



**GROWTH, DRY MASS AND ORGANICS SOLUTES ACCUMULATION IN
Cnidoscollus phyllacanthus (M. Arg) Pax & Hoffm.) SEEDLINGS UNDER SALINITY**

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ABSTRACT

Theoretical framework: The areas affected by salinity have increased in recent years, becoming a limiting factor for plants, negatively interfering with their growth and development. As a result of these effects, knowing species that tolerate this adverse salinity condition is fundamental, both from an environmental aspect and from a scientific point of view.

Objective: This research aimed to verify the effects of salinity on the growth and accumulation of organic solutes of *Cnidoscollus quercifolius* seedlings.

Method: The treatments consisted of five levels of NaCl (0, 50, 100, 200 and 400 mM), distributed in entirely randomized design, with four replications. The seedlings grew in 'Leonard' pots, containing washed sand and nutrient solution, kept under saline conditions for 60 days. The parameters analyzed were height; stem diameter; roots, stem, leaves, shoot, and total dry mass; and amino acids, proteins and total soluble sugars content.

Results and discussion: Salinity reduced linearly all parameters evaluated, especially at the highest levels of salt in the medium, indicating that the *C. quercifolius* seedlings were intolerant to the imposed salinity, not being able to adjust osmotically.

Research implications: Possibility of revegetation of areas with saline soil problems, reintegrating them into the production system.

Originality/value: To know the forest species capable of surviving in areas affected by salinization, enabling the adoption of recovery techniques and practices.

Keywords: Salt Stress, Organics Solutes Accumulation, Salt Tolerance, Semiarid, Caatinga Vegetation.

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CRESCIMENTO, ACÚMULO DE MASSA SECA E DE SOLUTOS ORGÂNICOS EM MUDAS DE FAVELEIRA *Cnidoscollus phyllacanthus* (M. Arg) Pax & Hoffm.) SOB SALINIDADE

RESUMO

Objetivo: Este trabalho teve como objetivo verificar quais os efeitos da salinidade no crescimento e no acúmulo de solutos orgânico das mudas de faveleira (*Cnidoscollus quercifolius*).

Referencial teórico: As áreas afetadas pela salinidade têm aumentado nos últimos anos, constituindo-se em fator limitante para as plantas, interferindo negativamente no seu crescimento e desenvolvimento. Como resultado desses efeitos, conhecer espécies que tolerem à essa condição adversa de salinidade é fundamental, tanto no aspecto ambiental como do ponto de vista científico.

Método: Os tratamentos consistiram de cinco níveis de NaCl (0, 50, 100, 200 e 400 mM), distribuídos em delineamento inteiramente casualizado, com 4 repetições. As mudas cresceram em vasos de Leonard, contendo areia lavada e solução nutritiva, mantidas sob salinidade durante 60 dias. Foram determinados os seguintes parâmetros: altura, diâmetro, massas seca das raízes, caule, folhas, parte aérea e total, e acúmulo de aminoácidos, proteínas e açúcares solúveis totais.

Resultados e conclusão: A salinidade causou redução linear em todos os parâmetros avaliados, principalmente nos níveis mais elevados de sal no meio, indicando que as mudas de *C. quercifolius* mostraram-se intolerantes à salinidade imposta, não sendo capazes de se ajustar osmoticamente.

Implicações da pesquisa: Possibilidade de revegetação de áreas com problema de solos salinos, reintegrando-as ao sistema produtivo.

Originalidade/valor: Conhecer as espécies florestais com capacidade de sobrevivência em áreas afetadas pela salinização, possibilitando a adoção de técnicas e práticas de recuperação.

Palavras-chave: Estresse Salino, Acúmulo de Solutos Orgânicos, Tolerância à Salinidade, Semiárido, Caatinga.

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1 INTRODUCTION

The arid, semi-arid and dry sub-humid climates, soils with poor drainage and subsurface waters rich in soluble salts cause salinization. However, this process can be established in environments previously free of salts at toxic levels, mainly due to inadequate soil management, use of poor quality water, use of fertilizers with a high saline index, high evapotranspiration rate and low precipitation, making the soils unproductive (Oliveira, 1997).

Currently, soil salinity has become one of the most serious problems for irrigated agriculture in different parts of the world (Rodrigues *et al.*, 2005). This problem in arid and semi-arid regions has become a matter of concern (Pedrotti *et al.*, 2015), mainly because it is concentrated in irrigated areas that have received high investments in infrastructure for its implementation.

