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Artigos

Growth and metabolism of *Pityrocarpa moniliformis* Benth. seedlings under water deficit

Crescimento e metabolismo de mudas de *Pityrocarpa moniliformis* Benth. sob *deficit* hídrico

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ABSTRACT

Pityrocarpa moniliformis Benth. has medicinal properties, forage potential, besides showing rusticity and rapid growth, which confer potential to recover degraded areas. In this context, the objective was to evaluate the growth and biochemical components of *Pityrocarpa moniliformis* seedlings under water deficit conditions. The design used was randomized blocks, with five treatments and four replicates, with the experimental plot consisting of twenty plants. Treatments were characterized by different periods of water deficit (0; 4; 8; 12 and 16 days without irrigation). At 44 days after sowing (DAS), when the seedlings had two pairs of fully formed true leaves, the treatments began to be applied. The development of the seedlings was evaluated until 60 DAS when they were collected for biometric and biochemical analyses. The variables analyzed were shoot height; collar diameter; number of leaves; shoot dry mass; root dry mass; root/shoot ratio; and Dickson's quality index. Contents of total chlorophyll, chlorophyll *a*, chlorophyll *b*, total free amino acids, total soluble sugar, and proline contents in the leaves were also determined. The treatment most affected by the lack of irrigation was 16 days of water deficit, which resulted in the death of 38.8% of the seedlings. This condition caused a decrease in shoot length, reducing it by approximately 29.2% compared to the control treatment. There was also a reduction in the production of new leaves from the eighth day after the differentiation of treatments. *Pityrocarpa moniliformis* seedlings can develop under the condition of water deficit for up to 8 days, even with chlorophyll degradation due to stress. *Pityrocarpa moniliformis* maintains its vegetative development by performing osmotic adjustment through the accumulation of biomolecules (sugars, proline, and amino acids).

Keywords: Water stress; Fabaceae; Seedling production; Catanduva

RESUMO

Pityrocarpa moniliformis Benth. possui propriedades medicinais, potencial forrageiro, além de apresentar rusticidade e rápido crescimento, o que lhe confere potencialidade de uso para recuperação de áreas degradadas. Nesse contexto, objetivou-se avaliar o crescimento e os componentes bioquímicos de mudas de *Pityrocarpa moniliformis* em condições de *deficit* hídrico. O delineamento foi em blocos casualizados, com cinco tratamentos e quatro repetições, sendo a parcela experimental composta por vinte plantas. Os tratamentos foram caracterizados por diferentes períodos de *deficit* hídrico (0; 4; 8; 12 e 16 dias sem irrigação). Aos 44 dias após a semeadura (DAS), quando as mudas apresentaram dois pares de folhas verdadeiras totalmente formadas, iniciou-se a aplicação dos tratamentos. O desenvolvimento das mudas foi avaliado até os 60 DAS, período em que ocorreu a coleta destas para as análises biométricas e bioquímicas. As variáveis analisadas foram: altura da parte aérea; diâmetro do colo; número de folhas; massa seca de parte aérea e raiz; relação entre raiz e parte aérea; e índice de qualidade de Dickson. Também foram determinados nas folhas os teores de clorofilas totais, *a* e *b*; aminoácidos livres totais; teor de açúcares solúveis totais; e teor de prolina. O tratamento mais afetado pela falta de irrigação foi o de 16 dias, o qual acarretou a morte de 38,8% das mudas. Essa condição ocasionou a diminuição no comprimento da parte aérea das mudas, com redução de aproximadamente 29,2%, quando comparado ao tratamento-controle. Houve também redução da emissão de novas folhas a partir do oitavo dia após a diferenciação dos tratamentos. Mudas de *Pityrocarpa moniliformis* conseguem se desenvolver em condição de *deficit* hídrico por até 8 dias, mesmo ocorrendo a degradação de clorofilas devido ao estresse. A manutenção do desenvolvimento vegetativo de *Pityrocarpa moniliformis* ocorre devido à realização de ajustamento osmótico pelo acúmulo de biomoléculas (açúcares, prolina e aminoácidos).

Palavras-chave: Estresse hídrico; Fabaceae; Produção de mudas; Catanduva

1 INTRODUCTION

Pityrocarpa moniliformis Benth., known in Portuguese as ‘catanduva’, has timber, forage, and beekeeping potential, besides being a rustic and fast-growing species (PEREIRA *et al*., 2016). Therefore, the production of seedlings of native species with these characteristics has been increasingly valued for use in reforestation projects and recovery of degraded areas (FELIX *et al*., 2020; MEDEIROS *et al., 2020*).

