

## Water restriction as a strategy for growing *Talinum fruticosum* (L.) Juss. (Talinaceae)

## Restrição hídrica como estratégia no cultivo de *Talinum fruticosum* (L.) Juss. (Talinaceae)

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**ABSTRACT** - *Talinum fruticosum* is a species confirmed to occur in the semi-arid region, considered an unconventional food plant with a high protein content and tolerant to temporary periods of water restriction. This study aimed to evaluate water deficit as a strategy for growing *Talinum fruticosum*, covering physiological and biochemical aspects. The experimental design adopted was entirely randomized, using six treatments and eight replications. The treatments were defined as water availability (WA) 0 (no water replenishment), 20, 40, 60, 80, and 100%, conducted in a greenhouse, with water replenishment every 7 days. After 21 days, the centesimal composition, water relations, gas exchange, and biochemical aspects were evaluated. The energy value of the dry biomass of *T. fruticosum* leaves was directly influenced by the water content in the soil, and the water deficit led to an increase in protein. Water restriction compromised the species' water status and performance, mainly limiting gas exchange by reducing the water content in the soil, compromising CO<sub>2</sub> gain. It also led to increased biomolecules, with the highest contents seen in the treatments without water replacement (0%) and for 20% WA. Therefore, reducing the WA to 40% could be a strategy adopted to increase the protein content in the leaves of *T. fruticosum*, promoting an increase in the centesimal composition of proteins, a reduction in gas exchange, and an increase in the content of biomolecules.

**RESUMO** - *Talinum fruticosum* é uma espécie de ocorrência confirmada na região semiárida, considerada uma planta alimentícia não convencional com alto teor de proteínas e tolerante a períodos temporários de restrição hídrica. Assim, o objetivo do trabalho foi avaliar o déficit hídrico como estratégia no cultivo de *Talinum fruticosum*, abrangendo aspectos fisiológicos e bioquímicos. O delineamento experimental adotado foi o inteiramente casualizado, utilizando 6 tratamentos e 8 repetições, sendo os tratamentos definidos em níveis de disponibilidade hídrica (DH) 0 (sem reposição hídrica), 20, 40, 60, 80 e 100%, conduzidos em casa de vegetação, com reposição de água a cada 7 dias. Após 21 dias, avaliou-se a composição centesimal, relações hídricas, trocas gasosas e aspectos bioquímicos. O valor energético da biomassa seca das folhas de *T. fruticosum* foi diretamente influenciado pelo conteúdo de água no solo, sendo que, o déficit hídrico promoveu incremento de proteínas. A restrição hídrica comprometeu o status hídrico e desempenho da espécie, limitando principalmente as trocas gasosas com a redução do conteúdo de água no solo, comprometendo o ganho de CO<sub>2</sub>, também promoveu incremento das biomoléculas, sendo os maiores conteúdos verificados nos tratamentos, sem reposição hídrica 0% e para 20% de DH. Portanto, a redução da DH para 40%, pode ser uma estratégia adotada para elevar o conteúdo de proteínas nas folhas de *T. fruticosum*, resultando em incrementos de proteínas da composição centesimal, redução das trocas gasosas e aumento no conteúdo das biomoléculas.

**Keywords:** Biomolecules. Abiotic stress. Leaf gas exchange.

**Palavras-chaves:** Biomoléculas. Estresse abiótico. Trocas gasosas foliares.

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### INTRODUCTION

The Brazilian semi-arid region includes 1,427 municipalities, where low rainfall predominates, equal to or less than 800 mm, and a daily water deficit equal to or greater than 60% (BRASIL, 2021). In addition to the socio-economic damage, long periods of low water availability threaten food security, especially for the most vulnerable population (ALVALÁ et al., 2019), which requires strategies to live with this reality and involves the selection of tolerant germplasm, as well as the use of species with potential use (LEITE et al., 2021).

To overcome periods of water deficit, plants use a series of physiological mechanisms that enable metabolic adjustments (GONZÁLEZ-CHAVIRA et al., 2018), made possible by the accumulation of compatible solutes (LEITE et al., 2018) and changes in gas exchange (LEITE et al., 2018; SILVA et al., 2020; SOUZA et al., 2020), thus compromising carbon gain.

