

## **GROWTH AND PHYSIOLOGY OF THREE FORAGE CACTUS GENOTYPES** INTERCROPPED WITH LEGUMES AND IRRIGATED WITH REUSE WATER

CRESCIMENTO E FISIOLOGIA DE TRÊS GENÓTIPOS DE PALMA FORRAGEIRA CONSORCIADOS COM LEGUMINOSAS E IRRIGADOS COM ÁGUA DE REÚSO

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Abstract: Forage palm stands out as a food base for ruminant herds, especially in periods of drought, common in the semiarid region, its cultivation intercropped with legumes and irrigated with reuse water can bring both quantitative and qualitative gains. In this sense, the objective was to evaluate the growth, physiology and productivity of cladodes of forage cactus irrigated with reuse water and intercropped with legumes. The trial design consisted of a randomized block with subdivided plots, with three palm varieties in each plot: Miúda, Baiana and Orelha de Elefante Mexicana and in the subplots, four legumes: algaroba, sabiá, cunhã and gliricídia, besides one control treatment (without consortium). The variables plant height and width, number of cladodes per plant, stomatal conductance, transpiration rate CO<sub>2</sub> uptake rate, internal CO<sub>2</sub> concentration, instantaneous water use efficiency and instant carboxylation efficiency were determined in the forage palm. The variety Miuda was higher in height, plant width and number of cladodes per plant. When not intercropped, both varieties of forage cactus presented higher levels of transpiration rate. The intercropping with algaroba, gliricidia and thrush increased CO<sub>2</sub> uptake, instantaneous water use efficiency and carboxylation of forage palm, regardless of variety.

Keywords: Opuntia ssp., Legumes, Photosynthesis, Irrigation, Water Treatment

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**Resumo:** A palma forrageira se destaca como base alimentar de rebanhos ruminantes, principalmente em períodos de estiagem, comum no Semiárido. O seu cultivo consorciado com leguminosas e irrigado com água de reúso pode trazer ganhos quantitativos e qualitativos. Nesse sentido, objetivou-se avaliar o crescimento, fisiologia e produtividade de cladódios de palma forrageira irrigados com água de reúso e consorciados com leguminosas. O delineamento experimental consistiu em blocos casualizados com parcelas subdivididas, com três variedades de palmeiras em cada parcela: Miúda, Baiana e Orelha de Elefante Mexicana e nas subparcelas, quatro leguminosas: algaroba, sabiá, cunhã e gliricídia e sabiá, Além de um tratamento testemunha (sem consórcio). As variáveis altura e largura da planta, número de cladódios por planta, condutância estomática, taxa de transpiração, taxa de absorção de CO<sub>2</sub>, concentração interna de CO<sub>2</sub>, eficiência instantânea do uso da água e eficiência instantânea da carboxilação foram determinadas na palma forrageira. A variedade Miuda foi maior em altura, largura de planta e número de cladódios por planta. Quando não consorciadas, ambas as variedades de palma forrageira apresentaram maiores taxas de transpiração. O consórcio com algaroba, gliricídia e sabiá aumentou a absorção de CO<sub>2</sub>, a eficiência instantânea do uso da água e a carboxilação da palma forrageira, independente da variedade.

Palavras-chave: Opuntia ssp., Leguminosas, Fotossíntese, Irrigação, Tratamento de Água

## Introduction

Forage cactus is indispensable for the food maintenance of semi-arid ruminant herds, especially in periods of drought. Its abundance in energy, water and minerals, especially calcium, make this cactus the main xerophilic crop grown in Brazil. This plant is very versatile, as it can produce high forage indices regardless of the system and management used <sup>(1; 2)</sup>.

The use of intercropping crops is a practice widely used worldwide, having as one of the main advantages the high efficiency in land use, an indispensable situation in the semiarid region of Brazil, where most of the properties are small. The use of the consortium between palm and woody legumes can bring great benefits to the soil and the ecosystem, since the superficial root system of this cactus in association with the greater reach of the roots of the legumes will enable improvements in aeration and mainly edaphic porosity. In addition, leaf senescence and known biological nitrogen fixation by legumes increase organic matter and soil fertility, respectively. This makes the use of intercropped species complementary, reducing the import of forage palms that are more fibrous and concentrated by the producer <sup>(3; 4)</sup>. Thus, the use of forage plants and efficient cropping systems is essential to improve agricultural sustainability <sup>(5)</sup>.

