

Morphophysiological and biochemical changes in *Enterolobium contortisiliquum* seedlings under abiotic stresses¹

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ABSTRACT - Among the main problems that compromise the development of forest species are water and salt stresses, especially in the early stages of development, when seedlings are more sensitive. Thus, the objective of this study was to evaluate the morphophysiological and biochemical changes in *Enterolobium contortisiliquum* seedlings subjected to abiotic stresses. The experimental design was randomized complete blocks (RCB), composed of five treatments, with four replicates, and the experimental plot consisted of twenty plants. From the 31st day after sowing, when the seedlings reached approximately 15 cm in height, they began to receive the treatments, which consisted of different levels of electrical conductivity of irrigation water (0.3; 2; 4; 6; 8 dS m⁻¹) for salt stress and periods of water restriction (0; 4; 8; 12 and 16 days without irrigation) for water stress. The traits evaluated were: plant height, root length, stem diameter, number of leaves, leaf area, total dry matter, Dickson quality index, height/shoot dry matter ratio, total soluble sugars, starch, total amino acids and proline. An increase in the electrical conductivity of irrigation water and days without irrigation compromised the development of *E. contortisiliquum* seedlings, which was intensified from 2 dS m⁻¹ and four days without irrigation for salt and water stresses, respectively. The mechanism of survival of *E. contortisiliquum* to abiotic stresses involves reduction in the growth and quality of seedlings, with activation of biochemical defense mechanisms of the species.

Key words: Fabaceae. Dry forests. Semi-arid region. Salinity. Hydrical stress.

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INTRODUCTION

Enterolobium contortisiliquum (Vell.) Morong is a tree species, belonging to the Fabaceae family and commonly known in Brazil as ‘tamboril’, reaching more than four meters in height. Currently, the species is used in programs of reforestation and recovery of degraded areas, as well as in the production of secondary metabolites in the pharmaceutical industry (ABDEL-MAGEED *et al.*, 2019).

Plants live in constantly changing environments, which are often unfavorable or stressful for their growth and development. The adverse effects of these stresses are intensified by climate change, which causes extreme conditions to occur more frequently (BEZERRA *et al.*, 2020). Among the adverse conditions, excess salts in soil and water deficit are the main environmental factors that affect the geographical distribution of plants in nature, affecting yield and limiting the production of energy that will be used in processes related to survival and adaptation (SILVA; AZERÊDO, 2022; ZHU, 2016).

Among the main adaptations for survival to abiotic stresses, those of biochemical nature related to osmotic adjustment mechanisms of plants stand out, such as the accumulation of soluble carbohydrates and free proline (LEITE *et al.*, 2020), all of this to maintain the water potential of the plant favorable to water absorption and assist in post-stress recovery. However, these mechanisms are activated with energy expenditure, negatively affecting plant development.

In recent years, studies have been conducted to obtain information on the initial development of forest species under abiotic stresses. Among these, the ones conducted by Leal *et al.* (2019) with *Combretum leprosum* Mart., Avrella *et al.* (2019) with *Mimosa scabrella* Benth., Leite *et al.* (2020) with *Poincianella pyramidalis* (Tul.) L.P. Queiroz, Ferreira *et al.* (2021) with *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson and Leite *et al.* (2022) with *Erythrina velutina* Willd. stand out. However, studies related to the behaviour of *E. contortisiliquum* under water stress or salt stress are scarce.

In view of the above, the objective of this study was to evaluate the morphological and biochemical responses of *E. contortisiliquum* seedlings subjected to abiotic stresses.

MATERIAL AND METHODS

The study was conducted in laboratory and greenhouse at the Federal University of Rio Grande do Norte (UFRN), Campus de Macaíba (5°53'10.0" S, 35°21'52.5" W and 56 m altitude). *E. contortisiliquum*

seedlings were produced from seeds supplied by the Ecology and Environmental Monitoring Center of the Federal University of the São Francisco Valley (UNIVASF), Petrolina, PE (9°19'10.47" S, 40°33'48.91" W and 375 m altitude).

For each experiment of water and salt stress, the experimental design was randomized complete blocks (RCB), composed of five treatments, with four replicates and experimental plot consisting of 20 plants.

For water stress, the treatments consisted of periods of water restriction (4; 8; 12 and 16 days without irrigation), with daily irrigation in the control. For the imposition of salt stress, different levels of electrical conductivity of irrigation water (2; 4; 6 and 8 dS m⁻¹) were obtained by adding sodium chloride (NaCl) to local-supply water (0.3 dS m⁻¹), which was used as a control.

The experimental units consisted of 1.5 dm³ polyethylene bags, filled with substrate obtained from the mixture of soil and bovine manure (1:1), whose chemical analysis indicated the following characteristics: pH = 7.5; Ca = 2.6 cmol_c dm⁻³; Mg = 1.3 cmol_c dm⁻³; Na = 1.66 cmol_c dm⁻³; K = 2.7 cmol_c dm⁻³; N = 1.89 g kg⁻¹; P = 241.1 mg dm⁻³; EC = 1.36 dS m⁻¹; ESP = 20%. Prior to sowing, the seeds were manually scarified in the opposite region to the hilum using sandpaper n° 100. Two seeds per container were sown in a moist substrate (total saturation) at 2.0 cm depth. At 15 days after sowing, thinning was performed, leaving one plant per container.