Although the impact of water deficit is well documented for different species, mainly limiting carbon gain and promoting metabolic adjustments through the accumulation of organic solutes such as sugars, proteins, and amino acids in the plant cell, it is necessary to understand whether these adjustments result in changes in food composition. Thus, studies that verify the final result of water deficit on centesimal composition are important, making it possible to



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evaluate the energy content and strategic use of irrigation management and provide subsidies that corroborate food security, considering climate change projections.

In this perspective, *Talinum fruticosum* (L.) Juss., popularly known as maria gorda, beldroega, língua of vaca, and caruru, stands out as an unconventional food plant with the possibility of being put to better use, being underexploited, permeates most different environments, including those with low water availability, making it possible to infer that this is an important genetic resource for the semi-arid region, especially in scenarios of water deficit. Although studies verifying its performance in the semi-arid region are scarce, the potential for cultivation under water restriction conditions has been observed (HERRERA; BALLESTRINI; MONTES, 2015). Among the attractions for encouraging the use of the resource, we highlight its protein content (BIOLTIF; EDWARD, 2020; EDEMA-EYENA et al., 2023), medicinal properties (IKEWUCHI; IKEWUCHI; IFEANACHO, 2016) and how it can be used: cooked in beans, sautéed and with duck eggs and sprouts (KINUPP; LORENZI, 2014). In Bahia, reports from family farmers also confirm the plant's use in preparing caruru and animal feed.

Knowing the importance of harnessing the genetic potential of species capable of establishing themselves in the

semi-arid region, it is necessary to understand better the performance of *T. fruticosum* under water deficit conditions. This study aimed to evaluate water deficit as a strategy for growing *Talinum fruticosum*, covering physiological and biochemical aspects.

## MATERIAL AND METHODS

The study was conducted at the Experimental Forest Garden Unit, the State University of Feira de Santana (UEFS), at 12°16'7.2" S, 38°56'21.6" W, and 258 m above sea level. The experiment was conducted in a greenhouse, with 50% shading, in a completely randomized design, with six treatments and eight replications. The seeds used were collected on the UEFS campus (12°12'12.1" S, 38°58'11.1" W, and an altitude of 237 meters). A sample of the species was deposited in the institution herbarium (HUEFS), voucher n° 254825.

The soil was collected from the subsurface layer (0 to 20 cm), with known chemical and physical characteristics (Table 1) and a clay loam texture. Thus, there was no mineral fertilization considering the plant hardiness, the levels of mineral elements in the soil, and the base saturation.

**Table 1.** Chemical and physical soil analysis.

Chemical analysis															
In H <sub>2</sub> O		mg dm <sup>-3</sup>							cmol dm <sup>-3</sup>			g.kg <sup>-1</sup>		%	
pH	P	K	S	Fe	Zn	Cu	Mn	B	Ca	Mg	H+Al	O.M.	V		
7.1	56.0	230.0	5.0	104.0	8.4	0.4	26.0	0.3	5.0	1.2	1.3	24.0	84.1		
Physical analysis															
g.kg <sup>-1</sup>															
Coarse sand				Fine sand				Silt				Clay			
586				174				60				180			

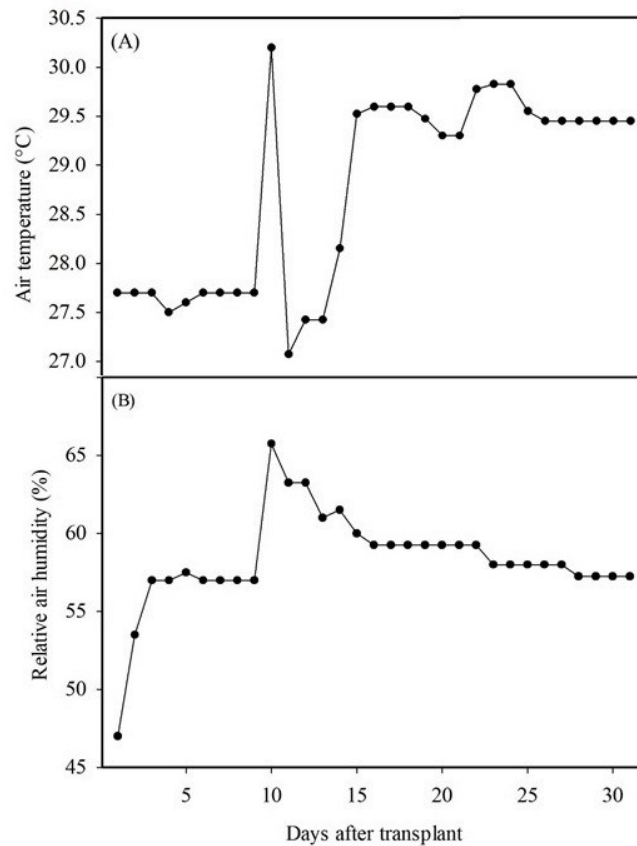
The water availability (WA) conditions applied were 0% (no water replenishment), 20, 40, 60, 80, and 100%, based on the field capacity of the soil in the pot. To determine these treatments, three pots were filled with air-dried soil (known weight), saturated and sealed at the top (PVC), and suspended for drainage. They were weighed again after 24, 48, and 72 hours, and the field capacity of the soil in the pot was obtained from the difference in weight.