In Brazil, the predominance of areas with salinity problems is in the regions of the irrigated perimeters of the Northeast (Sá, 2016) and it is estimated that the salinized area exceeds nine million hectares (Miranda *et al.*, 2002).

This region has problems with frequent dry periods, making it difficult to leach salts, in addition to the fact that most soils have a high concentration of salts in the soil, becoming a limiting factor for plant development (Pedrotti *et al.*, 2015). In addition, the use of saline water



and the indiscriminate use of fertilizers can aggravate this adverse situation, making the soil unsuitable for agriculture.

This accumulation of salts in the soil causes stress in plants, resulting in changes in several biochemical reactions, affecting physiological processes such as photosynthesis, antioxidant metabolism, osmolyte accumulation, hormonal signaling, enzymatic activities and seed germination (Siddiqui et al., 2010; Khan et al., 2012).

In addition to effects of an osmotic nature, restricting the availability of water and nutrients to plants, excess ions in the protoplasm, mainly Na^+ and Cl^- , can cause **toxicity** or disturbances in plant nutrition, directly reflecting on metabolism, growth and development establishing them (Shannon, 1997; Chusman, 2001; Munns, 2002; Navroski et al., 2018; França et al., 2023).

When plants are exposed to salinity, there is an increase in the production of reactive oxygen species (ROS), such as singlet oxygen ($\frac{1}{2}\text{O}_2$), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) (Ashraf; Harris, 2004). These compounds cause oxidative damage, such as enzymatic inactivation, lipid peroxidation, protein degradation and DNA damage (Zushi; Matsuzoe, 2009).

In order to survive under high salinity conditions, it is essential that plants develop tolerance mechanisms, which can be simple or extremely complex (Esteves; Suzuki, 2008). Plants, when subjected to high levels of salt, promote an increase in the cellular concentration of solutes, as a form of protection, among which are sugars, polyols, amino acids, betaines and related compounds (Hasegawa et al., 2000). These contribute to water absorption and cell turgor, ensuring vital physiological processes such as stomatal opening, photosynthesis and cell expansion (Sakamoto; Murata, 2002; Serraj; Sinclair, 2002).

In this context, the search for species that tolerate these adverse conditions is essential, and it is necessary to know species with potential for cultivation in saline environments and, even with limitations, these salinized areas can be explored (Cruz et al., 2020). However, it is essential to use species native to the region affected by salinity, since they are already adapted to its edaphoclimatic conditions (França et al., 2023).

Cnidoscollus quercifolius Pohl, known as faveleira or favela-de-cachorro, belonging to the Euphorbiaceae family, is native and endemic to Brazil, with confirmed occurrences in the states of Bahia, Ceará, Paraíba, Pernambuco, Piauí, Rio Grande do Norte, Sergipe and Minas Gerais (Flora do Brasil, 2023). It is a species of multiple use, and can be used for recovery of degraded areas, animal and human food, medicine, sawmill and energy, biodiesel, among others (Nóbrega, 2001; Maia, 2004).

Due to its economic and ecological importance and due to the lack of information about its behavior in relation to salinity, this work aimed to verify the effects of this adverse condition on the growth of seedlings, as well as on the accumulation of organic solutes.

2 MATERIALS AND METHODS

2.1 Growth Conditions

The study was carried out in a protected environment at the Academic Unit of Forestry Engineering at the Federal University of Campina Grande, in the municipality of Patos - PB ($7^\circ 1' 32''$ S, $37^\circ 16' 40''$ W, altitude 221 m). According to the climate classification established by Köppen, the climate is type Aw' (Hot and humid with rains from summer to autumn), with a rainy season from January to April and average precipitation around 800 mm (Menezes et al., 2015).

The seeds were selected according to size and health in order to maintain uniformity and then they were disinfected in 5% sodium hypochlorite for 5 minutes and washed with



distilled water to remove excess hypochlorite. Three seeds were placed per container (Leonard pots), made with plastic PET bottles, containing 1 kg of washed coarse sand, as proposed by Vincent (1970).

Saline treatments corresponded to 0 (control); 50; 100; 200 and 400 mM of NaCl, distributed in a completely randomized design, with four replicates, giving a total of 20 pots.