For the two experiments, the seedlings were irrigated daily with local-supply water (0.3 dS m⁻¹) during the first 30 days after sowing, aiming to maintain the soil at field capacity. From the 31st day after sowing, when the seedlings were approximately 15 cm tall, the treatments began to be applied.

The seedlings were evaluated at 46 days after sowing, by means of nondestructive determinations (plant height - H, measured with a ruler graduated in millimeters; stem diameter - SD, obtained with a digital caliper; and number of leaves - NL, obtained by directly counting the number of leaves per plant) and destructive determinations (leaf area - LA; root length - RL, using a ruler graduated in millimeters; total dry matter - TDM, obtained by weighing the plant material on a precision scale (0.0001 g), after drying in a forced air circulation oven at 65 °C for 72 hours; Dickson quality index - DQI (DICKSON; LEAF; HOSNER, 1960); and height to shoot dry matter ratio (H/SDM).

Before the destruction of the plant material, the leaf area was determined, using 10 leaflets randomly collected in the morning from each plant. These leaflets were

placed in white polyethylene containers and immediately measured according to the linear dimensions method (Equation 1), using a digital caliper (0.01 mm).

Equation 1: $A = L.W$, expressed in (cm²), where: L = length in cm; W = width in cm (median position). Length corresponded to the distance between the distal base of the petiole and the final extremity, while width corresponded to the longest distance perpendicular to the length axis.

At the time of destructive analysis, leaf material was collected, stored in a freezer, and later used to obtain the base extract for biochemical determinations. Initially, samples containing 0.2 g of fresh leaf mass were placed in hermetically sealed tubes and macerated with 3 mL of 80% ethanol. After automatic maceration, the material was placed in water bath at 60 °C for 20 minutes. Then, the tubes were subjected to centrifugation (10,000 rpm for 8 min at 4 °C) and the supernatant was collected to determine the contents of soluble sugars, total amino acids and proline, while the precipitate was macerated again with 3 mL of 30% perchloric acid and then centrifuged, and the supernatant was collected for the determination of starch content.

The content of total soluble sugars was determined by the anthrone method (YEMM; WILLIS, 1954), and the results were expressed in g⁻¹ glucose mg of fresh matter. Amino acids were determined by the ninhydrin method, using glycine as the standard (YEMM; COCKING; RICKETTS, 1955), with the results expressed in mmol g⁻¹ of fresh matter. Proline was determined using the method for quantifying the free proline content proposed by Bates, Waldren and Teare (1973), with the results expressed in μmol g⁻¹ of fresh matter. Starch content was quantified according to Morris (1948) and Yemm and Willis (1954), by the anthrone method, with results expressed in mg g⁻¹ of fresh matter.

The results were subjected to analysis of variance by the F test at 5% probability level, using the statistical program *Sistema para Análise de Variância - SISVAR* (FERREIRA, 2011). In case of significance, the results were subjected to regression analyses.

RESULTS AND DISCUSSION

Salt stress

Salinity negatively affected all growth variables measured, with linear decrease as a function of the increase in the electrical conductivity of irrigation water (Figure 1). The highest restrictions of growth were observed at the highest salinity level (8 dS m⁻¹),

reaching reductions of 40% for plant height (Figure 1A), 61% for root length (Figure 1B), 54% for stem diameter (Figure 1C) and 54% for number of leaves (Figure 1D).

The effects of environmental stresses differ along the plant's life cycle, that is, under any stress exerted. This effect will depend on each organism, its adaptation, dose and duration of exposure of the individual to stress (AZEREDO; PAULA; VALERI, 2016). In seedlings of forest species, the reduction of growth traits under salinity conditions is widely reported in the literature, such as the reductions of plant height in *Erythrina velutina* (LOPES *et al.*, 2019), *Combretum leprosum* Mart. (LEAL *et al.*, 2019) and *Pityrocarpa moniliformis* (FERREIRA *et al.*, 2021), root length in *Acacia gerrardii* Benth. (AL-HUQAIL *et al.*, 2019), and stem diameter in *Erythrina velutina* (LOPES *et al.*, 2019) and *Mimosa scabrella* (AVRELLA *et al.*, 2019).

Reductions in growth variables should not always be treated as limiting factors, since they may represent strategies to facilitate the survival of seedlings in stressful environments. As an example, reduction in the number of leaves to a certain limit may be a strategy to minimize transpiration, due to the low water availability caused by the reduction in the osmotic potential of the soil solution, due to the presence of salts (TAIZ *et al.*, 2017).

