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Scientific Article

Water regimes on the morphophysiological and biochemical responses of *Croton blanchetianus* (Euphorbiaceae)

Regimes hídricos nas respostas morfofisiológicas e bioquímicas de *Croton blanchetianus* (Euphorbiaceae)

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ABSTRACT - Plant species occurring in the Caatinga biome, when subjected to different water fluctuations, may have their morphophysiological and metabolic performance altered. Depending on the level of stress, the production of quality seedlings is affected. Therefore, the objective of this study was to evaluate the responses to different water availability levels in the morphophysiological and biochemical development of *Croton blanchetianus* Baill. The experiment was carried out in a greenhouse, evaluating four water regimes: 25%, 50%, 75% and 100% of pot capacity, and conducted in a randomized block design, with a total 5 blocks. The variables analyzed were: branch height and branch diameter; leaf area, number of leaves, root volume; root dry mass, shoot dry mass, total dry mass and root/shoot ratio; relative water content, proline, total soluble proteins, malondialdehyde, hydrogen peroxide, and photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids). The species *Croton blanchetianus* Baill. shows drought tolerance mechanisms, demonstrated by the reductions of growth, leaf area, dry mass of seedlings and photosynthetic pigments. It adjusts osmotically with increments in the production of proline, which acts in osmoprotection when there is water deficit, and in the production of proteins under the lower and higher water regime. The species has full development of seedlings under water regimes between 75% and 80% and adapts to different water fluctuations.

Keywords: Water fluctuations. Forest species. Caatinga. Metabolism.

RESUMO - Espécies vegetais de ocorrência no bioma Caatinga quando submetidas a diferentes flutuações hídricas podem ter seu desempenho morfofisiológico e metabólico alterado. A depender do nível de estresse, a produção de mudas de qualidade é afetada. Logo, objetivou-se avaliar as respostas às diferentes disponibilidades hídricas no desenvolvimento morfofisiológico e bioquímico de *Croton blanchetianus* Baill. O experimento foi desenvolvido em casa de vegetação e foram avaliados quatro regimes hídricos: 25%, 50%, 75% e 100% da capacidade de vaso e conduzido em delineamento experimental em blocos ao acaso, totalizando 5 blocos. As variáveis analisadas foram: altura e diâmetro dos ramos; área foliar, número de folhas, volume radicular; massa seca da raiz, parte aérea, total e relação raiz/parte aérea; teor relativo de água, prolina, proteínas solúveis totais, malondialdeído, peróxido de hidrogênio e pigmentos fotossintéticos (clorofila ɑ, clorofila *b* e carotenóides). A espécie *Croton blanchetianus* Baill. apresenta mecanismos de tolerância à seca, demonstrados pela redução de crescimento, área foliar, massa seca das mudas e da redução dos pigmentos fotossintéticos. Se ajusta osmoticamente com aumento na produção de prolina que atua na osmoproteção quando há déficit hídrico e, aumentando a produção de proteínas no menor e maior regime hídrico. A espécie apresenta pleno desenvolvimento de mudas nos regimes hídricos entre 75% e 80% e se adapta as diferentes flutuações hídricas.

Palavras-chave: Flutuações hídricas. Espécie florestal. Caatinga. Metabolismo.

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INTRODUCTION

Water unavailability is the main abiotic stress that affects the water status of plants and, when associated with high temperatures, increases the severity of the negative effects (JUMRANI; BHATIA, 2018). These climatic characteristics are naturally present in the arid and semi-arid regions of northeastern Brazil, where the Caatinga Biome is predominant. In these regions, numerous species are found, including aromatic ones, with medicinal potential, used for the treatment of various diseases.

Species of the genus *Croton* are widespread for popular use, especially the leaves and bark, which are used for the treatment of gastrointestinal disorders, rheumatism, headache, edema, among others (NUNES et al., 2023; MALVEIRA et al., 2022). This genus is quite rich in bioactive natural products, notably alkaloids and terpenoids (AQUINO et al., 2017; BRITO JUNIOR et al., 2015). The species *Croton blanchetianus* Baill is a shrub known in Portuguese as ‘marmeleiro preto’, and its essential oil has larvicidal and antibacterial action. For being adapted to the conditions of the semi-arid region and having bioactive compounds with proven efficacy, it is an excellent option to be cultivated by small and medium-sized producers to serve the pharmaceutical industry (NUNES et al., 2023; AQUINO et al., 2017).

Despite the pharmacological importance of *C. blanchetianus*, research on the morphophysiological and biochemical behavior of this species under different water regimes is still incipient. Water restriction or excess can negatively influence the vegetative growth and number of leaves of this species, essential parts for the extraction of essential oil. In addition, water availability is essential for the production of high-quality, rustic seedlings, but the water requirement of the species is unknown and, as it is variable between species, there may be sensitivity to both water deficit and excess water (BUENO et al., 2020; LEITE et al., 2020; FREITAS; SILVA, 2018).

In *Jatropha curcas* L., the water regimes between 60 and 80% of the water retention capacity in *Latossolo Vermelho distrófico* (Oxisol) promote the best responses for physiological and growth parameters of the seedlings. In turn, water contents below 60% and above 80% of the water retention capacity in the soil induce signs of water stress (PADILHA et al., 2016). On the other hand, the species *Schizolobium parahyba* (Vell.) Blake, *Cytharexylum myrianthum* Cham. and *Ceiba speciosa* Ravenna need more water, because they showed higher growth when subjected to a 100% water regime (BUENO et al., 2020).

