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Gas exchange in genotypes of *Nopalea cochenillifera* in different seasons and evaluation times

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Abstract – This study aimed to evaluate gas exchange in genotypes of *Nopalea cochenillifera* Salm-Dyck in different seasons and at different times of day. The experiment was conducted with the varieties Miúda and Baiana at the Prof. Ignacio Salcedo Experimental Station, belonging to the National Semi-Arid Institute (INSA), in the municipality of Campina Grande, State of Paraíba, Brazil. The treatments were distributed in a 24 × 2 factorial arrangement, corresponding to gas exchange evaluations performed every hour for 24 hours in the rainy (June) and in the dry season (December). Analyzed were stomatal conductance, transpiration rate, CO_2 uptake, and internal CO_2 concentration, as well as instantaneous water-use efficiency, intrinsic water-use efficiency, and instantaneous carboxylation efficiency. In both dry and rainy seasons, the variety Baiana presented higher gas exchange intensity than the Miúda variety. In the rainy season, gas exchanges are potentialized in both varieties evaluated. In this period, the peak of CO_2 uptake occurs from 1:00 a.m. to 3:00 a.m. for the variety Baiana, and from11:00 p.m. to 2:00 a.m. for the variety Miúda, whereas, in the dry season, it occurs from 11:00 p.m. to 2:00 a.m. for both varieties, with these constituting the ideal intervals for measuring gas exchanges in the field.

Keywords: Brazil, Cactaceae, CO_2 uptake, forage cactus, semi-arid, xerophilus

Introduction

The forage basis of cattle-raising in the Brazilian semi-arid region is constituted by plants native to the Caatinga associated with the cultivation of exotic species. Rainfall variability in time and space in this region makes it impossible to cultivate most traditional crops, turning the forage use of perennial plants, especially xerophytic species, into an indispensable activity, given their natural tolerance to water stresses, as seen in the forage cactus genus *Opuntia* and in *Nopalea* spp. (Lima et al. 2011, Almeida et al. 2019).

However, due to the appearance of the prickly pear wild cochineal (*Dactylopius opuntiae* Cockrell) in the early 2000s, most forage cactus fields in the Northeast region were decimated, a consequence of the homogeneity of the genetic material of the plants, as well as of the high aggressivity and biotic potential of the pest (Lopes et al. 2009, Lacerda et al. 2011). The reconstitution of forage cactus fields with resistant varieties was a crucial step to solve this problematic situation. Among these, the forage cactus genotypes Miúda and Baiana, of the subgenus *Nopalea*, stood out for their satisfactory productive yield, high contents of dry matter, and carbohydrates, besides excellent palatability (Araújo et al. 2019).

Nobel (2009) states that, in general, cacti and agaves present a slow growth, which is verified in cacti native to the Caatinga; the forage cactus, however, is an exception, reaching yield values up to 800 Mg ha year^{-1}. These plants present a Crassulacean Acid Metabolism (CAM), a photosynthetic pathway characterized by nocturnal CO_2 fixation. CAM plants use strategies such as the increase in CO_2 assimilation about lost water and high water-use efficiency, especially in places with little water. Furthermore, some plants, such as the forage cactus, might present a facultative CAM, switching to C_3 depending on the conditions in which they are, turning the forage cactus into one of the most efficient plants in the world regarding water-use efficiency, an indispensable feature in arid and semi-arid regions (Yu et al. 2019, Santos et al. 2020).

Despite the great importance of studies on the physiology of this crop, little research has been done since the sig-

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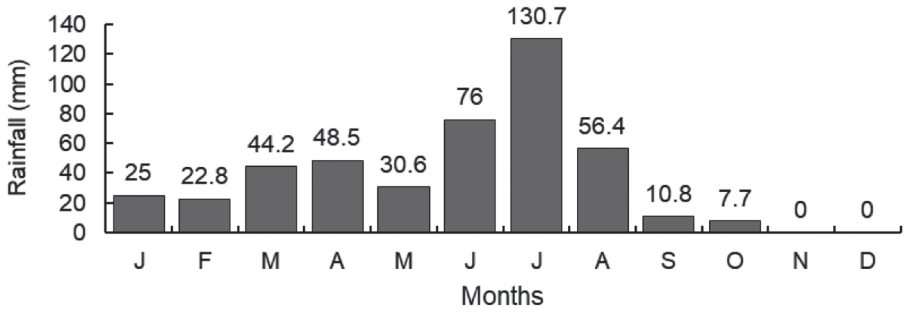