As observed for the number of leaves, the leaf area of *E. contortisiliquum* seedlings was also significantly affected by the increase in irrigation water salinity, with reductions of 17, 36, 57 and 76% for the levels of 2, 4, 6 and 8 dS m⁻¹, respectively, when compared with the control (Figure 2A). For Campelo *et al.* (2018), although the reduction of leaf area can be seen as an adaptation to stress conditions, the reduction of CO₂ assimilation is highly harmful to seedling development, causing reductions in photosynthesis and yield.

The result obtained for total dry matter (Figure 2B) reflects the reduction of seedling growth. For the highest level of salinity applied, *E. contortisiliquum* seedlings had a reduction of 29% in their dry matter compared to those of the control, highlighting the sensitivity of the studied species to salinity. Thus, as indicated by the Dickson Quality Index - DQI (Figure 2C), there was a reduction in the quality of seedlings irrigated with saline waters, which was proportional to the increase in irrigation water salinity. This decrease in the quality of seedlings irrigated with saline water of 2, 4, 6 and 8 dS m⁻¹ was equal to 14, 32, 48 and 65%, respectively, compared to the control. According to Gomes and Paiva (2013), the higher the DQI, the better the quality standard of seedlings and survival in the field, so DQI > 0.2 is recommended. Thus, despite the reduction in the quality of the seedlings, they still showed acceptable quality according to this index.

Figure 1 - Plant height (A), root length (B), stem diameter (C) and number of leaves (D) of *Enterolobium contortisiliquum* (Vell.) Morong seedlings irrigated with water of different salt concentrations

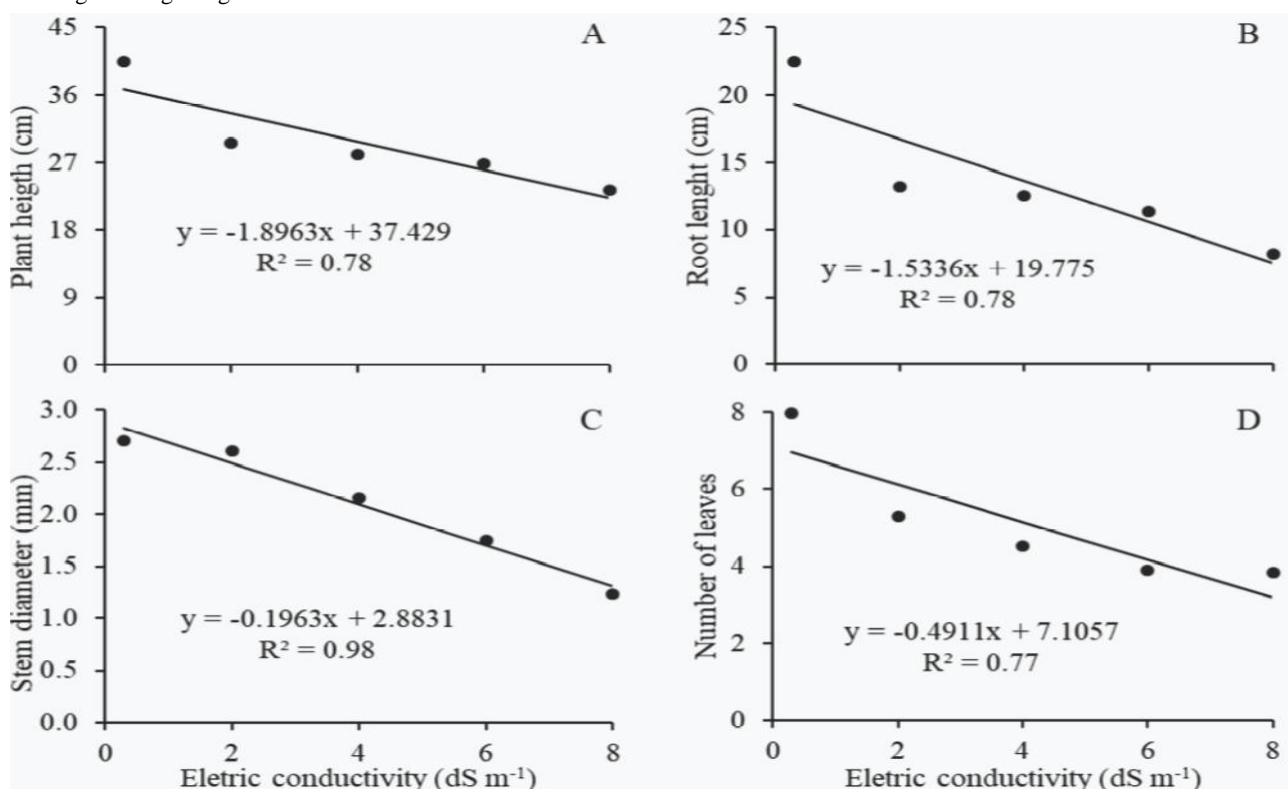
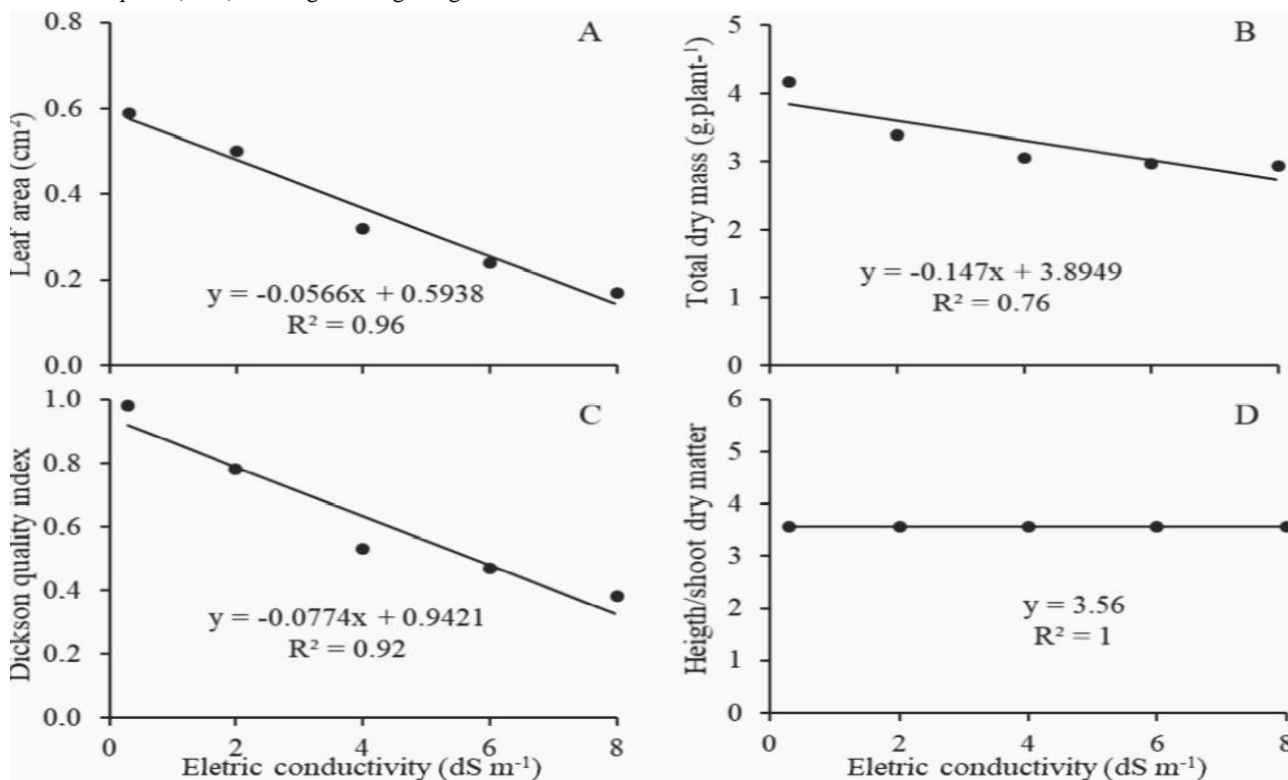