For being a pioneer species, *C. blanchetianus* is more adapted to semi-arid conditions and, consequently, to water restriction. However, it is necessary to know which water level is ideal for the production of quality seedlings. Therefore, the objective of this study was to evaluate the responses to different water availability levels in the morphophysiological and biochemical development of *C. blanchetianus*.

MATERIAL AND METHODS

The experiment was carried out under greenhouse conditions at the State University of Rio Grande do Norte (UERN), Mossoró/RN, Brazil. *C. blanchetianus* seedlings were obtained from cuttings collected from parent plants at the Central Campus of UERN, located at 5°11'2" South latitude and 37°20'52" West longitude. The climate of the region is BSwh (dry and very hot), according to Köppen's classification. There are two seasons: a dry season from June to January and a rainy season from February to May. The average annual temperature and rainfall are 27 °C and 673 mm, respectively; the relative humidity is 68%, and there are 241.7 hours of daylight per month (CARMO FILHO; OLIVEIRA, 1989).

Cuts for the production of the cuttings were made from the middle portions of the branches of the species, with 15 cm in length and approximately six millimeters in diameter, with a bevel cut at the base and apex. After collection, to stimulate rooting, the cuttings were immersed for 10 minutes in a solution of naphthaleneacetic acid (NAA), at a concentration of 600 mg L^{-1} (concentration previously determined). The *C. blanchetianus* cuttings were planted in pots with capacity of 8 L containing substrate composed of: natural soil, taken from the area of occurrence of the plant species in the surface layer of 0.02 m depth in the same place of occurrence of the species, near the UERN (05°12'10" S and 37°18'57" W) in the municipality of Mossoró, RN, and classified as a *Neossolo Quartzarênico* (Quartzipsamment); plus polyfértil® fertilizer in a 2:1 ratio. The soil samples were characterized for physical and chemical attributes according to the methodology of EMBRAPA (2009) (Table 1).

Table 1. Chemical and physical analysis of the soil used in the experiment.

pH	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	CEC	V	ESP
	($g\ kg^{-1}$)	-----($mg\ dm^{-3}$)-------			------------------------- ($cmol_c\ dm^{-3}$) -------------------------					----- % -----	
5.3	15.98	37.4	156.9	91.9	3.7	2.10	0.05	1.16	7.76	85	5.0
Texture[1]	BD $g\ cm^{-3}$		Sand			Silt			Clay		
		--- ($g\ kg^{-1}$) ---									
Loamy sand	1.53		824			138.5			37.5		

OM - Organic matter; CEC - Cation exchange capacity; V - Base saturation; ESP - Exchange sodium percentage; BD - Bulk density.

After 75 days, time required for the establishment of the seedlings, the water regimes were differentiated as follows: 25%, 50%, 75% and 100% field capacity, corresponding to 200, 600, 400 and 800 ml of water, respectively. The experimental design was randomized blocks (RBD), totaling 5 blocks, with a total of 20 experimental units, each of which consisting of one seedling per pot.

Pot capacity was established by the capillarity method, where the pot containing the dry substrate was weighed and placed in a larger container a known volume of water, which ascended by capillarity to saturate the substrate. The pot was covered with plastic wrap to prevent evaporation and allow for more accurate results. Upon reaching complete saturation, after the water drained, pot capacity was determined using Equation 1.

$$\mathrm{FCpot} = \frac{(\mathrm{WSW} + \mathrm{CO}) - (\mathrm{DSW} + \mathrm{CO})}{\mathrm{DSW}} \text{ x } 100 \qquad (1)$$

Where: FCpot = field capacity of the pot (%); WSW = Wet soil weight (g); CO = Container weight at the time of weighing (g); DSW = Dry soil weight (g).

Three repetitions of each treatment were weighed daily to determine the amount of water for each established pot capacity and, subsequently, the average was calculated for watering, performed at the same time using a graduated container.

Branch height and branch diameter were measured before applying the treatments and at the end of the experiment, on the 30th day. At the end of the experiment, leaf area, number of leaves and root volume were evaluated.

Height was measured using a tape measure on the main branches, with subsequent calculation of the average, and the data were expressed in cm. Diameter of the branches was measured using a digital caliper at 2 cm above the insertion point of the branch in the main segment, with subsequent calculation of the average, and the data were expressed in mm. For leaf area, 5 leaf discs per leaf were collected using a hole punch, with known diameter, dried in the oven and then weighed. Equation 2 was used to determine leaf area.

$$LA=\frac{(LDM \text{ x } DA)}{DDM} \qquad (2)$$

Where: LA – leaf area; LDM – leaf dry mass; DDM – disc dry mass; DA – disc area.

Number of leaves was determined by simply counting the fully expanded green leaves of each plant. Root volume (RV) was determined using a 1000 mL graduated cylinder containing a known volume of water, corresponding to the displacement of the water column as a function of the volume of roots. The greater the displacement, the greater the root volume.

After the growth analysis, the seedlings were sectioned into leaves, stems and roots, placed in kraft paper bags, dried in an oven with forced air circulation, at 70 °C until reaching constant weight, and weighed on an analytical balance (0.0001 g), with the results were expressed in g. These data were then used to calculate the root/shoot ratio (R/S).