The seedlings were grown in 0.2 dm<sup>3</sup> cups, using the same soil used in the pots, and transplanted to their final location after 25 days. The definitive site consisted of individual units (pots) arranged at a spacing of 1.0 m between rows and 0.5 m between plants, with a capacity of 8 dm<sup>3</sup>, containing 8 kg of air-dried soil. After transplanting, the

seedlings were kept at 80% WA for 10 days, and the treatments were applied after this period.

Water was replenished to the WA level of each treatment at 7-day intervals, disregarding the increase in fresh matter mass. This interval was chosen because of preliminary tests, in which it was possible to observe, based on the phenotype, that well-hydrated plants initially began to show signs of dehydration, such as leaf curling after this period.

Data on the microclimate at the experiment site was obtained using a digital thermos-hygrometer positioned inside the room, from which temperature and relative humidity data were collected daily (Figure 1). Evaluations were conducted 21 days after the treatments were applied.



**Figure 1.** Microclimate inside the greenhouse: average air temperature (A) and average relative air humidity (B).

The leaves were collected and dried in a forced air circulation oven at 60 °C until they reached a constant weight. They were then crushed and stored in an airtight container for later determination in the food engineering laboratories at UEFS. The moisture content (M, %) of the dry matter of the leaves was determined in a forced air circulation oven at 105 °C for 30 hours. Ash content (A, %) was determined by incinerating the material in porcelain capsules for 5 hours in a muffle furnace. The lipid content (L, %) was obtained by treating the samples in a mixture of chloroform, methanol, and water in a ratio of 1:2:0.8 (BLIGH; DYER, 1959) and the protein content (P, %) by sequential processes of digestion, distillation, and titration, using a factor of 6.25 to convert nitrogen into protein. The carbohydrate content (CB, %) was obtained by the difference between the respective elements, according to Equation (1) below:

$$CB = [100 - (M + A + L + P)] \quad (1)$$

The relative water content (RWC) of the fully expanded leaves of the middle third of the plants was assessed using five leaf discs with a diameter of 5 mm for each repetition, using the following data: mass of fresh matter (MFM, g), mass of turgid matter (MTM, g), and mass of dry matter (MDM, g). Turgid mass was obtained by placing the disks in a petri dish with distilled water for 5 hours and dry mass by drying in a forced air circulation oven at 60 °C until constant weight, according to Equation (2) below:

$$RWC = [(MFM - MDM) / (MTM - MDM)] \times 100 \quad (2)$$

Leaf succulence (S) was determined based on the mass of fresh and dry matter and leaf area (A, cm<sup>2</sup>), expressed in g H<sub>2</sub>O cm<sup>-2</sup>, according to Equation (3) below:

$$S = [(MFM - MDM) / A] \quad (3)$$

The efficiency of the photosynthetic process was assessed between 9 a.m. and 12 p.m. on fully expanded leaves from the middle third of the plants in duplicates, for 60 seconds for each repetition, using portable IRGA equipment (Infrared Gas Analyzer, PPSystems, Amesbury, USA, model CIRAS-3), photosynthetically active photon flux of 800 μmol m<sup>-2</sup> s<sup>-1</sup>, reference CO<sub>2</sub> 400 μmol mol<sup>-1</sup> and a temperature of 25 °C. We quantified CO<sub>2</sub> assimilation (A) expressed in mmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, stomatal conductance (gs) in mmol H<sub>2</sub>O cm<sup>-2</sup> s<sup>-1</sup>, and transpiration (E) in mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>. Based on this data, water use efficiency (WUE) was estimated in mmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O, according to Equation (4) below:

$$WUE = A/E \quad (4)$$

The biochemical determinations were conducted in triplicates, using fully expanded leaves from the middle third of the plants and extracts obtained by macerating 0.5 g of the mass of fresh leaf matter in porcelain mortar, adding 10 ml of 0.1 M phosphate buffer solution pH 7.0 and then centrifuged at 11,000 rpm for 15 minutes at 4 °C, using the supernatant for the evaluations.