Figure 2 - Leaf area (A), total dry matter (B), Dickson quality index (C) and height/shoot dry matter ratio (D) of *Enterolobium contortisiliquum* (Vell.) Morong seedlings irrigated with water of different salt concentrations



Reduction of dry biomass is one of the main responses observed in plants under salinity conditions. In this context, the results found by Avrella *et al.* (2019) with *Mimosa scabrella*, Leal *et al.* (2019) with *Combretum leprosum* Mart. and Ferreira *et al.* (2021) with *Pityrocarca moniliformis* corroborate those obtained in the present study. The seedlings of these species had their biomass reduced with the increase in the salt concentration in the irrigation water, reaching, in the case of *C. leprosum*, a reduction of 100% for those irrigated with salinized water of 6.5 dS m⁻¹.

The results found for the contents of soluble sugars (Figure 3A) and starch (Figure 3B) showed an inverse relationship between these variables. While the content of soluble sugars increased 35% at the highest salinity level, starch decreased by 60% for this same level of stress, compared to the control treatment.

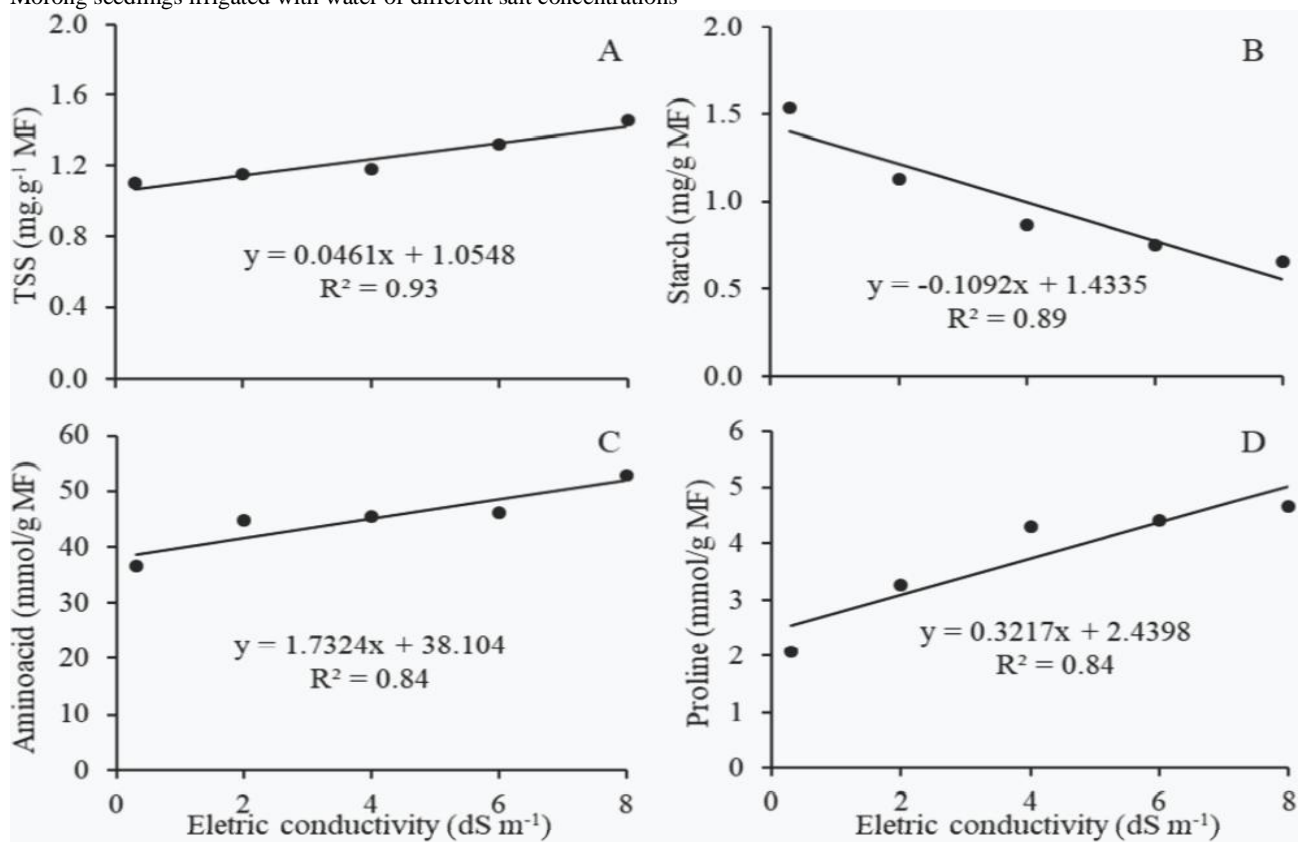
Consumption of starch (raffinose and sorbitol) and accumulation of soluble sugars were verified in the present study. This occurs as a way to protect the photosynthetic apparatus from the accumulation of reactive oxygen species and provide carbon for the maintenance of cellular processes (MITCHELL *et al.*, 2013). Similarly, Dietze *et al.* (2014)

stated that starch synthesis and degradation are controlled by interdependent regulatory networks that ultimately allow balancing the supply of carbon via photosynthesis with its use for other activities.

The contents of amino acids (Figure 3C) and proline (Figure 3D) increased linearly with increasing salinity levels. Increase in amino acid content is directly related to the increase in proline, an amino acid whose accumulation is linked to salt stress. For proline, the content at the highest salinity level (8 dS m⁻¹) was almost twice as high (1.97) as that observed for seedlings in the control treatment.

The accumulation of amino acids reveals that there was protection of plant cells from possible damage caused by salt stress. Increased protease activity has the function of breaking down reserve proteins as stress time increases (TAIZ *et al.*, 2017). According to these authors, it consequently increases the content of total soluble amino acids in order to regulate intracellular osmotic potential and accelerates the recovery of plants under adverse conditions. The main amino acid involved in this process is proline, which is related to the mitigation of post-stress damage. This break-down allows reducing agents, which

Figure 3 - Total soluble sugars (A), starch (B), total amino acids (C) and proline (D) contents of *Enterolobium contortisiliquum* (Vell.) Morong seedlings irrigated with water of different salt concentrations



participate in mitochondrial oxidative phosphorylation, to produce ATP for the recovery and restoration of stress-induced damage (LEHMANN *et al.*, 2010).

Water stress

The height (Figure 4A) and root length (Figure 4B) of *E. contortisiliquum* seedlings decreased linearly with the increase in the period of water restriction. The highest and lowest values were obtained for these variables in treatments of 0 and 16 days without irrigation. Similarly, stem diameter (Figure 4C) decreased as the period without irrigation increased. For this variable, there were reductions of 5, 10, 14 and 19% for seedlings from treatments of 4, 8, 12 and 16 days without irrigation, respectively, compared to those of the control. According to Campelo *et al.* (2018), the decrease in height, root length and stem diameter of plants may reflect the reduction of protein synthesis, as it affects the speed of mitotic processes, culminating in the interruption of cell division and expansion, caused by the lower availability of water, since it is indispensable for the occurrence of these events (TAIZ *et al.*, 2017).