Relative water content (RWC) was obtained using 3 leaf discs with known diameter, collected with a cork borer, at 7 a.m. The discs were immediately weighed to determine the fresh weight and then placed in a Petri dish on filter paper soaked with distilled water. The Petri dishes were placed in germination chambers at 25 °C and 80% relative humidity, in the dark, and after 24 h the discs were weighed to determine the turgid weight. The discs were then dried in an oven at 70 ° C until reaching constant weight. Dry weight was determined and RWC was calculated based on the formula of Barrs and Weatherley (1962), expressed in Equation 3.

$$RWC=\left(\frac{FW-DW}{TW-DW}\right) \text{ x } 100 \qquad (3)$$

Where: FW - fresh weight; TW - turgid weight; and DW - dry weight.

Proline content was determined according to the method proposed by Bates, Waldren, and Teare (1973). In test tubes containing 1 mL of the plant extract, 1 mL of acid ninhydrin and 1 mL of glacial acetic acid were added. Proline contents were estimated using a proline standard curve as a reference and using toluene as blank. Each extract and its replicates were measured in triplicates, and colorimetric readings were taken in a spectrophotometer at 520 nm. The results were expressed in µmol g^{-1} DM.

Protein concentration was quantified according to Bradford's (1976) method and using bovine serum albumin (BSA) as standard. A total of 200 µL of a 1 mg mL^{-1} BSA solution was prepared and, from this solution, serial dilutions in H_2O were performed to obtain the other concentrations (0.5 mg mL^{-1}, 0.25 mg mL^{-1} and 0.125 mg mL^{-1}). Absorbance readings were performed in a spectrophotometer at 595 nm. Protein contents were determined using a standard curve of known BSA concentrations by linear regression.

Malondialdehyde (MDA) was determined according to the Heath and Packer's (1968) method, using 200 mg of plant material (shoots) macerated in 2 mL of trichloroacetic acid (0.1%), containing approximately 20% PVPP. After homogenization, the material was centrifuged at 10,000 rpm for 5 minutes. 1 mL of trichloroacetic acid (20%, containing 0.5% thiobarbituric acid) was added to 0.25 mL of the supernatant, and the mixture was maintained for 30 minutes at 95 ºC. After being removed from the water bath, the samples were subjected to rapid cooling on ice. In order to separate some residue during heating and to clarify the sample, centrifugation was performed at 10,000 rpm for 10 minutes. Readings were performed in a spectrophotometer at 535 and 600 nm.

Hydrogen peroxide (H_2O_2) was determined by following the Alexieva et al. (2001) method, using 200 mg of plant material (shoots) macerated in 2 mL of trichloroacetic acid (0.1%), containing approximately 20% PVPP. After homogenization, the material was centrifuged at 10,000 rpm for 5 minutes. Subsequently, 200 µL of the supernatant was transferred to another Eppendorf flask and then 800 µL of potassium iodide and 200 µL of phosphate buffer were added. This mixture was kept on ice for an hour. After this period, it was removed from the ice and left at rest for 20 minutes for stabilization of the reaction and then the reading was performed in a spectrophotometer at 390 nm.

Chlorophyll and carotenoid contents (photosynthetic pigments) were determined by the method of extraction with 80% acetone, followed by absorbance readings of the extracts obtained in UV-VIS spectrophotometer at wavelengths of 663, 645 and 470 nm for chlorophyll *a*, chlorophyll *b* and carotenoids, respectively. Then, the concentrations of each pigment were calculated according to the equations described by Lichtenthaler and Buschmann (2001), and the results were expressed in g kg^{-1} FM.

Laboratory analyses were carried out at the Laboratory of Plant Physiology and Biochemistry (LFBP) – UERN.

The data were subjected to analysis of variance, F-test, and in case of significance, regression analysis was applied at 5% probability level. Statistical analyses were performed with the aid of the computer program System for Analysis of Variance – SISVAR 5.7 (FERREIRA, 2019).

RESULTS AND DISCUSSION

Effects of water regimes were significant ($p < 0.01$) on plant height, leaf area, root dry mass, root/shoot ratio, relative water content, and root volume (Table 2).

Table 2. Analysis of variance by the F test for branch height (BH), branch diameter (BD), leaf area (LA), number of leaves (NL), root dry mass (RDM), shoot dry mass (SDM), root/shoot ratio (R/S), relative water content (RWC) and root volume (RV) of *Croton blanchetianus* seedlings under different water regimes, at thirty days after the imposition of the water regimes.

Sources of variation	DF	BH	BD	LA	NL	RDM	SDM	R/S	RWC	RV
		Pr > Fc								
		(cm)	(mm)	(cm^2)		-----(g plant^{-1})-----			(%)	(ml)
Water regimes	3	0.0085	0.1285	0.0000	0.4401	0.0000	0.0765	0.0022	0.0001	0.0000
Block	4	0.4118	0.7053	0.2620	0.4431	0.9143	0.9220	0.9049	0.5263	0.4590
Error	12									
CV (%)		25.8	15.9	4.57	7.27	7.9	9.8	11.0	8.3	6.7

DF = degrees of freedom; CV = coefficient of variation.