Amino acid content (mg g<sup>-1</sup> FM) was determined, based on the ninhydrin method, using glycine as a standard, with the readings being taken on a spectrophotometer at

$\lambda = 570$  nm (YEMM; COCKING, 1955), total soluble sugars ( $\text{mg g}^{-1}$  MF), using the Anthrone method  $\lambda = 620$  (YEMM; WILLIS, 1954) and reducing sugars ( $\text{mg g}^{-1}$  MF), using the dinitrosalicylic acid (DNS) method,  $\lambda = 540$  nm (MILLER, 1959). The free proline content ( $\text{mmol g}^{-1}$  DM) was also determined,  $\lambda = 520$  nm, using the mass of dry matter (0.1 g), macerated in 10 ml of sulfosalicylic acid (3%), centrifuged for 15 minutes at 11,000 rpm, based on the provisions of Bates, Waldren and Teare (1973).

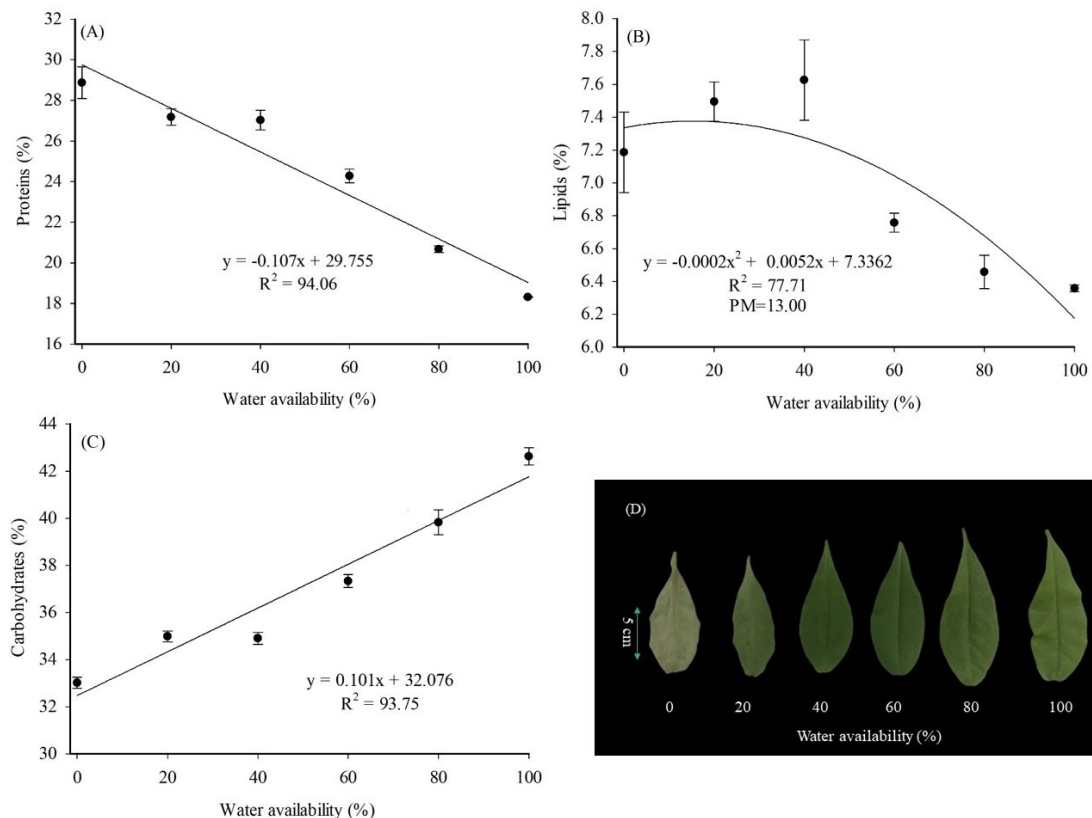
The data obtained in this experiment was subjected to analysis of variance using the F-test, and when a significant difference was found, regression analysis was conducted using the R software to process the data (R DEVELOPMENT CORE TEAM, 2021).