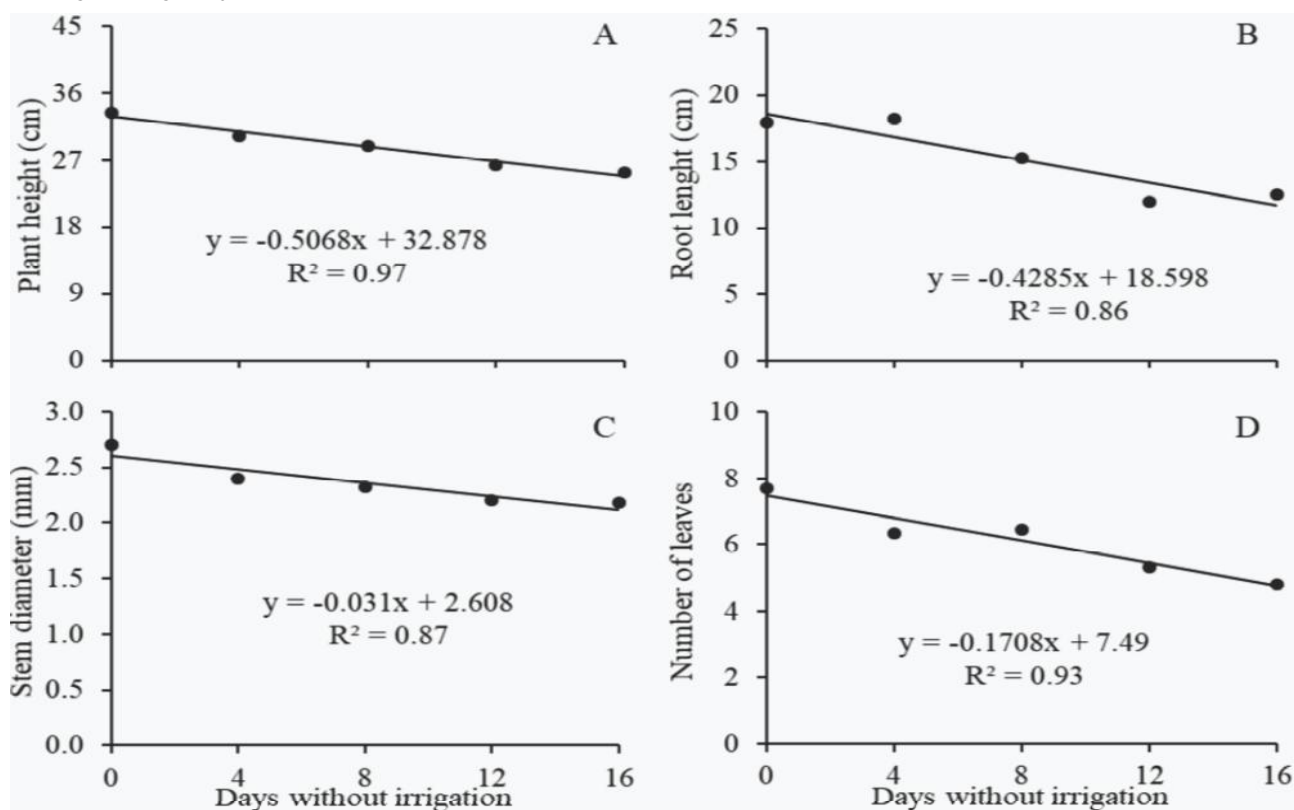
The number of leaves gradually decreased as the number of days without irrigation increased (Figure 4D). This reduction occurred due to the fall and lower production of

leaves in the treatments of higher water restriction. Similar results were also found by Lúcio *et al.* (2017) in *Erythrina velutina* and *E. contortisiliquum*. The authors verified that water stress caused marked leaf senescence and restriction to the emergence of new leaves, and the reduction in the number of leaves was proportional to the stress level.

The leaf area of *E. contortisiliquum* seedlings decreased significantly with water restriction (Figure 5A). Plants of treatments with 4, 8, 12 and 16 days without irrigation had reductions of 17, 35, 52 and 70%, respectively, compared to those of the control. Reduction of leaf area was also verified by França *et al.* (2017) in seedlings of *Calophyllum brasiliense* Cambess under water stress. According to these authors, the decrease in leaf area is a response to low water potential in the leaves, functioning as an adaptation to prevent water loss through stomata and cuticular transpiration.

For total dry matter, a linear reduction was observed in *E. contortisiliquum* seedlings with the increase in days without irrigation (Figure 5B). Plants of the control treatment had on average 12.6 g of dry matter, while the longest period of water restriction resulted in a reduction of 55% of dry matter, with an average of 5.7 g of dry matter per plant.

Figure 4 - Plant height (A), root length (B), stem diameter (C) and number of leaves (D) of *Enterolobium contortisiliquum* (Vell.) Morong seedlings subjected to water deficit



The closure of stomata, with consequent reduction of photosynthetic activity, is one of the first responses to water deficit, culminating in severe reduction in biomass production (TAIZ *et al.*, 2017). Thus, plants maintained under water stress conditions tend to accumulate less biomass and show underdevelopment when compared to those maintained under adequate water availability.