The branches of *C. blanchetianus* seedlings reached the greatest height of 16.3 cm with a water regime of 75.6% pot capacity. The lowest pot capacity, 25%, led to lower growth (Figure 1A). There was no statistical difference in branch diameter, with an average of 2.01 mm, regardless of the water regime (Figure 1B). For leaf area, the water regime of 61.3% promoted the highest value, 1437.8 cm², whereas the lowest water regime, 25%, led to the smallest leaf area, corresponding to 1005.8 cm^2 (Figure 1C). The number of leaves of *C. blanchetianus* was not affected by the different water regimes, with an average of 35 leaves per seedling (Figure 1D).

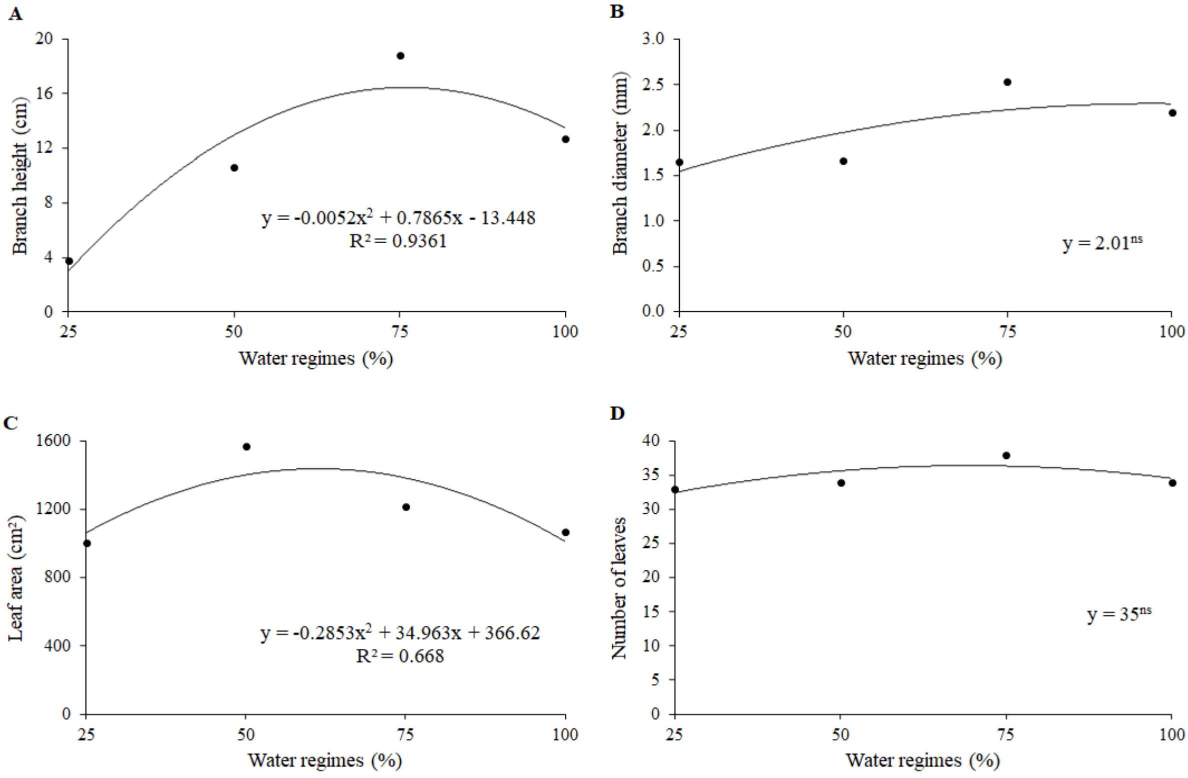

Figure 1. Branch height (A), branch diameter (B), leaf area (C) and number of leaves (D) of *Croton blanchetianus* seedlings subjected to different water regimes, at thirty days after the imposition of water regimes.

The 25% water regime led to lower results for branch height, leaf area, root dry mass and root volume in *C. blanchetianus* seedlings. It is possible to observe that the lowest (25%) and highest (100%) pot capacity (PC) caused reduction in leaf area. Leaf area expansion is associated with the entry of water into the plant cell, and the turgor pressure favors cell elongation, a process primarily inhibited under water deficit conditions (TAIZ et al., 2017). In the different water regimes, the species maintained the number of leaves as an adaptive strategy, which may have favored photosynthetic activity. A similar behavior was observed by Andrade et al. (2023) in *C. blanchetianus* under salinity levels, as the leaves were maintained during the experiment and the accumulation of salts did not cause leaf abscission.

The highest root dry mass was obtained with a water regime of 76.3%, resulting in 36 g plant^{-1}. The lowest root dry mass, 13.6 g plant^{-1}, occurred in the 25% water regime (Figure 2A). Although there was no significant effect on shoot dry mass, the highest water regime promoted greater dry mass accumulation in C. *blanchetianus*, 31.2 g plant^{-1} (Figure 2B). The water regime of 55.6% promoted the highest value, 1.4, for the root/shoot ratio, while the lowest value was obtained with the highest water regime, corresponding to 0.63 (Figure 2C).