Fig. 1. Rainfall in the experimental area during 2019.

nificant advances achieved for the crop are related to questions such as spacing, intercropping with annual and perennial crops, irrigation, and especially the discovery of genotypes resistant to cochineal. However, the mechanisms and functions of the CAM metabolism are directly related to gas exchanges, in which the knowledge of factors such as the interval and intensity of gas exchanges might be decisive for the good establishment of the crop. In this context, this study aimed to evaluate the gas exchanges of genotypes of *Nopalea cochenillifera* Salm-Dyckin in different seasons and at different evaluation times.

Materials and methods

The experiment was conducted at the Ignácio Salcedo Experimental Station, belonging to the National Semi-Arid Institute – INSA, located in the municipality of Campina Grande, in the Agreste Meso region of the state of Paraíba, in the geographic coordinates 07º13'50" S, 35º52'52" W and an elevation of 551 m a.s.l. The climate of the region is classified as Aw', according to the climate classification by Köppen, and is considered dry and sub-humid, with a water deficit most of the year (Alvares et al. 2013). The rainfall during 2019 was 452.7 mm, as seen in Fig. 1.

The study was conducted with three-year-old plants in a forage cactus field with a planting density of 17.391 plants ha^{-1} composed of the cochineal-resistant varieties Miúda and Baiana, both belonging to the same species (*Nopalea cochenillifera*). The gas exchange evaluations were performed using a 24 × 2 factorial arrangement with five replications, corresponding to an evaluation every hour during 24 hours in the rainy (June) and in the dry (December) season. These months were chosen because they fall within the rainy and dry seasons, respectively.

The following variables were measured in two secondary cladodes per plant: stomatal conductance (gs) (mol m^{-2} s^{-1}), atmospheric CO_2 uptake (A) (µmol m^{-2} s^{-1}), transpiration rate (E) (mmol H_2O m^{-2} s^{-1}), and internal CO_2 concentration (Ci) (µmol CO_2 mol^{-1}). Based on the data, the instantaneous water-use efficiency (EUA) was calculated by relating the net photosynthesis to the transpiration (A/E); the intrinsic water-use efficiency (EIUA) was calculated by relating the net photosynthesis to the stomatal conductance (A/gs), and the instantaneous carboxylation efficiency (EiC) was calculated by relating the net photosynthesis to the internal carbon concentration (A/Ci).

A portable infrared gas analyzer (IRGA) was used for the evaluations: model LCpro+, manufactured by BioScientific LLC. The protocol for the evaluations with the IRGA was as follows: air relative humidity, airflow, and atmospheric CO_2 concentration, with a leaf chamber dimension of 6.25 cm^2. The photosynthetically active radiation, the ambient temperature, and the temperature on the surface of the cladode during both seasons are seen in Fig. 2.

The results were subjected to analysis of variance by the F-test. If the values were significantly different, Tukey's test was applied at a 5% level of probability. The statistical software SAS - Statistical Analysis System® was used for data processing (Cody 2015).

Results

The stomatal conductance (gs) of the varieties Miúda and Baiana (Fig. 3) behaved similarly in the distinct seasons evaluated: in the rainy season, both varieties exhibited greater stomatal opening at 1:00 a.m., with 0.54 and 0.44 mol m^{-2} s^{-1}, respectively (Fig. 3A). In the dry season, the peak of stomatal conductance was also observed at this time, with 0.22 and 0.21 mol m^{-2} s^{-1}, respectively (Fig. 3B). In the rainy season, the gs of the Miúda variety was statistically higher than the gs obtained by the Baiana variety from 11:00 p.m. to 3:00 a.m. When comparing according to seasons, it is observed that the gs of these varieties in the rainy season is 145.5 and 109.5% greater than in the dry season. Another factor to be considered is the permanence of the gs: in the rainy season, the variety Baiana remains with its stomata opened from 4:00 p.m. to 6:00 a.m. (15 hours), while for the variety Miúda this condition occurs from 3:00 p.m. to 6:00 a.m. (16 hours). In the dry period, this interval is from 5:00 p.m. to 5:00 a.m. (12 hours) and from 4:00 p.m. to 5:00 a.m. (13 hours), respectively. As well as having a higher gs intensity in the rainy season, the stomata remain open for three more hours, on average.