Economic growth in the northeastern semi-arid region of Brazil is mainly based on the exploitation of natural resources, which has led to the degradation of ecosystems (GONG *et al*., 2016). This process is intensified by climate change, which can cause severe damage to plants, especially those related to abiotic stresses, which interfere with plant development (GOMES *et al*., 2020; RIBEIRO *et al.,* 2021).

Reduction in the amount of water in the soil decreases water potential, which hampers absorption by the roots, causing plant cells to further decrease their water potential, so that they can maintain turgor and metabolism (LUM *et al*., 2014; TAIZ *et al*., 2017). Osmotic adjustment is the primary plant defense mechanism capable of reducing cell water potential. It is performed by accumulating biomolecules (sugars, amino acids, proline, etc.) in the cytosol without intoxicating the cell (BUTT *et al*., 2017). Thus, the cell water balance will allow the plant to absorb the water needed for its development.

Knowledge of the level of water stress tolerance on the development of native forest species is fundamental for seedling production (BUENO *et al*., 2020). No reports were found about the eco-physiological responses of *Pityrocarpa moniliformis* subjected to water deficit.

In this context, the objective was to evaluate the tolerance of *Pityrocarpa moniliformis* seedlings in situations of water deficit, as well as the physiological and biochemical changes caused by this stress.

2 MATERIAL AND METHODS

The experiment was conducted in a seedling nursery at the Federal University of Rio Grande do Norte (UFRN), in the Academic Unit Specialized in Agrarian Sciences, Jundiaí, Macaíba, RN, Brazil (5°53’09.77” S, 35°21’52.47” W and 15 m altitude). *Pityrocarpa moniliformis* seeds were collected from parent trees existing at the Rafael Fernandes Experimental Farm, belonging to the Federal Rural University of the Semi-Arid Region (UFERSA), Mossoró, RN, Brazil (5°11’S, 37°20” W and 18 m altitude).

The initial quality of the seeds was evaluated by the germination test with four replicates of 50 seeds. Pruning scissors were used to cut off the part of the seed coat opposite to the hilum aiming to overcome seed dormancy. Subsequently, sterilization was performed with sodium hypochlorite solution (10% concentration), followed by washing in running water. The substrate consisted of Germitest paper moistened with water in the proportion of 2.5 times its dry weight. After sowing, the seeds were placed in a biochemical oxygen demand (B.O.D.) germination chamber at 25 °C and counted at 7 and 21 days after sowing (BRASIL, 2013). After evaluation of the initial quality, the experiment was installed.

The experiment used randomized complete blocks (RCB) design, formed by five treatments, with four replicates, and the experimental plot consisted of twenty plants. The treatments consisted of periods of water deficit (0; 4; 8; 12 and 16 days without irrigation).

Sowing was performed directly in polyethylene bags (1.2 L), with one seed per container. The substrate consisted of soil collected in the experimental area (Table 1). During the first 44 days after sowing (DAS), irrigation was performed once a day equally for all plots. The supply of 200 mL of water per seedling daily was sufficient for saturating the substrate. At 44 DAS, when the seedlings had two pairs of fully formed true leaves, the treatments began to be applied. Despite the size of the seedlings, this period was determined from pre-tests performed, in which they managed to establish themselves at 60 days.

Table 1 – Chemical analysis of the soil used in the production of *Pityrocarpa moniliformis* Benth. seedlings

Sample	N	pH	OM	P	K^+	Na^+	Ca^{2+}	Mg^{2+}	Al^{3+}	(H+Al)	SB	V	ESP
	g/kg	(water)	g/kg	mg/dm³			cmolc/dm³					%	
Substrate	0.42	7.1	-	0.7	0.11	0.02	1.1	0.4	0	0	1.63	100	2

Source: Soil Fertility and Plant Nutrition Laboratory - UFERSA. DCAT/UFERSA (2019)

In where: pH = hydrogen potential; OM = organic matter; ESP = exchangeable sodium percentage.