The Hoagland and Arnon's nutrient solution (1950) was used in half ionic strength with the proper concentrations of sodium chloride. The saline treatments began 30 days after thinning, adding 50 mM NaCl daily until reaching the desired concentration. The solutions were changed every five days in order to maintain the salinity level and the adequate concentration of nutrients, ending the experiment 60 days after the beginning of the treatments.

2.2 Morphological Attributes

After 60 days, plants were uprooted carefully, washed with water, and cut into roots and shoots. The root and shoot lengths were measured using measuring rod, while their fresh and dry (after oven drying at for 72 h at 65 °C)

2.3 Organics Solutes Quantification

The extracts for the quantification of sugars, amino acids and proteins were performed with methanol, chloroform, and water (MCW) in 12:5:3 ratio (v/v/v) (Bieliski; Turner, 1966). Sugar determination assays according to the anthrone method (Yemm; Willis, 1954), using glucose as standard. The proteins quantified according to Bradford's methodology (1976), using BSA (bovine serum albumin). For total amino acids determination assays according the methodology proposed by Yemm, Cocking and Ricketts (1955), with glycine as standard.

2.4 Statistical Analysis

Statistical analyses and regressions were performed using SISVAR statistical program (Ferreira, 2000).

3 RESULTS AND DISCUSSIONS

Increasing the salt concentration in the solution resulted in a linear reduction in the height and diameter of the stem in the plants (Figure 1). At the lowest salt concentrations (50 and 100 mM), reductions of 23% and 32% in plant height were observed, compared to the non-saline treatment (0 mM NaCl), increasing to a 57% reduction when added 400 mM NaCl (Figure 1A). Thus, it appears that the effects of moderate salinity levels exert little influence on the height of *C. quercifolius* seedlings.

However, on stem diameter, the salt effects were smaller, with this average reduction of 29%, in all saline treatments compared to non-saline treatment.

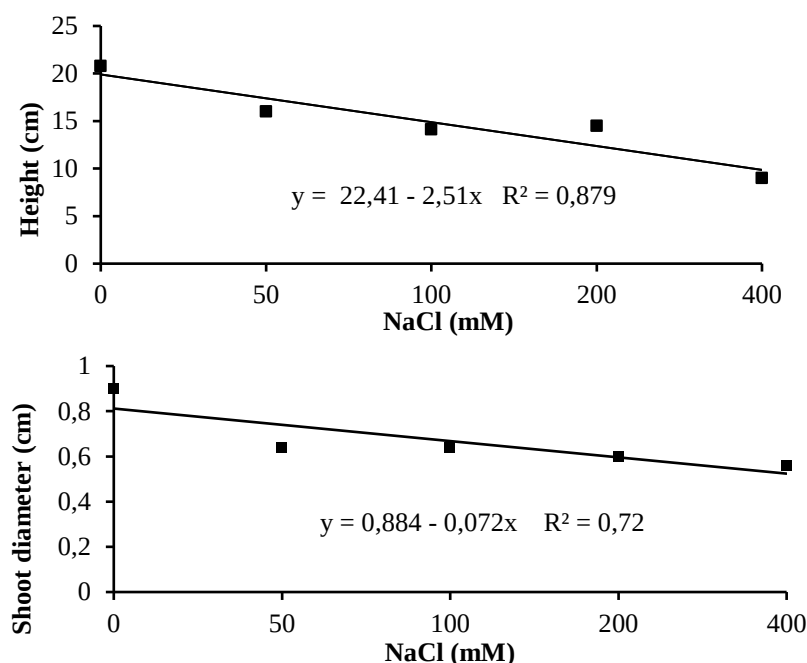


Figure 1. Height and shoot diameter of *C. quercifolius* seedlings under NaCl concentrations.
Source: authors.

According to Freire, Rodrigues and Miranda (2010), the effect of salinity on plant growth varies according to the species and salt concentration used. Thus, the sensitivity to the existence of higher or lower levels of salts in the soil is a characteristic of each type of plant (Pedrotti et al., 2015).