CO_2 uptake showed to be different in the varieties and times studied: while in the rainy season the forage cactus variety Baiana reached a maximum of 6.06 µmol CO_2 m^{-2} s^{-1} (Fig. 4A), the variety Miúda reached 3.98 µmol CO_2 m^{-2} s^{-1} (Fig. 4A), both at 2:00 a.m. This trend remains in the dry

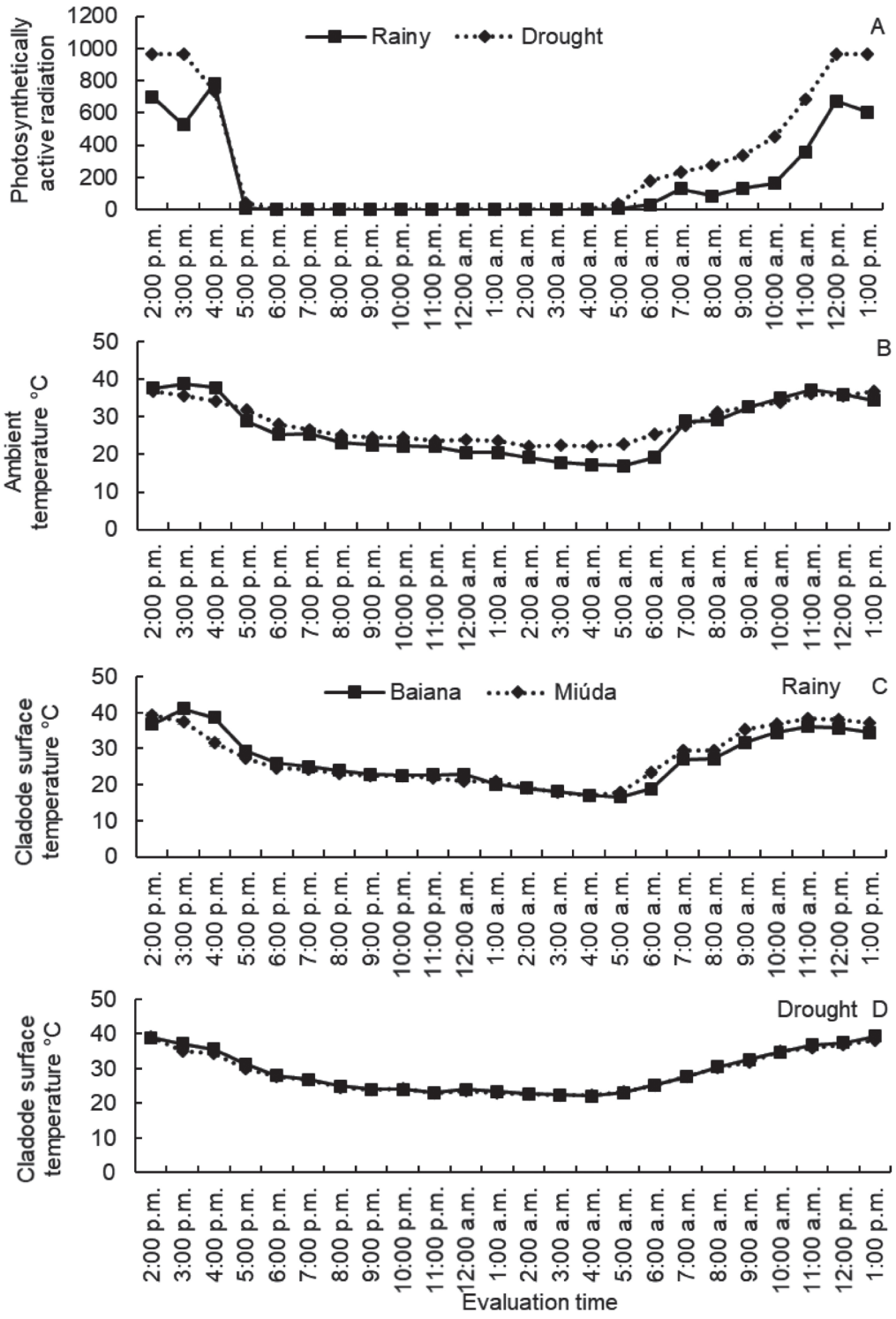

Fig. 2. Photosynthetically active radiation (A), atmospheric temperature (B), and temperature on the surface of the cladodes in the forage cactus varieties Baiana (C) and Miúda (D) in different evaluation times in the rainy and dry seasons.

period, with the varieties Baiana and Miúda reaching 2.95 and 2.05 μmol CO_2 m^{-2} s^{-1}, respectively (Fig. 4B), and the new peak time was 00:00. The Baiana variety obtained statistical superiority in terms of CO_2 uptake over the Miúda palm from 19:00 to 04:00 in both evaluation times.

