Agronómica**, Palmira, v. 66, n. 4, p. 549-557, out. 2017.

OGBURN, R. M.; EDWARDS, E. Quantifying succulence: a rapid, physiologically meaningful metric of plant water storage. **Plant, Cell and Environment**, Bethesda, v. 35, n. 9, p. 1533-1542, set. 2012.

OJEDA, H.; RUBILAR, R. A.; MONTES, C.; CANCINO, J.; ESPINOSA, M. Leaf area and growth of *Chilean radiata* pine plantations after thinning across a water stress gradient. **New Zealand Journal of Forestry Science**, New York, v. 48, p. 1-11, jul. 2018.

POORTER, H.; NIINEMETS, Ü.; POORTER, L.; WRIGHT, I. J.; VILLAR, R. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. **New Phytologist**, Lancaster, v. 82, n. 2009, p. 565-588, abr. 2009.

RIBEIRO, J. E. S.; LEITE, A. P.; NÓBREGA, J. S.; ALVES, E. U.; BRUNO, R. L. A.; ALBUQUERQUE, M. B. Temperatures and substrates for germination and vigor of *Erythroxylum pauferrense* Plowman seeds. ***Acta Scientiarum, Biological Sciences****, Maringá,* v. 41, p. 1-7, nov. 2019.

SCALON, S. P. Q.; MUSSURY, R. M.; EUZÉBIO, V. L. M.; KODAMA, F. M.; KISSMANN, C. Estresse hídrico no metabolismo e crescimento inicial de mudas de mutambo (*Guazuma ulmifolia* Lam.). **Ciência Florestal**, Santa Maria, v. 21, n. 4, p. 655-662, out. 2011.

SHABALA, S. **Plant Stress Physiology**. Oxford: CAB International, 2012. 318 p.

SOARES, L. A. A.; FERNANDES, P. D.; LIMA, G. S.; BRITO, M. E. B.; NASCIMENTO, R.; ARRIEL, N. H. Physiology and production of naturally-colored cotton under irrigation strategies using salinized water. **Pesquisa Agropecuária Brasileira**, Brasília, v. 53, n. 6, p. 746-755, jun. 2018.

TAIZ, L.; ZEIGER, E.; MØLLER, I.; MURPHY, A. **Fisiologia e Desenvolvimento Vegetal**. Porto Alegre: Artmed, 2017. 888 p.

TOUNEKTI, T.; AL-TURKI, T. A.; MAHDHI, M.; KHEMIRA, H. Leaf functional trait variation associated with salinity tolerance in *Salvadora persica*. **Indian Journal of Pure & Applied Biosciences**, Kota, v. 5, n. 2, p. 14-21, abr. 2017.

ZEGAOUI, Z.; PLANCHAIS, S.; CABASSA, C.; DJEBBAR, R.; BELBACHIR, O. A.; CAROL, P. Variation in relative water content, proline accumulation and stress gene expression in two cowpea landraces under drought. **Journal of Plant Physiology**, Bethesda, v. 218, p. 26-34, nov. 2017.

Authorship Contribution

1 João Everthon da Silva Ribeiro

Dr. in Agronomy, Professor, Researcher

https://orcid.org/0000-0002-1937-0066 • j.everthon@hotmail.com

Contribution: Conceptualization; Data curation; Formal analysis; Methodology; Writing – original draft; Writing – review & editing

2 Ester dos Santos Coêlho

MSc. in Phytotechnics, Doctoral student, Researcher

https://orcid.org/0000-0002-5541-1937 • estersantos12@hotmail.com

Contribution: Data curation; Formal analysis

3 Francisco Romário Andrade Figueiredo

Dr. in Phytotechnics, Researcher

https://orcid.org/0000-0002-4506-7247 • romarioagroecologia@yahoo.com.br

Contribution: Investigation; Data curation

4 Francisco Thiago Coelho Bezerra

Dr. in Agronomy, Professor, Researcher

https://orcid.org/0000-0002-9185-2641 • bezerra_ftc@yahoo.com.br

Contribution: Validation; Writing – review & editing

5 Thiago Jardelino Dias

Dr. in Agronomy, Professor, Researcher

https://orcid.org/0000-0002-7843-6184 • thiagojardelinodias@gmail.com

Contribution: Writing – review & editing

6 Mário Luiz Farias Cavalcanti

Dr. in Agricultural Engineering, Professor, Researcher

https://orcid.org/0000-0002-9505-417X • mariolfcavalcanti@yahoo.com.br

Contribution: Writing – review & editing

7 Manoel Bandeira de Albuquerque

Dr. in Biology, Professor, Researcher

https://orcid.org/0000-0003-1871-0046 • bandeira1977@hotmail.com

Contribution: Supervision; Writing – original draft

How to quote this article

RIBEIRO, J. E. S.; COÊLHO, E. S.; FIGUEIREDO, F. R. A.; BEZERRA, F. T. C.; DIAS, T. J.; CAVALCANTI, M. L. F.; Albuquerque, M. B. Morphophysiological characteristics in Erythroxylum pauferrense Plowman plants under water stress. Ciência Florestal, Santa Maria, v. 33, n. 2, e65886, p. 1-25, 2023. DOI 10.5902/1980509865886. Available from: https://doi.org/10.5902/1980509865886.