## RESULTS AND DISCUSSION

Significant changes were observed in the allocation of energy content in the dry matter mass of *T. fruticosum* leaves

subjected to water deficit, with protein, lipid, and carbohydrate values showing sensitivity to the level of water availability. There was a significant increase in protein, with a decreasing linear adjustment. Plants grown without water replenishment had the highest protein content (28.87%), while plants grown at maximum WA (Figure 2A) had the lowest protein content (18.31%). Notably, plants grown at WA levels of 20 and 40% also had high protein contents of 27.18% and 27.03%, respectively, the phenotype of which showed greater market potential for the leaves of plants grown at 40% (Figure 2D).

The lipid content showed a quadratic adjustment, with the highest contents seen in the most restrictive treatments 0, 20, and 40% WA, corresponding to 7.19%, 7.49%, and 7.63% respectively (Figure 2B). For carbohydrates, a significant increase was observed in plants irrigated for maximum WA, which showed a carbohydrate content of 42.64%, with a positive linear fit (Figure 2C). There was no significant effect on ash content and moisture of leaf dry matter mass.



**Figure 2.** Centesimal composition of the dry biomass of leaves and leaves of *Talinum fruticosum* grown at different levels of water availability. Proteins (A), lipids (B), carbohydrates (C), and fresh leaves collected for drying and analysis of the centesimal composition of each treatment (D). Values are presented as mean  $\pm$  standard error.

Thus, water restriction in *T. fruticosum* plants altered energy storage, which can be seen in the centesimal composition, protein, and lipid analyses in the most restrictive treatments (Figure 2). The accumulation of organic compounds may contribute to osmotic regulation and protection against oxidative stress during the water restriction period, which explains the increase in centesimal composition. In addition, carbon compounds formed from

$\text{CO}_2$  capture can be converted into stored polysaccharides or oxidized to meet energy demands (MUNNS; MILLAR, 2023).

The increase in protein in *T. fruticosum* leaves under conditions of water deficit highlights the importance of including the species in the food menu, actions aimed at disseminating information in the semi-arid region and encouraging the use of genetic resources, considering that in

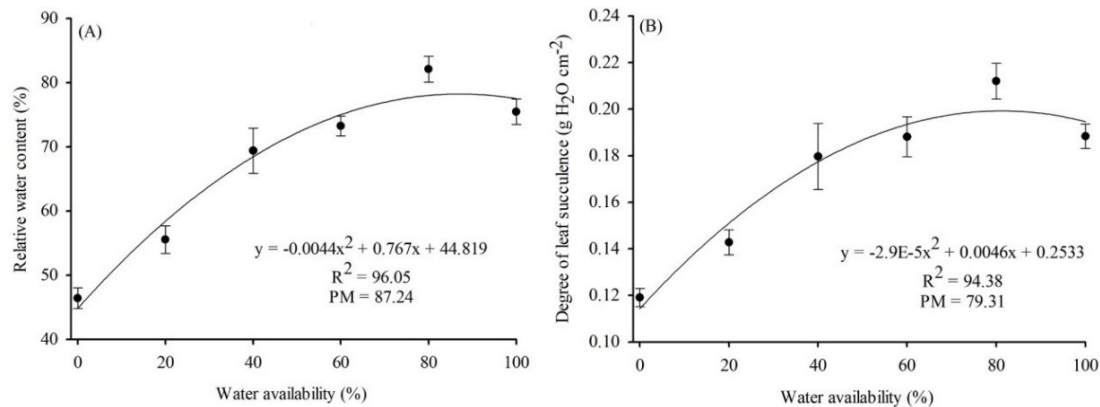


times of drought, the socio economic problems of the population in this region threaten food security. In addition, *T. fruticosum* could be used as animal feed, especially during drought and low forage production.

However, this study found the highest carbohydrate content in plants with the maximum WA. In this sense, it has been observed changes in extracts of the mass of fresh matter of leaves of plants grown under conditions of water deficit, such as the accumulation of soluble and reducing sugars (LEITE et al., 2018), were not reflected in an increase in carbohydrates concerning the plants at low WA, as observed

in our study (Figures 2 and 5).