The development of the seedlings was evaluated until 60 DAS when they were collected for biometric and biochemical analyses. For this, six central plants were collected per replicate and subjected to the following evaluations: shoot height - measured with a ruler graduated in millimeters; collar diameter - obtained with a digital caliper; number of leaves - obtained by directly counting the number of leaves per plant; shoot dry mass, root dry mass and root/shoot ratio - the plant materials were placed in paper bags, dried in a forced ventilation oven at 65 ºC for 72 h and weighed on an analytical precision scale (0.0001 g); and Dickson quality index - determined according to the equation proposed by Dickson, Leaf and Hosner (1960).

For biochemical analyses, shoot tissues were collected, placed in plastic bags, and stored in a freezer for subsequent analysis: contents of total chlorophyll, chlorophyll *a,* and chlorophyll *b* - performed by the spectrophotometric method; total free amino acids - performed by the method of Peoples et al. (1989), using the ninhydrin reagent; content of total soluble sugars - determined by the method of Yemm and Willis (1954); and proline content - determined by following the methodology proposed by Bates Waldren and Teare (1973).

The results were submitted to analysis of variance and regression by F test at 5% probability level after confirming the normal distribution of residuals by the Shapiro-Wilk test. For all statistical analyses, the System for Analysis of Variance software - SISVAR (FERREIRA, 2011) was used.

3 RESULTS AND DISCUSSION

The biometric variables were affected by water deficit (Table 2). Treatments with 8 and 12 days of water deficit caused deaths of 5 and 6.3% of *Pityrocarpa moniliformis* seedlings; however, the most affected treatment was 16 days, which resulted in the death of 38.8% of the seedlings. For the other treatments, the seedlings did not suffer from water deficit and showed a good appearance until the end of the experiment.

Treatments with higher levels of water deficit resulted in a considerable decrease in shoot length (Figure 1A). There was a reduction from 4 days without irrigation, becoming more intense at 16 days, which contributed to a reduction of approximately 29.2% compared to the control treatment. Similar results were obtained by Scalon *et al.* (2011) when evaluating the initial growth of *Guazuma ulmifolia* Lam. seedlings. These authors found that shoot growth was reduced by 50% at the lowest water availability compared to the other treatments. Reduction in shoot growth occurs due to the decrease in the amount of water available in the shoots, which affects turgor pressure, decreasing cell elongation (IVANOV *et al*., 2019).

Table 2 – Mean squares for shoot length (SL), number of leaves (NL), collar diameter (CD), total dry mass (TDM), root/shoot ratio (R/S), and Dickson quality index (DQI) of *Pityrocarpa moniliformis* Benth. seedlings subjected to water deficit

SV	DF	Mean squares					
		SL	NL	CD	TDM	R/S	DQI
Treatment	4	0.767**	24.690**	0.060**	1.644**	34.596*	0.069**
Error	15	0.09	2.297	0.004	0.179	5.146	0.009
CV(%)	-	9.67	21.11	7.11	16.73	17.15	15.49

Source: Authors (2020)

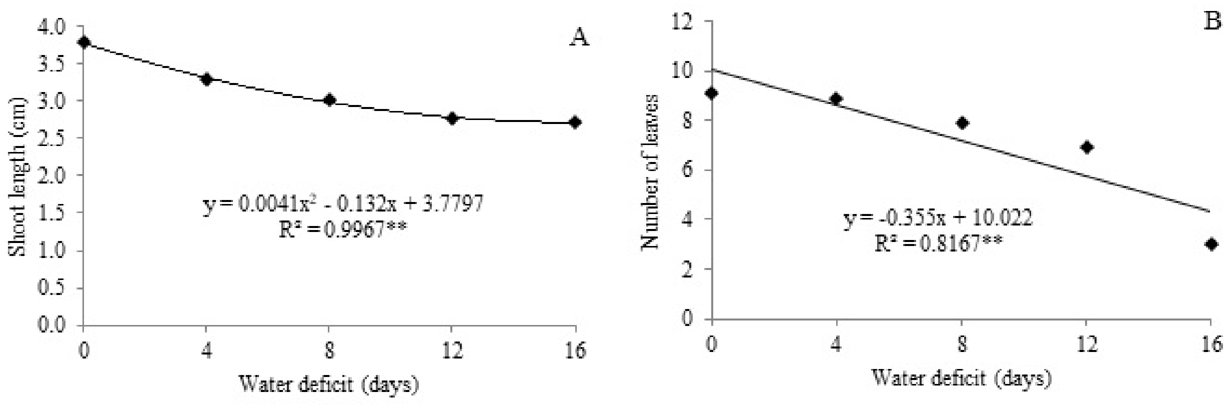
In where: SV = source of variation; DF = degrees of freedom; CV = coefficient of variation; *, **; Significant effect at 5% and 1% probability levels, respectively.