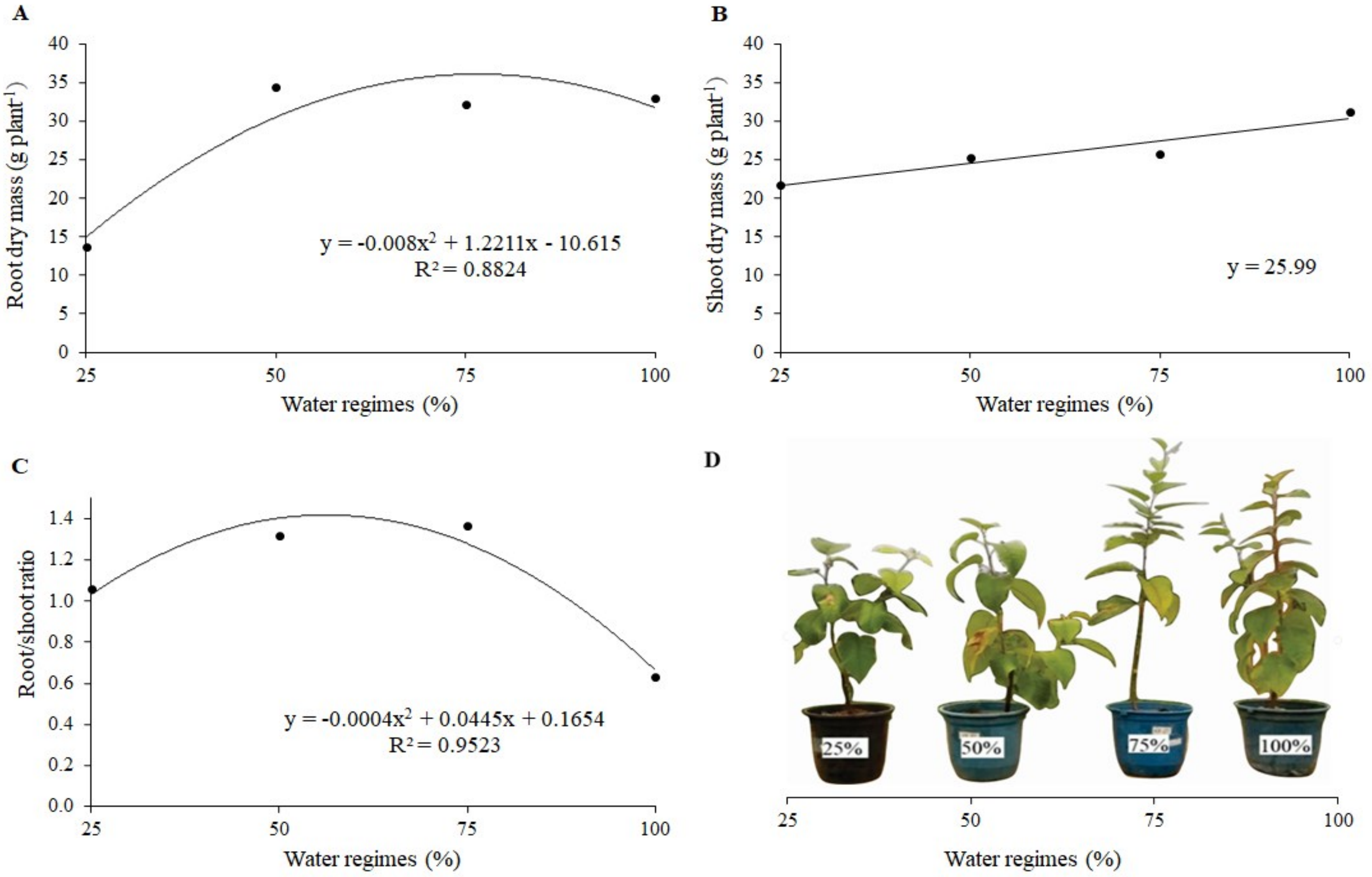

Figure 2. Root dry mass (A), shoot dry mass (B), root/shoot ratio (C) and *Croton blanchetianus* seedlings (D) subjected to different water regimes, at thirty days after the imposition of the water regimes.

In the 100% water regime, the reduction of leaf area and branch height occurs without affecting dry mass production and root volume, which indicates that the species has directed its reserves to root production (Figure 2D). Some studies have shown that soil-plant hydraulic conductivity may have been affected in response to inadequate oxygen supply caused by excess water and/or physical limitation imposed by the pot. According to Loose et al. (2017), the inability of roots to meet leaf water demand causes leaf wilting, as observed in sunflower (*Helianthus annuus* L.).

In association, the physical limitations imposed by the pot may have contributed to the reduction of growth of *C. blanchetianus* seedlings under the highest water regime, which affects morphophysiological processes and influences gas exchange and, consequently, cell respiration (OLIVEIRA et al., 2020). Corroborating the results obtained in this study, seedlings of *Tabebuia serratifolia* (yellow ipe) when subjected to a greater volume of irrigation water had their growth compromised (SILVA et al., 2019).

The relative water content (RWC) in C. *blanchetianus* seedlings increased linearly according to the water regimes. For 100% pot capacity, RWC reached 68%, while 25% pot capacity led to RWC equal to 46.3% (Figure 3A). The highest root volume was obtained when the water regime was 80%, with 128.22 mL, and the lowest root volume was 54 mL, obtained under the lowest water regime (Figure 3B).