There was also an influence on the level of hydration of leaf tissues in *T. fruticosum* plants, showing that the plant water status is altered under water deficit conditions. The RWC and the degree of leaf succulence fitted quadratically according to the regression analysis (Figure 3). In this sense, there was an increase in RWC as the WA increased (Figure 3A), with plants irrigated up to 80% of the WA showing the highest RWC of 82.10%. A decrease in RWC with the imposition of water restriction was reported in *T. fruticosum* (PIETERS; TEZARA; HERRERA, 2003).



**Figure 3.** Water relations in *Talinum fruticosum* plants grown at different levels of water availability. Relative water content (A) and degree of leaf succulence (B). Values are presented as mean  $\pm$  standard error.

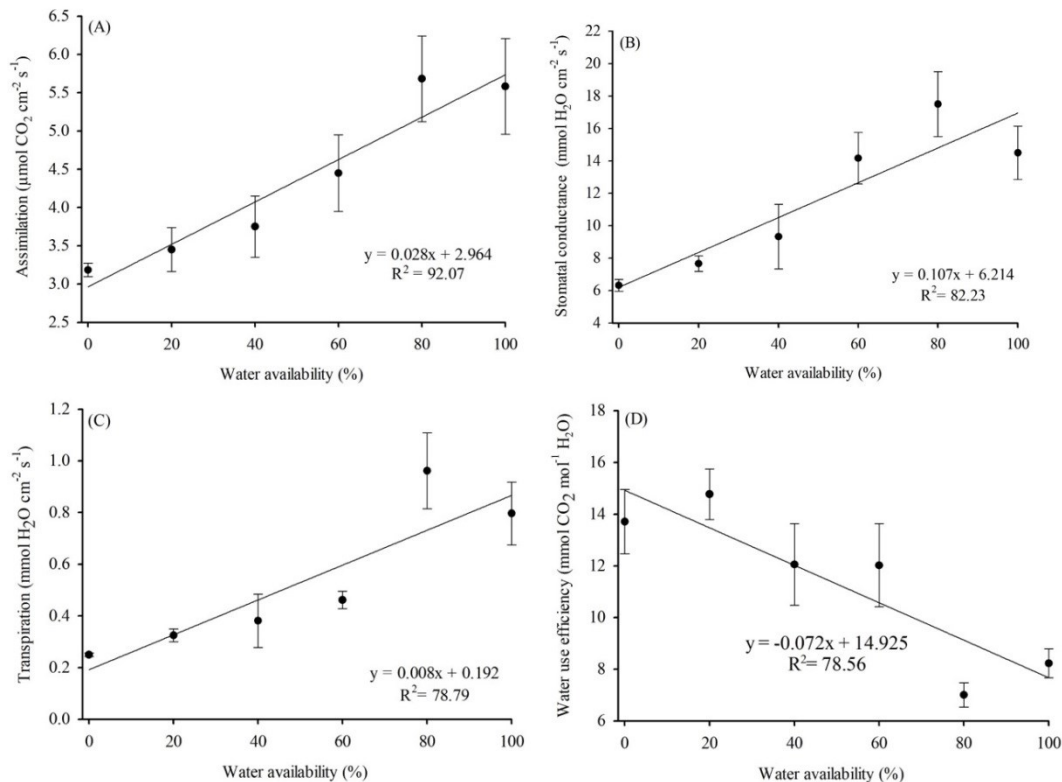
In addition, there was an increase in the degree of leaf succulence for the treatments that received the highest water content (Figure 3B), in which the plants grown at 80% of WA showed the highest degree of leaf succulence ( $0.212 \text{ g H}_2\text{O cm}^{-2}$ ). This strategy, characterized by the allocation of water in living tissues, is used by plants to make this resource available during periods of low WA (GRIFFITHS; MALES, 2017). In addition, the chemical and physical properties of water justify the allocation of the resource, as water has a high specific heat, high latent heat of vaporization, and high thermal conductivity; these aspects allow water to absorb a high amount of energy without a significant increase in temperature and also contribute to cooling the leaves in the transpiration process. These properties can be explained by the high efficiency of water use under water deficit conditions.