Water reuse is a long-established practice in several countries and regions with water shortages around the world. The practice of water reuse has evolved significantly in Israel,



covering more than 50% of its demand for agricultural water with recovered water <sup>(6)</sup>. Beneficial effects in relation to agricultural crops irrigated with domestic sewage effluents have been found by several researchers, such as the cultivation of pastures by Cruvinel *et al.* <sup>(7)</sup>; quality of papaya fruits by Batista *et al.* <sup>(8)</sup> and also in forage palm cultivation <sup>(9)</sup>.

Wastewater in most municipalities is abundantly available in the urban environment, in most cities it returns in the form of sewage and without proper treatment. Studies on the viability of agricultural production associated with water reuse have been widely researched and analyzed in some regions of the Northeast <sup>(10)</sup>.

As this region is essentially livestock, the cultivation of forage irrigated with effluents of this nature can increase pasture stocking capacity, productive efficiency of rural properties, generating a positive impact on the local economy. In this sense, the aim was to evaluate the growth, physiology and yield of cladodes of forage cactus irrigated with reuse water and intercropped with legumes.

## Methodology

The research was carried out for 24 months in the municipality of Frei Martinho-PB, Eastern Seridó Microregion, under the geographical coordinates: 6°24'09"S and 36°37'13.67"O. The climate is classified as BSh type, according to the Koppen climate classification, being considered low latitude dry semi-arid <sup>(11)</sup>.

In November 2018, a forage palm research/multiplication field was implemented in an area of 01 hectare, near the Sewage Treatment Station – STS belonging to the municipality, which receives the effluents from the urban population. The experimental design was a randomized block with subdivided plots, with three palm genotypes in the plots: Miúda (*Nopalea cochenillifera* salm-dyck), Baiana (*Nopalea cochenillifera* salm-dyck) and Orelha de Elefante Mexicana (OEM) [*Opuntia stricta* (Haw.) Haw.]; and in the subplots, four legumes: Two timbers Algaroba (*Prosopis juliflora*) and Sabiá (*Mimosa caesalpinifolia*) and two forragers Asian pigeonwings (*Clitoria ternatea* and Gliricidia (*Gliricídia sepium*), besides one control treatment (without consortium). The planting of forage cactus was carried out at a spacing of 1.50 m x 0.50 m x 0.50 m for cultivation in double rows. The legumes were planted in a consortium system, between the double rows of the palm, in the spacings: 2 m x 1 m for the gliricidia; 2 m x 0.7 m for the pigeonwings; 2 m x 0.7 m for the pigeon pea; 2 m x 2 m for the sabiá and 2 m x 3 m for the algaroba.



The irrigation was performed via drip, with a water depth of 7.5 L/linear meter and frequency of 07 (seven) days, which corresponds to 1.87 liters/plant/week, that corresponds to 5 mm per week. The water used for this purpose comes from the municipal STS (100% of the treated effluent), which goes through the treatment process with two ponds, anaerobic and stabilization (Frame 1). After that, the water is pumped, passing through a sand filter, to a reservoir of 20 thousand liters, and from this to the field, being filtered again by a disc filter. The drip irrigation system promotes the availability of water to specific sectors avoiding direct contact with plants. Cultural treatments were performed whenever necessary. In the sabiá and algaroba species periodically, the conduction pruning was carried out, in order to remove lateral sprouting, promoting a rectilinear growth of the plants.

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Parameters					
рН	7.7 ± 0.8				
Color (uC)	882 ± 140				
Turbidity (NTU)	124 ± 74				
Salinity (g/L)	1.25 ± 0.13				
Total dissolved solids (TDS) (mg/L)	1615 ± 213				
Electric conductivity (mS/cm)	2.57 ± 0.24				
Chemical Oxygen Demand (COD) (mg/L)	412 ± 291				
Ammonia (mg/L)	74.3 ± 25.6				
Phosphor (mg/L)	13.5 ± 5.7				
Sodium (mg/L)	201 ± 117				
Potassium (mg/L)	63 ± 36				
<i>E. coli</i> (NMP/100 mL)	4.96E+05				
helminth eggs (eggs/L)	0				
Source: Authors (2022)					

## Frame 1 - Analysis of wastewater used in irrigation

Source: Authors (2022).