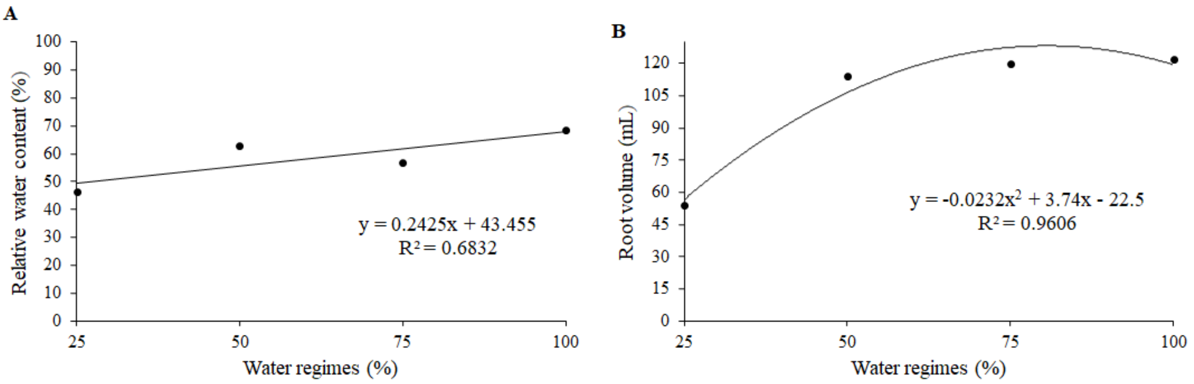

Figure 3. Relative water content (A) and root volume (B) of Croton blanchetianus seedlings subjected to different water regimes, at thirty days after the imposition of water regimes.

On the other hand, the water regimes of 76.3 and 80% promoted increments in root dry mass and root volume, respectively. The increase in soil water availability favored the increase of water volume in the leaf tissues, confirmed by the increase in RWC. The results prove the species' ability to adapt to the temporal and spatial fluctuations of rainfall that occur in arid and semi-arid regions. Similar behavior was also observed in seedlings of *Jatropha curcas* L. (MOURA et al., 2016); however, for *Aspidosperma pyrifollium*, the behavior was the opposite. To ensure a deep root system and maintain the influx of water, the species invested in root growth under more severe water deficit conditions, and the RWC remained constant under the different water regimes (FREITAS; SILVA, 2018). The results show that the behaviors are different among the species that occur in the Caatinga.

Effects of water regimes were significant ($p < 0.05$) on soluble proteins, significant ($p < 0.01$) for lipid peroxidation, hydrogen peroxide, free proline, chlorophyll *a* and total chlorophyll, and not significant for chlorophyll *b* (Table 3).

Table 3. Analysis of variance by the F test for soluble proteins (ProT), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), free proline (ProL), chlorophyll *a* (C*a*), chlorophyll *b* (C*b*), total chlorophyll ($C_{(a+b)}$) and carotenoids (CAR) in seedlings of *Croton blanchetianus* under different water regimes, at thirty days after imposition of water regimes.

Sources of variation	Pr > Fc								
	DF	ProT	MDA	H_2O_2	ProL	C*a*	C*b*	$C_{(a+b)}$	CAR
		(mg/ml)	(umol g^{-1})	(mol g^{-1})	(umol g^{-1})	--------------------(g kg^{-1} FM) --------------------			
Water regimes	3	0.0132	0.0056	0.0000	0.0000	0.0006	0.1298	0.0008	0.0056
Block	4	0.5261	0.3026	0.8470	0.9624	0.0766	0.2937	0.0187	0.0762
Error	12								
CV (%)		10.31	12.28	9.59	7.45	9.04	17.93	7.69	12.72

DF = degrees of freedom; CV = coefficient of variation.

The 50% water regime caused reduction in the soluble protein content, with value of 0.0634 mg/ml, while the highest protein content was obtained in the 100% water regime (Figure 4A). For membrane lipid peroxidation, the 41% water regime led to reduction in the MDA level to 0.196 µmol g^{-1}, whereas the 100% water regime promoted an increase in lipid peroxidation to 0.349 µmol g^{-1} (Figure 4B). Hydrogen peroxide production was reduced to 3.47 mol g^{-1} in the 62% water regime. In the lowest and highest water regimes, hydrogen peroxide production increased to 7.0 and 7.13 mol g^{-1}, respectively (Figure 4C). For proline, there was a decreasing linear behavior as the water regime increased up to 100%, resulting in a reduction of its content to 30.09 µmol g^{-1}, while the lowest water regime favored the increase of this amino acid to 48.89 µol g^{-1} (Figure 4D).

For *C. blanchetianus* seedlings, water deficit and excess water promoted increments in malondialdehyde and hydrogen peroxide, which indicates stress. To acclimatize to the stressful conditions, *C. blanchetianus* seedlings produced more protein. According to Santos et al. (2014), water unavailability stimulates the synthesis of soluble proteins so that osmotic adjustment occurs in plant cells, besides acting as a signal for water stress, favoring the stabilization of cell membranes. A similar behavior was observed in *Lippia grata*, which showed increased soluble protein content when subjected to water deficit (PALHARES NETO et al., 2020).

Although the behavior was quadratic for malondialdehyde production, there was a 25% reduction in plasma membrane damage in the 25% water regime, when compared to the 100% water regime. Excess water caused greater lipid peroxidation, which is one of the first signs that the plant is under oxidative stress, occurring when there is an imbalance between the production of antioxidative enzymes and the reactive oxygen species, such as hydrogen peroxide, which was produced in large quantity under the lowest and highest water regime for *C. blanchetianus* (AQUINO et al., 2017; CHOUDHURY et al., 2017).