A study conducted by Oliveira et al. (2016) found that water deficit in *Erythrina velutina* Willd plants resulted in a 32% reduction in shoot growth compared to those that did not undergo water deficit. Usually, when plants are subjected to lower water availability, their photosynthetic activities are compromised, causing low production of photoassimilates, which are necessary to maintain growth (BUTT *et al.,* 2017).

Concerning the number of leaves (Figure 1B), water deficit led to a reduction in the production of new leaves from the eighth day after the differentiation of treatments. However, the control treatment had the most significant value for this variable, with a 56.7% higher production than seedlings under a higher water deficit.

Reduction in the number of leaves is a physiological strategy of plants to survive under a condition of greater water deficit because the plant reduces the transpiration area of its tissues (ILYAS *et al*., 2020). On the other hand, a reduction in the number of leaves directly interferes with the photosynthetic capacity of plants, causing lower biomass accumulation (BELTRAMIN *et al*., 2020). The effect of water deficit also reduced the number of leaves in *Poincianella pyramidalis* Tul. seedlings (LEITE *et al.,* 2020).

Figure 1 – Shoot length (A) and number of leaves (B) of *Pityrocarpa moniliformis* Benth. seedlings subjected to water deficit



Source: Authors (2020)

In collar diameter, there was a linear reduction as the period of water deficit increased and, at the most drastic level (16 days), the reduction was equal to 25.8%, compared to seedlings under continuous irrigation (control) (Figure 2A). Seedlings subjected to water stress may exhibit anatomical and morphological changes, such as reduced stem diameter; in response to the lack of water, turgor pressure is reduced—consequently, the sap flow through conducting vessels decreases (BHUSAL; HAN; YOON, 2019).

The total dry mass of *Pityrocarpa moniliformis* seedlings was inversely proportional to the days of water deficit; the longer the period without irrigation, the lower the dry mass accumulation (Figure 2B). Thus, the control treatment showed a higher value than the other treatments, equal to 3.15 g. The treatments with 12 and 16 days of restriction resulted in lower values, 2.03 and 1.66 g, respectively, causing reductions on the order of 35.6 and 47.3% compared to the control. This result is related to the decrease in the number of leaves, which is a direct response of the plant to the water scarcity, verified in the present study. Conversely, under adequate soil water conditions, the plant can increase biomass accumulation through photosynthesis, a result evidenced by Leite et al. (2020) when studying this variable in *Poincianella pyramidalis* seedlings.