Studies with other Caatinga species also obtained reductions in stem height and diameter in response to increased salinity, such as *Mimosa caesalpiniaefolia* (Silva et al., 2009), *Erythrina velutina* (Silva et al., 2019), *Libidibia ferrea* (Bezerra et al., 2020) and *Mimosa tenuiflora* (França et al., 2023). These results are corroborated by other works carried out, which also observed significant effects with an increase in salinity, causing a decrease in the initial growth of seedlings, such as in *Gliricidia sepium* (Farias et al., 2009), *Caesalpinia ferrea* (Freitas et al., 2010) and *Delonix regia* (Nogueira et al., 2012). The negative effects of salinity have also been visible in fruit species, as reported in the study developed by Coelho et al. (2015), evaluating the effects of saline water on the growth of *Carica papaya* seedlings.

This is clearly verified when comparing the previously mentioned studies with the work developed by Veras et al. (2011), in which there was no significant effect of salinity on height and stem diameter of *Jatropha curcas* plants.

There are several factors that may have negatively affected the growth of *C. quercifolius* seedlings, among them the decrease in water availability can be highlighted, being retained in the saline solution, mainly as the concentrations of salts increase, it becomes water less and less available (Esteves; Suzuki, 2008). In addition to the osmotic effect, seedling growth may also have been affected by the toxic effect caused by the concentration of ions in the protoplasm (Taiz et al., 2017).

The dry mass of plants decreased with increasing NaCl concentrations in the nutrient solution (Figure 2). Compared to non-saline treatment (0 mM NaCl), there were decreases in roots dry mass (RDM) (86%), shoot dry mass (SDM) (85%), and plant dry mass (TDM) (83%) when plants were submitted to highest salt concentration (400 mM NaCl).

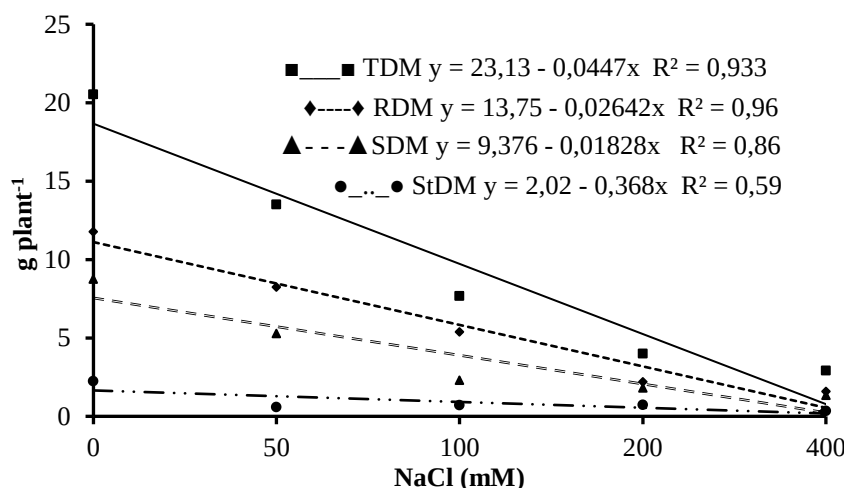


Figure 2. Total (TDM), roots (RDM), shoot (SDM), and stem dry mass (StDM) of *C. quercifolius* seedlings under NaCl concentrations.

Source: authors.

Comparing seedlings subjected to 50 mM NaCl, there was a reduction of 74%, 64%, 63% and 63%, respectively in StDM, RDM, SDM and TDM, both compared to those that did not receive NaCl. When increasing the salt level to 100 mM, the reductions were 71%, 68%, 68% and 60%, respectively for RDM, StDM, TDM and SDM.

There are several aspects that negatively affect the growth and dry mass production of plants when kept under saline conditions (França et al., 2023). The reduction in plant mass at the highest levels of salinity was probably due to the increase in the osmotic pressure of the solutions, caused by saline stress and the consequent decrease in water absorption by plants, a fact commonly reported in the literature (Katerji et al., 2003 ; Bie; Ito; Shinohara, 2004).

Dawalibi et al. (2015) studied the salt effects in two *Tamarix* species, and observed that salt had a highly negative effect on plant height increment of *Tamarix. aphylla*, which was reduced by 27.3%, while it did not show any significant effect on *T. jordanis*. The shoot dry weight of both species was not negatively affected by salt stress in the short period of the experiment, yet shoot dry weight of *T. jordanis* under salt stress was even higher than the control. Similarly, roots of both species were not affected by salt stress.