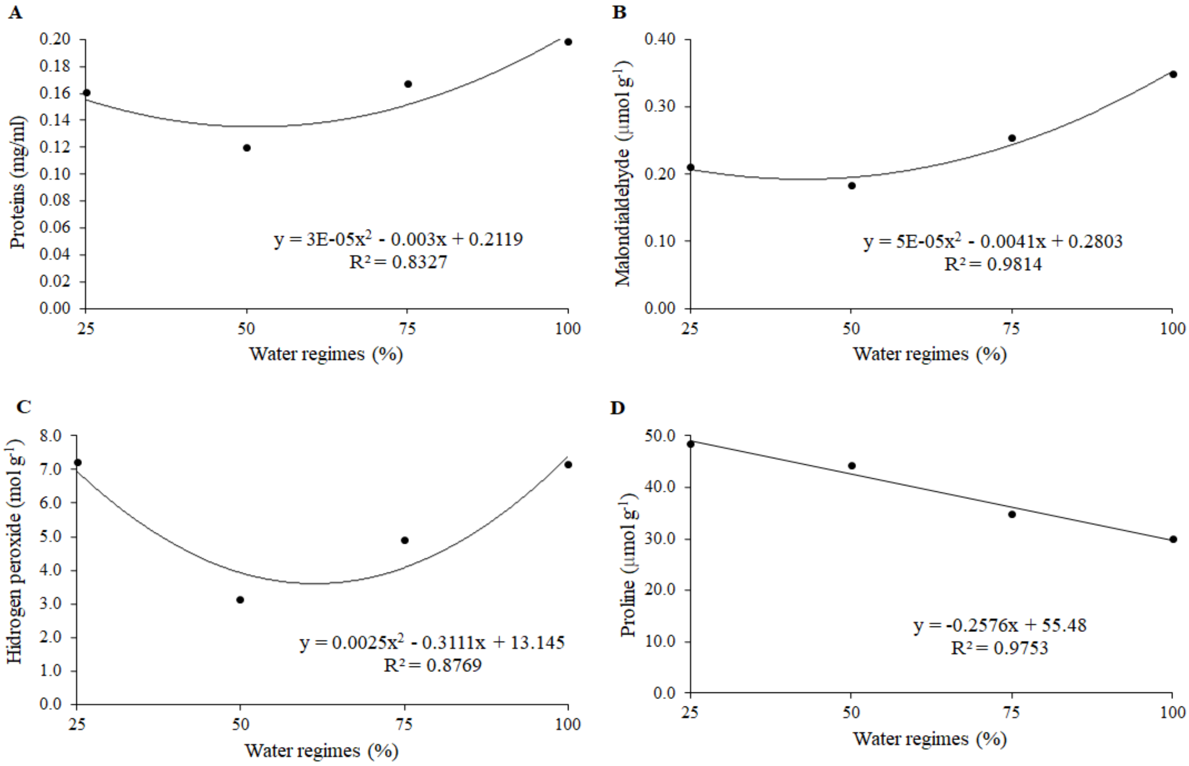

Figure 4. Proteins (A), malondialdehyde (B), hydrogen peroxide (C) and proline (D) in *Croton blanchetianus* seedlings subjected to different water regimes, at thirty days after the imposition of the water regimes.

To tolerate the effects of water stress, 25%, the species produced a higher content of free proline. The increase in the production of this amino acid is related to the osmotic adjustment mechanism, plays an important role in protecting against enzymatic denaturation and may have influenced the reduction of hydrogen peroxide, contributing to minimizing the effects of oxidative stress (SANTOS JÚNIOR; OLIVEIRA; SILVA, 2020). In this type of adjustment, there is a reduction in the osmotic potential at the cellular level. Water balance occurs between the vacuole and the cytoplasm, without reducing the turgidity of plant cells, allowing the continuity of physiological processes that are essential for plant growth and development (LEITE et al., 2020). The result confirms that proline is an osmoprotective amino acid and plays an important role as a defense mechanism for C. *blanchetianus* species to acclimatize and survive even under abiotic stresses (ANDRADE et al., 2023).

The increase in water availability increased chlorophyll *a* and total chlorophyll quadratically. For chlorophyll *a*, this increase was from 0.74 g kg^{-1} FM under the 25% water regime to 1.0 g kg^{-1} FM under the 116% water regime (Figure 5A). For total chlorophyll, the 85% water regime caused increase to 1.2 g kg^{-1} FM (Figure 5C). For chlorophyll *b*, no difference was detected between water regimes, with an average of 0.28 g kg^{-1} FM (Figure 5B). Carotenoid content increased linearly, with 0.33 g kg^{-1} FM under the 100% water regime (Figure 5D).

For the photosynthetic pigments, although they are light-dependent, the water regimes influenced the activity of chlorophyll *a*, total chlorophyll and carotenoids. Lower water availability reduces the activity of photosynthetic pigments, being an adaptation of the species that occur in the Caatinga Biome, with the purpose of reducing metabolic activities under adverse conditions. However, the activities of photosynthetic pigments vary according to the species and water stress duration and severity (LEITE et al., 2020; OLIVEIRA et al., 2020).