DQI is used by several authors as an important indicator of seedling quality. According to Freitas *et al.* (2017), this index is a balanced formula that includes relations of morphological traits, considering the sturdiness and balance in the biomass distribution of the seedling. Considering this index, the higher its value, the better the quality standard of the seedlings. Thus, there was a reduction in the quality of *E. contortisiliquum* seedlings with the increase in the period without irrigation to which the seedlings were subjected (Figure 5C).

The result of the division of shoot height by the weight of shoot dry matter (H/SDM) is important to estimate the survival of the seedling in the field (GOMES; PAIVA, 2013). These same authors also point out that the lower this index, the more lignified the seedling, resulting in greater capacity for survival

under unfavorable conditions. Thus, corroborating the results found for DQI, the H/SDM ratio indicated reduction in the quality of *E. contortisiliquum* seedlings under water stress condition (Figure 5D). Thus, the capacity for survival in the field was gradually reduced as the period without irrigation increased.

As observed for salt stress, there was a reduction in starch content (Figure 6B) and an increase in amino acid content (Figure 6C) as a function of the greater number of days without irrigation. The reduction in starch content was 39%, while the increase in amino acid content was 22%, when comparing the highest level of water stress with the control treatment. However, the contents of soluble sugars (Figure 6A) and proline (Figure 6D), variables that proved to have great importance for the adaptation of the species to salt stress, were not altered by water stress.

Regarding the defense mechanisms of plants under water deficit conditions, metabolic changes usually occur, increasing the activity of proteolytic enzymes, responsible for the transformation of proteins into amino acids, which accumulate by synthesis or degradation of other organic compounds, reducing the damage caused by water scarcity (SILVA *et al.*, 2013). Studies have shown

Figure 5 - Leaf area (A), total dry matter (B), Dickson quality index (C) and height/shoot dry matter ratio (D) of *Enterolobium contortisiliquum* (Vell.) Morong seedlings subjected to water deficit