Herrera, Ballestrini and Montes (2015), working with *T. fruticosum*, observed water accumulation in the leaves under growing conditions of 30% WA. Although succulence declined under conditions of soil water limitation after the treatments were imposed, the survival of the experimental units until the end of the experiment was influenced by the species' ability, albeit to a lesser extent, to store water in the tissues, as well as possible physiological changes from carbon capture ( $C_3$ ) to the Crassulacean acid metabolism (CAM facultative).

There was significant interference from water deficit on gas exchange and consequently on the species' performance (Figure 4). There was a positive linear

adjustment for  $\text{CO}_2$  assimilation (Figure 4A), stomatal conductance (Figure 4B), and transpiration (Figure 4C), which increased with increasing WA. On the other hand, water use efficiency showed a negative linear adjustment, with a decrease for the treatments that received the highest water content (Figure 4D). Thus, it was found that plants at 80% WA performed better in terms of  $\text{CO}_2$  assimilation ( $5.6833 \text{ mmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $17.50 \text{ mmol H}_2\text{O cm}^{-2} \text{ s}^{-1}$ ), and transpiration ( $0.9617 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-2}$ ).

The maintenance of gas exchange under conditions of water deficit demonstrates the plant plasticity in providing adjustments because *T. fruticosum* is a  $C_3$  plant and can present the Crassulacean acid metabolism (CAM facultative) when subjected to stress conditions, in which it also presents a reduction in  $\text{CO}_2$  assimilation (HERRERA; DELGADO; PARAGUATEY, 1991). In this study, the plants grown in WA above 60% showed higher values. It is suggested that the increase in water restriction-induced the accumulation of organic acids, probably in the vacuole of the plant cell, in response to the capture of carbon during the night by the enzyme phosphoenolpyruvate carboxylase. During the day, malate was decarboxylated in the cytoplasm and used as a substrate by ribulose 1.5 bisphosphate carboxylase oxygenase in the  $C_3$  photosynthetic pathway, supported by an analysis by Herrera (2009). Changes in gas exchange patterns have been reported in species of this genus under water deficit conditions, as observed in *T. paniculatum* (Jacq.) Gaertn. (GUERERE et al., 1996).



**Figure 4.** Gas exchange in *Talinum fruticosum* grown at different levels of water availability. CO<sub>2</sub> assimilation (A), stomatal conductance (B), transpiration (C), and water use efficiency (D). Values are presented as mean ± standard error.

Changes in gas exchange affected by abiotic factors are reported in different species (LEITE et al., 2018; SOUZA et al., 2020; SILVA et al., 2020). However, in CAM metabolism species, the impacts of WA on photosynthesis are complex, involving strategies to save water and gain carbon (HERRERA, 2009). According to Yang et al. (2015), using species with CAM metabolism can help agricultural expansion in semi-arid regions and reduce yield losses.

Plants at 20% WA showed the highest water use efficiency (14.77 mmol CO<sub>2</sub> mmol<sup>-1</sup> H<sub>2</sub>O) (Figure 4D). Herrera, Ballestrini and Montes (2015) also reported an increase in the WUE of the species under water restriction conditions. This strategy is used mainly by species capable of establishing themselves in regions with low rainfall, allowing plants to be maintained over time and space.