During water deficit, plant tissue undergoes a significant inhibition of photosynthesis, and CO_2 fixation is primarily affected due to stomatal closure. In this situation, the NADPH produced by the flow of electrons in the photochemical phase is possibly redirected to the production of proline (FAROOQ et al., 2017; MATHOBO; MARAIS; STEYN, 2017), as observed for *C. blanchetianus*. Among other adaptive responses, this is considered a protective mechanism with an essential function of mitigating the deleterious effects of water deficit on *C. blanchetianus*.

On the other hand, water regimes above 75% favored photosynthetic activity in *C. blanchetianus*. This behavior occurs in response to the increase of leaf water potential, which favors stomatal opening; consequently, the photosynthetic pigments become more efficient for photosynthesis and CO_2 capture.

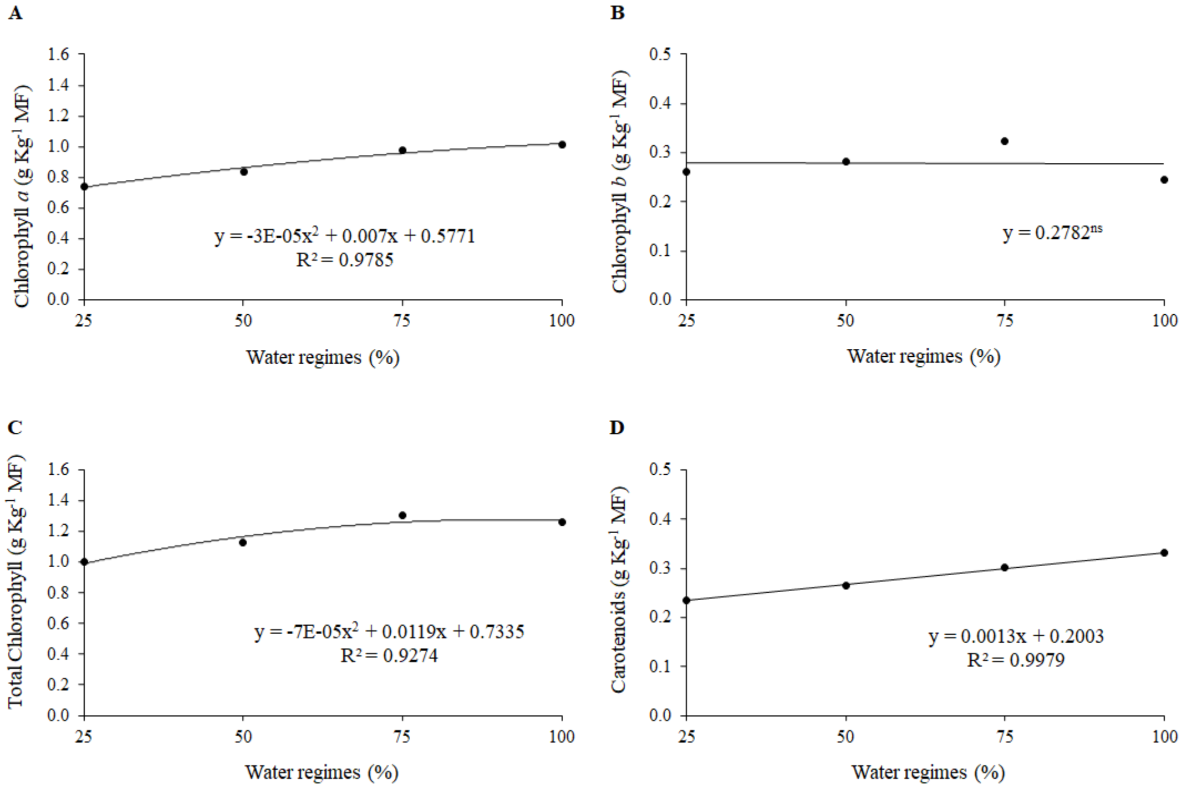

Figure 5. Photosynthetic pigments: chlorophyll *a* (A), chlorophyll b (B), total chlorophyll (C) and carotenoids (D) in Croton blanchetianus seedlings subjected to different water regimes, at 30 days after the imposition of water regimes.

Adequate water availability is essential for the development and establishment of *C. blanchetianus* seedlings. The results confirm that *C. blanchetianus* is adapted to water restriction and, therefore, restricts growth as a strategy to ensure permanence in the environment under water stress. The adaptive capacity of *C. blanchetianus* is due to the presence of anatomical characteristics in the species, commonly associated with water saving, such as leaves with simple epidermis, thicker cuticle, indumentum, and basal cell wall of trichomes impregnated with lipids and phenolic compounds (SOARES; BARROS, 2013).

Together with the morphological characteristics, plants have a mechanism of regulation or osmoregulation with increased production of proteins, amino acids, and other metabolites. Thus, they significantly reduce their metabolism through the reduction of photosynthesis, reduction of water relations, excessive increase of reactive oxygen species (ROS), such as hydrogen peroxide, and increase in membrane lipid peroxidation, with an increase in malondialdehyde (MDA) production.

CONCLUSION

The species *Croton blanchetianus* Baill. shows reductions of growth, leaf area, dry mass of seedlings and photosynthetic pigments as drought-tolerance mechanisms. It adjusts osmotically with an increase in the production of proline and proteins under the lowest and highest water regime that caused stresses. Water regime for full development of *C. blanchetianus* seedlings is between 75% and 80% pot field capacity. The results indicate that the species adapts to different water fluctuations.

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