Growth variables (plant height, width and number of cladodes per plant) were determined in the forage palm using a measuring tape, every 90 days after planting. In addition to the physiological variables of gas exchange, stomatal conductance (gs) (mol  $m^{-2} s^{-1}$ ),



transpiratory rate (E) (mmol de  $H_2O \text{ m}^{-2} \text{ s}^{-1}$ ),  $CO_2$  uptake rate (A) (µmol m<sup>-2</sup> s<sup>-1</sup>) and internal  $CO_2$  concentration (Ci) (µmol de  $CO_2 \text{ mol}^{-1}$ ). Based on the data, the instantaneous efficiency of water use (iWUE) was calculated, relating the  $CO_2$  uptake rate with transpiration (A/E), by the relationship between the  $CO_2$  uptake rate and stomatal conductance (A/gs), in addition to the instantaneous efficiency of carboxylation (iCE), relating the  $CO_2$  uptake rate with the internal concentration of carbon (A/Ci).

The determinations of gas exchange were carried out between 00:00 and 02:00 hours, due to the forage cactus being governed by the acid metabolism of the crassulacean (CAM), its CO<sub>2</sub> uptake being more intense in this interval. The measurements occurred in three plants per plot, totaling 12 plants of each variety per consortium, and the readings were performed in a mature cladode per plant, characterized by sustaining one or more young cladodes.

To carry out the evaluations, a portable infrared gas analyzer (IRGA) (model LI-COR 6400-XT, Lincon, USA) and tweezers adapted for forage palm were used. The protocol with IRGA was: PAR (photosynthetically active radiation) turned off; relative humidity between, air flow and ambient atmospheric  $CO_2$  concentration, with a leaf chamber size of 6.25 cm<sup>2</sup>.

The data were submitted to analysis of variance and the means were compared by Tukey's test at the 5% probability level, using the statistical software Sisvar <sup>(12)</sup>.

## **Results and Discussion**

The summary of the analysis of variance (Frame 2) showed that the growth variables Height and width of plant, in addition to the total number of cladodes, had a significant effect only as a function of the forage cactus variety used. While the physiological variables transpiratory rate (E), CO2 uptake rate (A), instantaneous water use efficiency (iWUE), instantaneous efficiency of carboxylation (iCE) were statistically influenced by the type of intercropping and by the interaction of cactus variety x specie of legume used.



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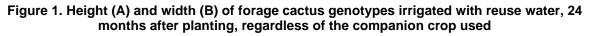
Frame 2 - Summary of analysis of variance for height and width of plant, transpiratory rate (E),  $CO_2$  uptake rate (A), instantaneous water use efficiency (iWUE), instantaneous efficiency of carboxylation (iCE) and number of cladodes per plant (NC) of forage palm genotypes irrigated with reuse water, 24 months after planting, depending on the intercropping approach used

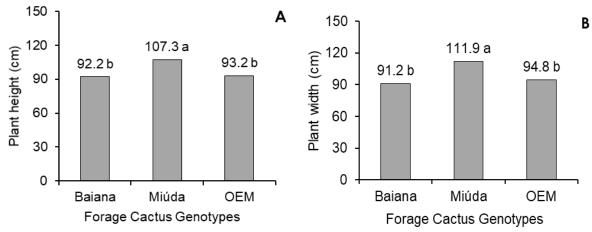
Source variation	of GL	Height	Width	E	A	iWUE	iCE	NC
Block	3							
Var (V)	2	260,23*	349,15*	0,08 <sup>ns</sup>	0,19 <sup>ns</sup>	0,83 <sup>ns</sup>	64,07 <sup>ns</sup>	736,17*
Con (C)	4	689,17 <sup>ns</sup>	243,22 <sup>ns</sup>	0,84*	4,12*	13,40*	377,69*	338,57 <sup>ns</sup>
V*C `́	8	257,28 <sup>ns</sup>	359,52 <sup>ns</sup>	0,31*	0,57*	4,20*	17,08*	87,48 <sup>ns</sup>
Residue	45	398,65	460,56	0,04	0,13	0,35	29,09	231,21
Total	59							
CV (%)		20,49	21,62	15,65	18,96	13,85	17,68	36,54

GL = Degree of freedom; Var = Variety; Con = Consortium; \*: significant at 5%; ns: not significant; CV: Coefficient of variation.