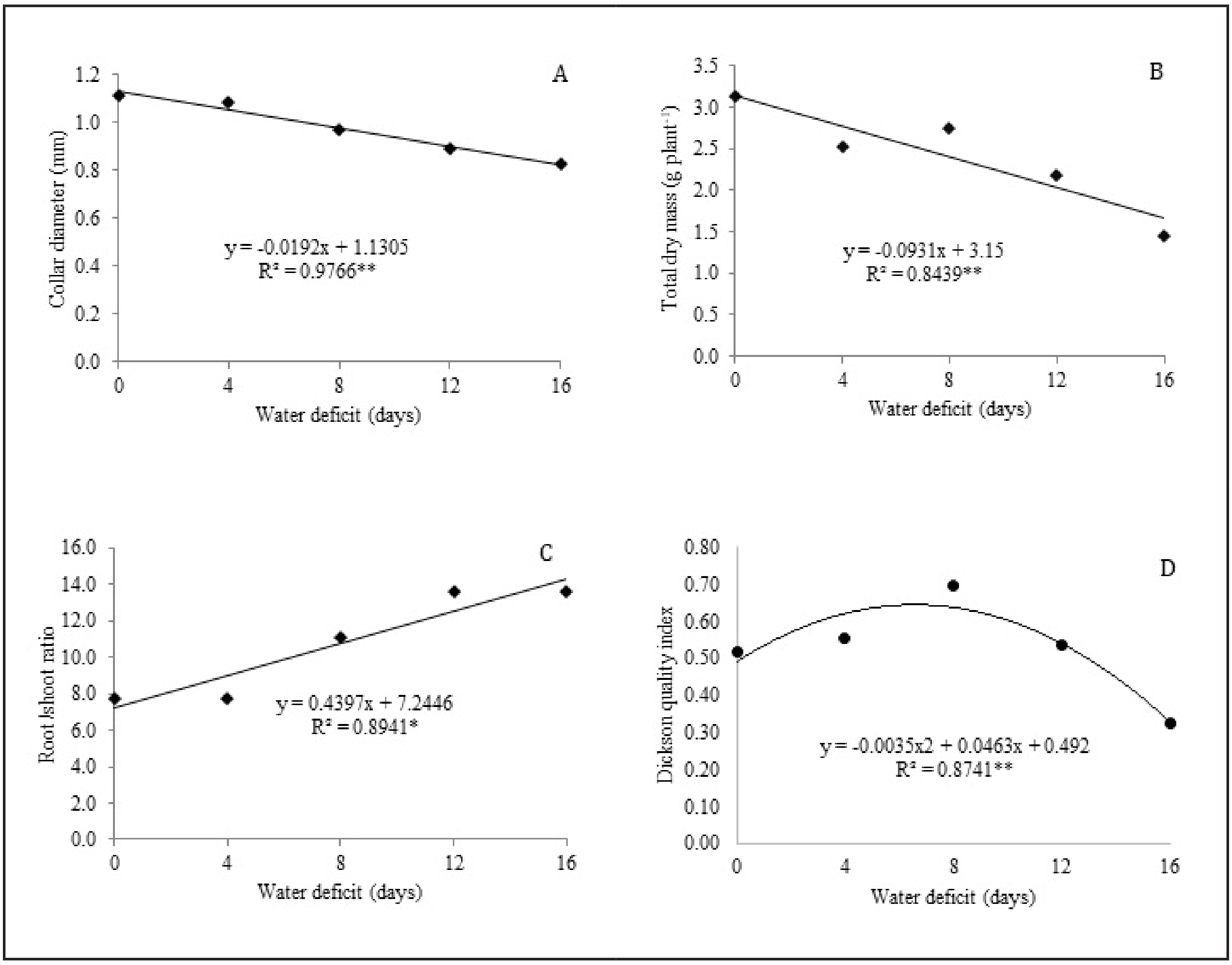
The root/shoot ratio showed an increasing linear trend, in which seedlings under longer periods of water deficit (12 and 16 days) obtained better results (Figure 2C). This behavior is related to the maintenance strategy because the translocation of a greater amount of photoassimilates to maintain the root system in plants that are under an adverse condition of water availability allows for the elongation of the roots, which increases the area of exploration and water absorption (BUTT *et al*., 2017).

This morphological strategy of the plants was observed in *Guazuma ulmifolia* seedlings, in which the root/shoot ratio was higher in the treatment with water availability corresponding to 25% field capacity, until 35 days, maximum tolerance limit of the species (SCALON *et al.,* 2011). In general, water stress causes changes in the morphology and metabolism of species that use mechanisms for their maintenance.

Regarding the Dickson quality index (DQI), the best result for *Pityrocarpa moniliformis* seedlings was obtained with 8 days of water deficit (Figure 2D). It was also observed that there was a reduction in this variable with the increase in the number of days without water. This response may be related to the accumulation of dry mass obtained at 8 days of deficit and the most significant root growth, expressed in the root/shoot ratio, directly influencing the DQI. It is noteworthy that the control treatment obtained a value lower than the maximum point of the curve.

According to Morais et al. (2012), the Dickson quality index allowed them to distinguish the most efficient treatments to produce *Schinus terebinthifolius* seedlings. However, the best result was obtained when the daily irrigation depth of 12 mm was used. These authors observed that the treatment that provided a greater amount of water daily led to lower results compared to the others, except for the one that used an 8 mm irrigation depth. Furthermore, according to the authors, this may be related to nutrient leaching. In the present work, the seedlings under water stress for 8 days performed metabolic changes in growth by accumulating dry mass until this period, with more severe damage in longer periods.

Figure 2 – Collar diameter (A), total dry mass (B) and root/shoot ratio (C), and Dickson quality index (D) of *Pityrocarpa moniliformis* Benth. seedlings subjected to water deficit



Source: Authors (2020)

All biochemical variables were also affected by water deficit, except for chlorophyll *a* (Table 3).