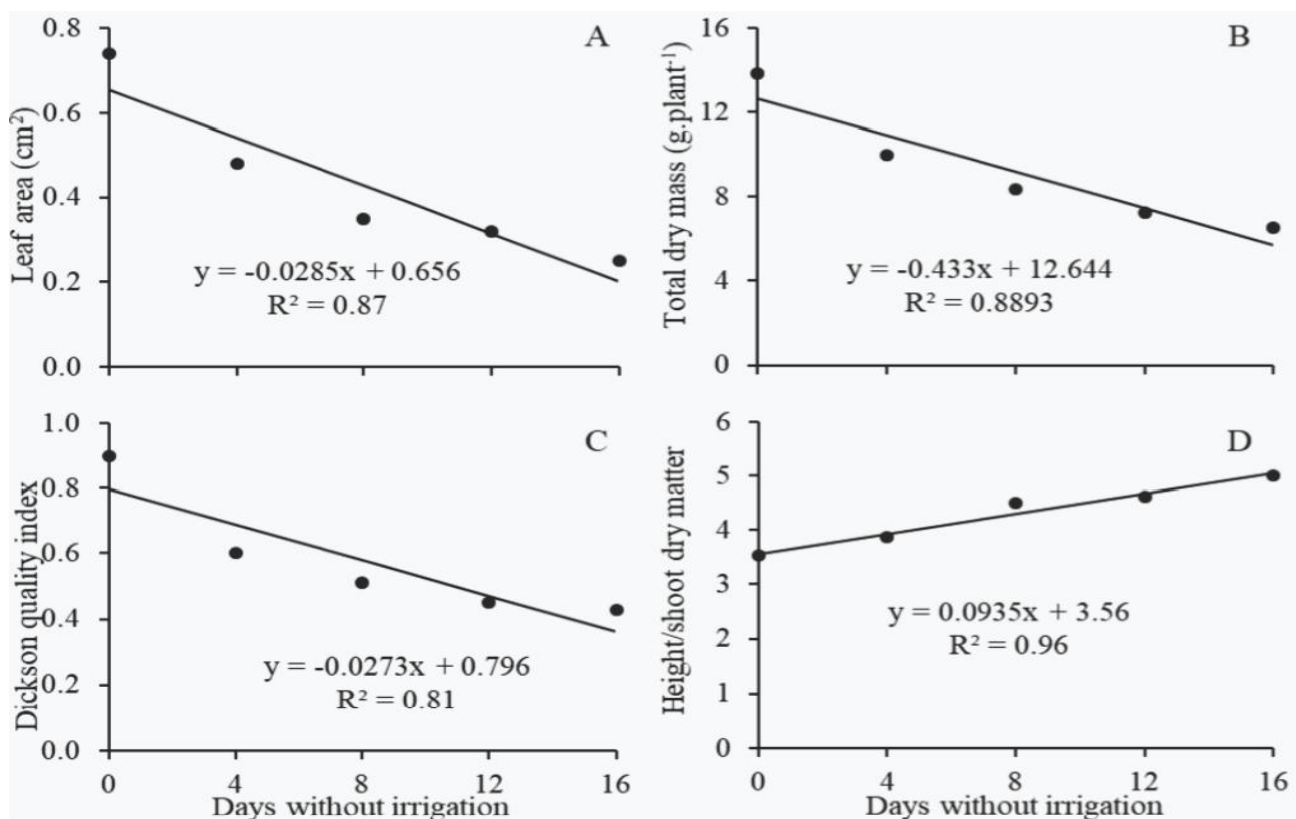
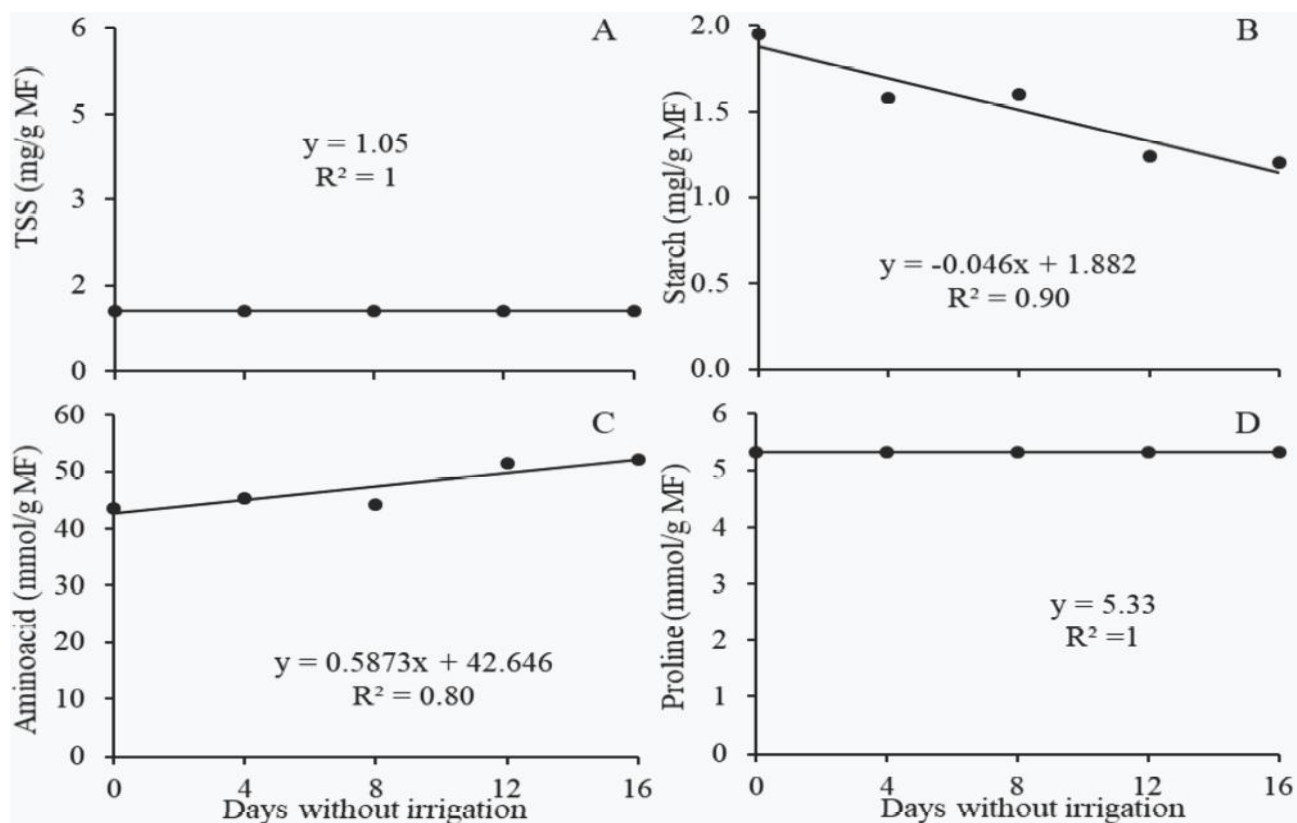


Figure 6 - Total soluble sugars (A), starch (B), total amino acids (C) and proline (D) contents of *Enterolobium contortisiliquum* (Vell.) Morong seedlings subjected to water deficit



accumulation of osmotically active solutes due to water deficit (LEITE *et al.*, 2020, 2022), and the amount and type of solute accumulated depend directly on the plant species and on the duration of the stress period.

Starch consumption and accumulation or maintenance of soluble sugar act by providing carbon for use in cellular processes and survival (NASCIMENTO; NASCIMENTO; GONÇALVES, 2019). This strategy contributes to the survival of plants under water deficit conditions.

CONCLUSION

Increase in the electrical conductivity of irrigation water and days without irrigation compromise the development of *E. contortisiliquum* seedlings, which was intensified from 2 dS m⁻¹ and four days without irrigation for salt and water stresses, respectively. The mechanism of survival of *E. contortisiliquum* to abiotic stresses involves reduction in the growth and quality of seedlings, with activation of biochemical defense mechanisms of the species.

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