Source: Authors (2022).

It is observed in Figure 1 A and B, that both in height and plant width, the Miuda genotype (107.3 and 111.9 cm, respectively) surpassed the Baiana genotypes (92.2 and 91.2 cm) by 16.3 and 22.7% and, OEM (93.2 and 94.8 cm) by 15.1 and 18%, respectively. This is possibly due to this material presenting more erect growth and higher cladodium emission (Figure 6), directly influencing its growth in height and width.





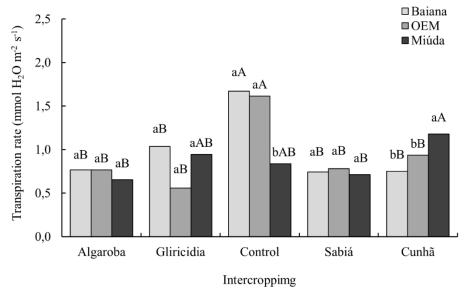
Means followed by the same lowercase letters are equal to each other by Tukey's test at 5% probability.

Source: Authors (2022).



Silva *et al.* <sup>(13)</sup> working with these materials in dry conditions 24 months after planting, found that the OEM variety obtained higher plant height, while for plant width there was no difference between the genotypes. However, Rocha *et al.* <sup>(14)</sup> in irrigated cultivation of the materials Miuda, OEM and IPA 20 at 16 months after planting, found that the Miuda obtained greater plant width, being similar to IPA 20 at the time. The greater perspiration occurred in the genotypes of forage cactus cultivated without consortium, possibly due to the greater exposure of these to the sun and especially to the wind, since, even when intercropped with pigeonwing and gliricidia, which are not very dense, they exert little shading and eventually do not create a microclimate, similar to sabiá and especially to algaroba. The transpiratory rate (E) of the varieties of forage cactus did not vary statistically when cultivating these with the same intercropping system, except in the absence of an intercropping approach, when the Baiana genotype and the OEM (1,7 e 1,6 mmol de H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>), exceeded the Miuda (0.8mmol of H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>), by 112.5 and 100%, respectively (Figure 2). However, the inverse occurred when the intercropping with Asian pigeonwing, where Miuda surpassed Baiana and OEM by 33.3 and 50%, respectively.

# Figure 2. Transpiratory rate of forage palm genotypes irrigated with reuse water, 24 months after planting, depending on the intercropping approach used



Means followed by the same lowercase letters between the intercropping type and capital letters between the forage cactus varieties are equal to each other by Tukey's test at 5% probability.

Source: Authors (2022).



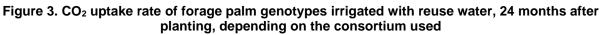
The Baiana and OEM genotypes obtained a higher transpiratory rate when cultivated in the absence of the intercropping approach (1.7 and 1.6  $H_2O~m^2~s^{-1}$ ), this physiological superiority is probably a consequence of the greater stomatal comfort of shading and root activity, obtained through intercropping with tree species (algaroba and sabiá). While the Miuda genotype obtained a higher transpiratory rate when intercropped with Asian pigeonwing, gliricidia and in the control treatment, respectively, 1.2; 0.9 and 0.8  $H_2O~m^2~s^{-1}$ .

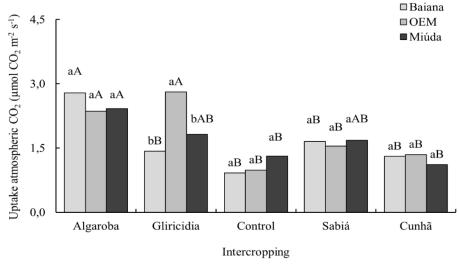
Souza *et al.* <sup>(14)</sup> found that through the use of mulch with vegetable materials, the transpiratory rate of forage cactus intercropped with annual forage was reduced. They found that soil shading is an essential factor, especially in forage cactus crops, considering that most of the root system of this xerophile acts on the layer up to 20 cm deep, this can also be considered in the present case, since greater shading by nurse tree can provide similar results, as shown in this study.