Regarding total sugars, the highest accumulation was observed between 12 and 16 days of water deficit, which led to accumulations of 53.1 and 70.8%, respectively, compared to the control treatment (Figure 3A). Increases in soluble sugars were also observed in *Myracrodruon urundeuva* Allemão when the seedlings were subjected to higher levels of water deficit (COSTA *et al*., 2015). The accumulation of soluble sugars is justified as a plant survival strategy to maintain a favorable internal water potential (BUTT *et al*., 2017).

Table 3 – Mean squares for total soluble sugars (TSS), total free amino acids (TFAA), proline (PRO) chlorophyll *a* (CHL a), chlorophyll *b* (CHL b), total chlorophyll (CHL total) of *Pityrocarpa moniliformis* Benth. seedlings subjected to water deficit

SV	DF	Mean squares					
		TSS	TFAA	PRO	CHL a	CHL b	CHL total
Treatment	4	35.2**	11.2 **	42.578 **	50.7 ^{ns}	134.33**	125.41**
Error	10	1.916	162.345	1.647	24.7	7.69	9.038
CV (%)	-	10.08	15.14	30.1	0.95	8.57	4.77

Source: Authors (2020)

In where: SV = source of variation; DF = degrees of freedom; CV = coefficient of variation; **, ^{ns}; significant at 1% probability level and not significant at 5% probability level, respectively.

About the total amino acids content (Figure 3B), there was a linear increase as the water deficit of the seedlings increased, and the most stressful treatment enabled an increase of 1.4 times in the quantity of total amino acids compared to the control. According to Yang *et al.* (2019), this may be related to the acceleration of protein degradation or the retardation of protein synthesis. During the stress period, some nitrogen metabolites tend to increase in plant tissues, and nitrogen assimilation is reduced under this condition. Thus, the plant with this mechanism can reuse amino acids to adapt to the adversities.

As observed for the concentration of total sugars, it was found that the water deficit also favored the increase of proline, as plants in the treatment with the highest level of stress (16 days without irrigation) were able to express a quantity of proline 32 times higher than that found in the control treatment (Figure 3C). The accumulation of this biomolecule was also verified in a situation of salt stress for the same species (FERREIRA *et al*., 2021).

Proline is an amino acid that constitutes the protein of the cell wall of plants. Its function is to contribute to osmoregulation processes, maintenance of membrane stability, and degradation of free radicals (LUM *et al.,* 2014). Thus, it is primarily linked to the response to various abiotic stresses, especially water deficit (MOURA *et al*., 2016).