The reduction in water absorption leads to the inhibition of cellular extensibility and a consequent reduction in leaf area, affecting the photosynthetic capacity of the leaves, providing a reduction in the production of carbohydrates, necessary for plant growth, as stated by Zidan, Azaizah and Neumann (1990) and Munns (2002). Photosynthesis involves a long chain of mechanisms (Esteves; Suzuki, 2008), being directly affected as there is an increase in saline concentrations, with significant changes in carbohydrate concentrations in the source tissues, characterized by the appearance of oligosaccharides from the raffinose group, verbascose, and also methylated inositol (Pharr et al., 1995). Thus, saline stress affects photosynthesis due to the reduction in water potential, indicating that for plants to tolerate salinity, they must increase water use efficiency (Esteves; Suzuki, 2008).

Furthermore, an imbalance in the partition of assimilates between the various parts of the plant, causing a reduction in the shoot/root ratio of plants under stress may occur as a result of salinity (Hanson; Hitz, 1982).

However, one cannot also disregard the effects that Na⁺ and Cl⁻ salts can have on plants, causing disturbances in nutrition and metabolism, causing damage to growth (Chusman, 2001; Munns, 2002). This is proven in a study developed by Natale et al. (2018) stating that the accumulation of dry mass in each plant organ is proportionally related to the accumulation of nutrients.



The roots behaviour under salinity conditions is one of the most important characteristics in studies of tolerance to salt stress, as they are in direct contact with salt and absorb water for cellular supply (Jamil; Rha, 2004). Demir and Arif (2003) observed that root growth was more affected by salinity than shoot growth. Jeannette, Craig, and Lynch (2002) reported that shoot and root fresh weight of crop accessions was reduced with increasing salt stress.

Silva et al. (2008) related that as the salinity level increased, there was a decrease in the growth and accumulation of dry mass of melon plants. The effects of salinity on plant growth and dry mass accumulation are also well demonstrated by Bessa et al. (2017), in which, studying the effects of salinity on six species from the Caatinga, they observed different degrees of tolerance, varying according to the intensity of saline stress and the species studied.

In relation to the accumulation of organic solutes, salinity had a significant influence only on sugar content (Figure 3).

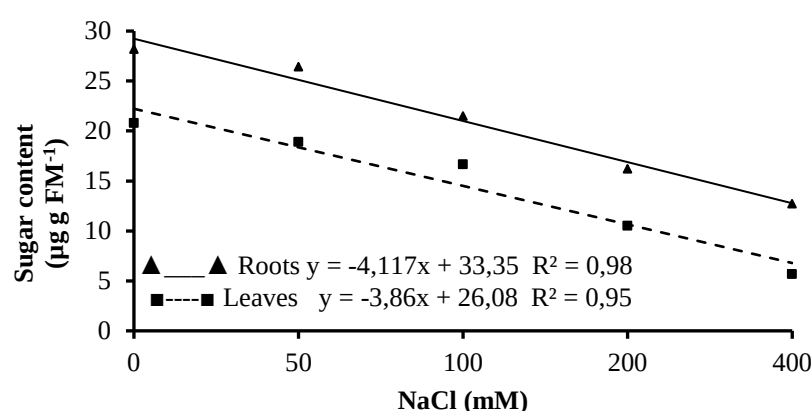


Figure 3. Sugar content in *C. quercifolius* seedlings under NaCl concentrations.

Source: authors.

There was a linear decrease in total sugar content with increasing salinity, which may have been due to the effects of salinity affecting photosynthesis, impairing the synthesis of solutes necessary for growth, since there was a reduction in height (Figure 1) and mass plant drought (Figure 2) with increased salinity.

In two *Tamarix* species under salt stress (*T. aphylla* and *T. jordanis*), Dawalibi et al. (2015) observed a drastic reduction of glucose (51%) and fructose (41.5%) content was detected *T. aphylla* plants.

It is therefore inferred that the *C. quercifolius* seedlings were not capable of developing osmotic adjustment to maintain cell turgidity and subsequent growth. Salinity-tolerant plants often develop osmotic adjustment by accumulating mainly sugars, amino acids, polyols, betaines and related compounds (Hasegawa et al., 2000). This accumulation contributes to the maintenance of water absorption and cell turgidity, ensuring vital physiological processes, such as stomatal opening, photosynthesis and cell expansion (Sakamoto; Murata, 2002; Serraj; Sinclair, 2002).

4 CONCLUSION

The seedlings were strongly affected by the imposed salinity, resulting in a reduction in growth and accumulation of dry mass, not being able to adjust osmotically.



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