The atmospheric CO<sub>2</sub> uptake rate of forage cactus genotypes in the same intercropping system obtained statistical difference only for OEM when cultivated with gliricidia (2.8  $\mu$ mol m<sup>2</sup> s<sup>-1</sup>) superiority of 100 and 55.5% compared to Baiana and Miuda materials, in this order (Figure 3).

When comparing the influence of the different consorts with the forage palm materials, it was found that the Baiana palm captured more  $CO_2$  when grown under the algaroba (2.8 µmol m<sup>2</sup> s<sup>-1</sup>), an increase of at least 75% in relation to the other intercropped species. The OEM obtained higher  $CO_2$  uptake when intercropped with algaroba and gliricidia (2.3 and 2.8 µmol m<sup>2</sup> s<sup>-1</sup>), while the algaroba, gliricidia and sabia cultivation provided a higher rate of atmospheric  $CO_2$  uptake to the Miuda genotype (2.4 and 1.8 and 1.7 µmol m<sup>2</sup> s<sup>-1</sup>).







Means followed by the same lowercase letters between the intercropping type and capital letters between the forage cactus varieties are equal to each other by Tukey's test at 5% probability.

Source: Authors (2022).

The highest rates of CO<sub>2</sub> uptake obtained by palm varieties when intercropped with larger legumes (algaroba, gliricidia and sabiá), possibly occurs due to the microclimate created by them, which ends up benefiting positively this cactus. <sup>(15)</sup> points out that in a forage palm intercropped with C<sub>3</sub> and C<sub>4</sub> crops both can be benefited, because during the night these plants are breathing, that is, releasing CO<sub>2</sub> to the atmosphere, creating a richer CO<sub>2</sub> environment in the space between the soil and the plant top.

<sup>(16)</sup> points out that the plants governed by CAM have a maximum photosynthetic rate of 7.6  $\mu$ mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, while the rate is usually 2.5, these low photosynthetic rates in most cases cause the plant to grow very slowly. However, it is found that with the use of the intercropping approach with plants governed by other metabolisms, the forage cactus obtained more intensity of CO<sub>2</sub> uptake, remaining at adequate levels.

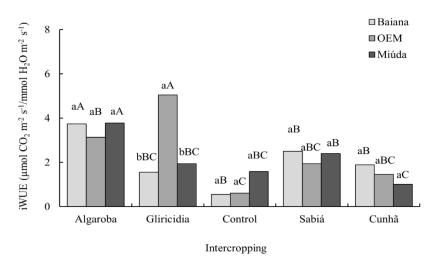
The instantaneous water use efficiency (iWUE) behaved similarly to CO<sub>2</sub> uptake, when in consortium with gliricidia, the OEM genotype (5.0  $\mu$ mol m<sup>2</sup> s<sup>-1</sup>/ mmol of H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>) exceeded the Baiana and Miuda genotypes by 212.5 and 163.1%, respectively (Figure 4). This iWUE was also the highest for this palm genotype in comparison with the other nurse plants, while Baiana obtained the highest iWUE when intercropping with algaroba (3.7  $\mu$ mol m<sup>2</sup> s<sup>-1</sup>/ mmol



of H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>) and Miuda was positively influenced by intercropping with algaroba (3.8  $\mu$ mol

m<sup>2</sup> s<sup>-1</sup>/ mmol of H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>) and sabiá (2,4  $\mu$ mol m<sup>2</sup> s<sup>-1</sup>/ mmol of H<sub>2</sub>O m<sup>2</sup> s<sup>-1</sup>).

# Figure 4. Instantaneous water use efficiency of forage cactus genotypes irrigated with reuse water, 24 months after planting, depending on the intercropping approach used



Means followed by the same lowercase letters between the intercropping type and capital letters between the forage cactus varieties are equal to each other by Tukey's test at 5% probability.

Source: Authors (2022).