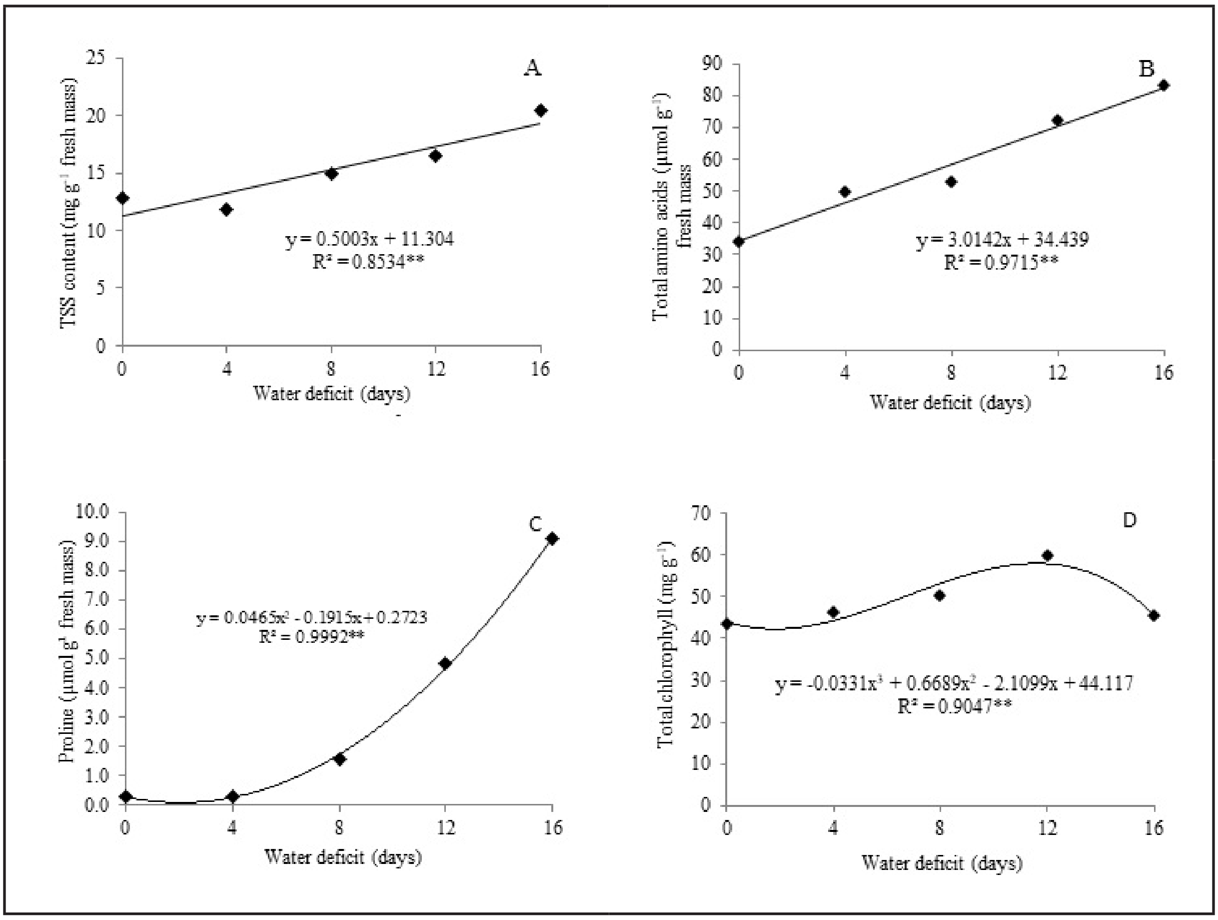
In addition, the proline levels in cells also have a significant effect on gas exchange and the activities of enzymes involved, as antioxidants, besides helping to maintain turgor pressure (BUTT *et al*., 2017).

Regarding total chlorophyll, there was an increase as a function of the periods of water deficit, with the highest results obtained for seedlings under 12 days of water deficit (Figure 3D). Chlorophylls are photosynthetic pigments located in chloroplasts with an unstable chemical structure, hence being easily degraded, leading to cell dysfunction or death (SHARMA *et al*., 2012). The degradation of chlorophylls generates smaller metabolites that can be used in osmotic adjustment. As a prevention against this degradation, antioxidant systems protect chlorophylls and other photosynthesizing pigments (SANDMANN, 2019). Typically, plants respond to water deficit by reducing chlorophyll content (OLIVEIRA *et al.,* 2014). However, some species, such as ornamental pineapple, increase the chlorophyll content in a stress situation, which is associated with the mechanism of protection of the photosynthetic apparatus (MENDES *et al.,* 2011).

It can be verified that seedlings of *Pityrocarpa moniliformis* tolerated water deficit for 8 days under the studied conditions. The plants used mechanisms of adaptation to stress through the development of the root system, leaf loss, and osmotic adjustment. Also, the seedlings performed the accumulation of sugars, proline, and free amino acids. However, shoot growth is hindered, as is the maintenance of chlorophylls.

Although there is good development of the seedlings of *Pityrocarpa moniliformis* up to 8 days under water deficit, in periods after this, the establishment is significantly affected. However, it is possible to produce these seedlings under conditions of intermittent irrigation, with a smaller water deficit interval. In addition, the use of this water management to produce seedlings can save resources, consequently improving the production cost of this species. However, future studies will be needed to prove these hypotheses.

Figure 3 – Total soluble sugars (TSS) (A), total amino acids (B), proline (C), and total chlorophyll (D) of *Pityrocarpa moniliformis* Benth. seedlings subjected to water deficit



Source: Authors (2020)

4 CONCLUSIONS

The growth of *Pityrocarpa moniliformis* seedlings is impaired with the period of water deficit.

Pityrocarpa moniliformis seedlings can develop under water deficit for up to 8 days, despite slowing their growth rate and the occurrence of chlorophyll degradation due to stress.

Pityrocarpa moniliformis maintains its vegetative development by performing osmotic adjustment through the accumulation of biomolecules (sugars, proline, and amino acids).

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