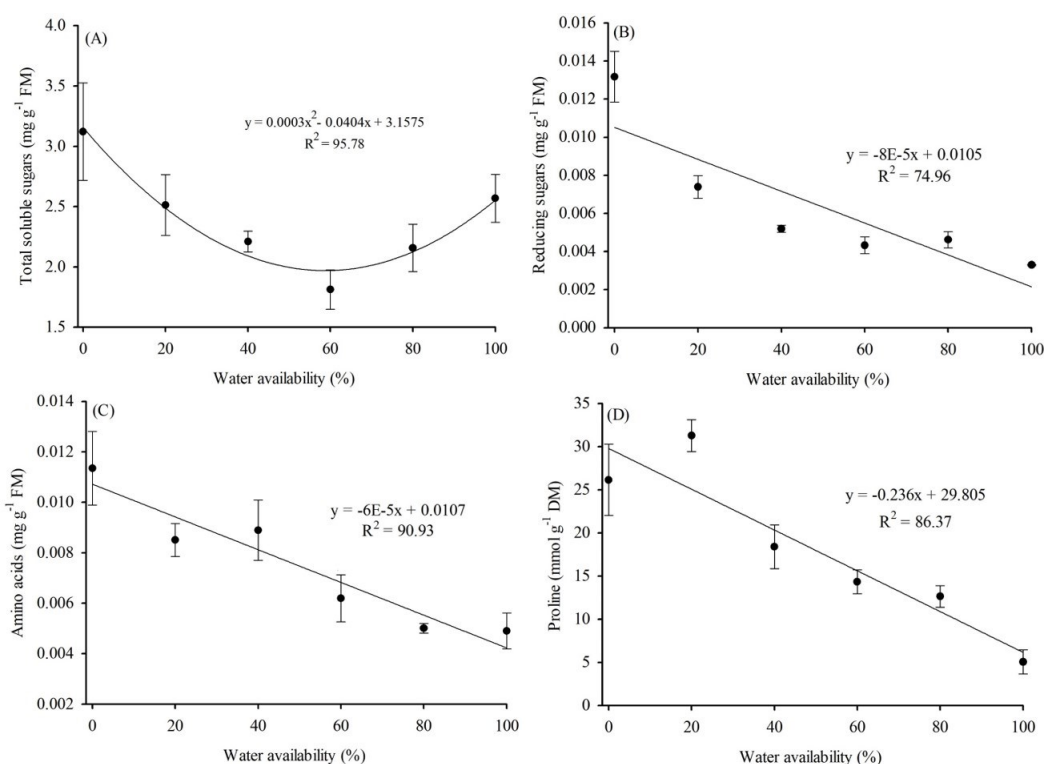
In short, the impact of water deficit on the photosynthetic performance of *T. fruticosum* is complex and dependent on the soil water conditions, and the species plasticity is a fundamental mechanism for overcoming temporary periods of low WA. Establishing a condition of tolerance to water deficit was observed, with the strategy of increasing efficiency in using the water resource as the WA in the soil became increasingly limiting.

Significant metabolic adjustments enabled the species to maintain itself in the unfavorable conditions imposed by the low WA in the soil. Quadratic performance was observed for the analysis of soluble sugars and linear for reducing sugars, amino acids, and free proline (Figure 5).

The accumulation of soluble sugars showed quadratic performance, in which it was observed that low WA in the

soil resulted in a greater increase (3.1 mg g<sup>-1</sup> FM) for plants grown without water replacement (Figure 5A). On the other hand, the content of reducing sugars showed a negative linear adjustment, with a decrease as the WA increased (Figure 5B). In this sense, plants without water replacement had a higher content (0.013 mg g<sup>-1</sup> FM). The increase in the content of total soluble sugars was also observed in genotypes of *Jatropha curcas* L. (CAM facultative) under water deficit conditions (SILVA et al., 2019). These mechanisms align with the study under water deficit conditions (LEITE et al., 2018). In addition, the accumulation of organic solutes enables tolerance to abiotic stress (MA et al., 2017).

Other biomolecules also performed significantly and may have contributed to maintaining the leaf RWC at a level that enabled the plants to survive the water deficit. This accumulation was pronounced in plants grown without water replacement, where the content of amino acids was reduced with increasing levels of WA (Figure 5C). Amino acids contribute to osmoregulation and are fundamental units for protein formation. Proline also decreased as WA increased, with the highest content observed for plants whose WA was 20% (31.29 mmol g<sup>-1</sup> DM) (Figure 5D). An increase in this biomolecule was also observed in bean seedlings grown under water deficit conditions (ARAÚJO et al., 2021). In conditions of low WA, it is common for proteases to intensify the degradation of proteins, increasing the content of amino acids (HUANG; JANDER, 2017). Amino acids, in turn, can also be degraded and used in metabolic processes (HILDEBRANDT et al., 2015).



**Figure 5.** Accumulation of compatible solutes in leaves of *Talinum fruticosum*, grown at different levels of water availability. Total soluble sugars (A), reducing sugars (B), amino acids (C), and proline (D). Values are presented as mean  $\pm$  standard error.

From this perspective, the water deficit in *T. fruticosum* plants resulted in decreased gas exchange, increased water use efficiency, and accumulated organic solutes, which may have contributed to maintaining the plant's water status.

## CONCLUSIONS

Water deficit changes the energy content of the dry biomass of *T. fruticosum* leaves, and irrigation at 40% of water availability can be used strategically to increase protein.

Water restriction reduces gas exchange in *T. fruticosum*, limiting carbon gain, increasing water use efficiency and biomolecule accumulation.

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