The iWUE levels obtained in this study are mostly well above those achieved by <sup>(14)</sup> in a consortium with annual forages, such as buffel grass and forage watermelon (1.33 and 1.10  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>)/(mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>, respectively). This demonstrates the superiority of CO<sub>2</sub> uptake in response to higher transpiration rates, determining an increase in this variable.

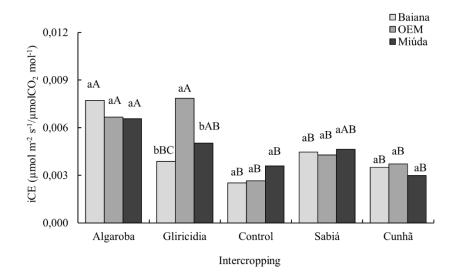
The greater physiological activity of forage cactus genotypes, when intercropped with nurse trees and shrubs, is probably due to the microclimate created by them, which reduces the local temperature, increases environmental humidity, increases stomatal conductance and intracellular CO<sub>2</sub> concentration.

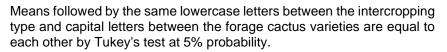
As for the instantaneous efficiency of carboxylation (iCE), it was found that only when cultivated with gliricide there was a significant effect of forage cactus genotypes, where OEM (0.008 mol  $CO_2$  m<sup>-2</sup> s<sup>-1</sup>/mol de  $CO_2$  mol<sup>-1</sup>) was superior to Baiana and Miuda in 100 and 60%, respectively (Figure 5). Analyzing the strongest influencing intercropping, we find the Sabia and tree species increased the iCE of the forage palm, where the algaroba acted positively on



the iCE of both materials, 0.008 for the Baiana palm and 0.007 mol of  $CO_2 \text{ m}^{-2} \text{ s}^{-1}/\text{mol} \text{ de } CO_2 \text{ mol}^{-1}$  for OEM and Miuda. While gliricidia also elevated the iCE of the OEM 0.008 and Miuda 0.005 mol of  $CO_2 \text{ m}^{-2} \text{ s}^{-1}/\text{mol}$  of  $CO_2 \text{ mol}^{-1}$ , the Miuda was also influenced by intercropping with Sabia (0.005 mol  $CO_2 \text{ m}^{-2} \text{ s}^{-1}/\text{mol}$  of  $CO_2 \text{ mol}^{-1}$ ).

# Figure 5. Instantaneous efficiency of carboxylation of forage cactus genotypes irrigated with reuse water, 24 months after planting, depending on the intercropping approach used



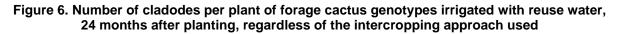


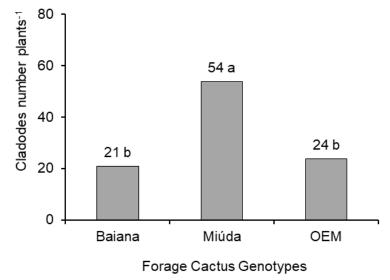
Source: Authors (2022).

Figure 6 shows that the Miuda genotype obtained 54 cladodes plant<sup>-1,</sup> a relative increase of 157.1 and 125% compared to the Baiana and OEM materials, which obtained 21 and 24 cladodes plant<sup>-1</sup>, respectively.

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Means followed by the same lowercase letters are equal to each other by Tukey's test at 5% probability.

Source: Authors (2022).

Rocha *et al.* <sup>(17)</sup> observed that the Miúda genotype presented at 16 months 44.2 cladodes plant<sup>-1</sup>, while the OEM and IPA 20 in the same period presented 19.7 and 11.05 cladodesplant<sup>-1</sup>, respectively. Lira *et al.* <sup>(4)</sup> found in a forage palm consortium system with annual crops that the OEM and Miúda genotypes produced 224.8% and 176.1% more leaves per plant compared to the Baiana variety.

### **Final considerations**

The Miúda genotype was higher in height, plant width and number of cladodes per plant; With no intercropping approach, both genotypes of forage cactus presented higher transpiration rates; Intercropping with algaroba, gliricidia and sabiá increased CO<sub>2</sub> uptake, instantaneous water use efficiency and carboxylation of forage palm in comparison with the consortium with cunhã and the control treatmentre, gardless of variety.



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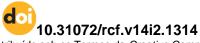


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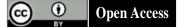
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