The transpiration rate varied greatly (Fig. 5) in the evaluated seasons, oscillating in the rainy season from -3.90 (11:00 a.m.) to 2.07 (7:00 p.m.) and from -5.52 (10:00 a.m.) to 1.88 (4:00 p.m.) mmol m^{-2} s^{-1} (Fig. 5A), while in the dry season this variation took place from –1.78 (12:00 p.m.) to 0.86 (12:00 a.m.) and from –1.38 (2:00 p.m.) to 0.73 (9:00 p.m.) in the varieties Baiana and Miúda, respectively (Fig. 5B).

The behavior of the internal CO_2 concentration (Fig. 6) was similar in the two varieties; however, the variety Baiana

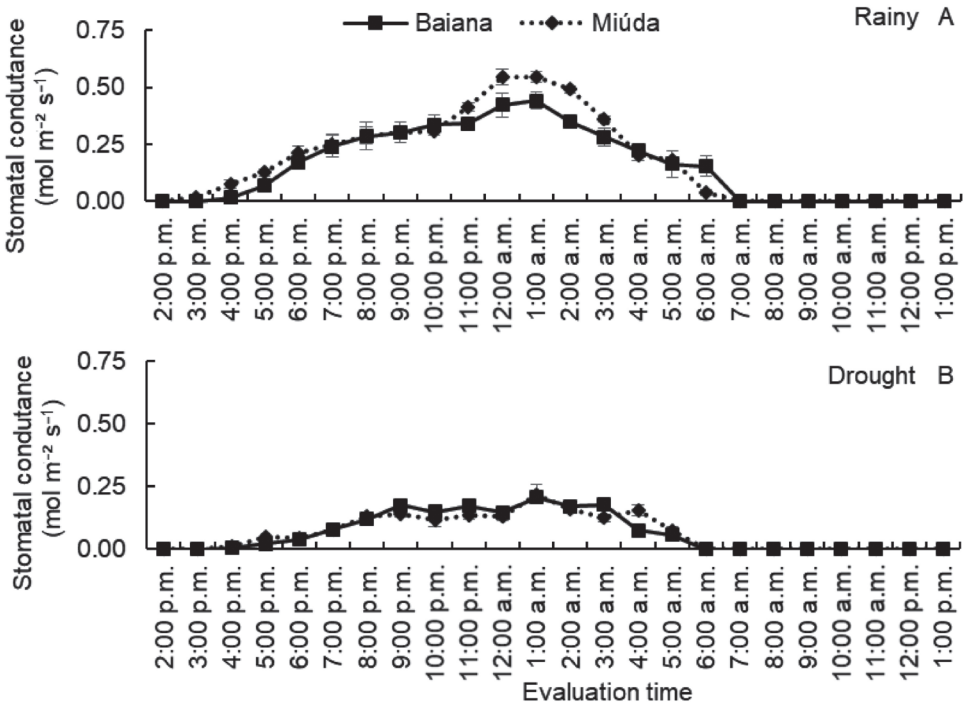

Fig. 3. Stomatal conductance of the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) and drought (B) seasons.

expressed higher values, especially in the dry season, varying from 372 at 6:00 p.m. to 2,074.4 μmol mol^{-1} at 12:00 p.m. (Fig. 6B). In the rainy period, this concentration varied from 330.2 at 4:00 a.m. to 1,207.2 μmol mol^{-1} at 12:00 p.m. (Fig. 6B). For the variety Miúda, the CO_2concentration oscillated from 224.8 at 2:00 p.m. to 831.4 at 10:00 a.m. and from 376.4 at 5:00 p.m. to 1,674.6 μmol mol^{-1} at 11:00 a.m., in the rainy and drought seasons, respectively (Fig. 6A, B).

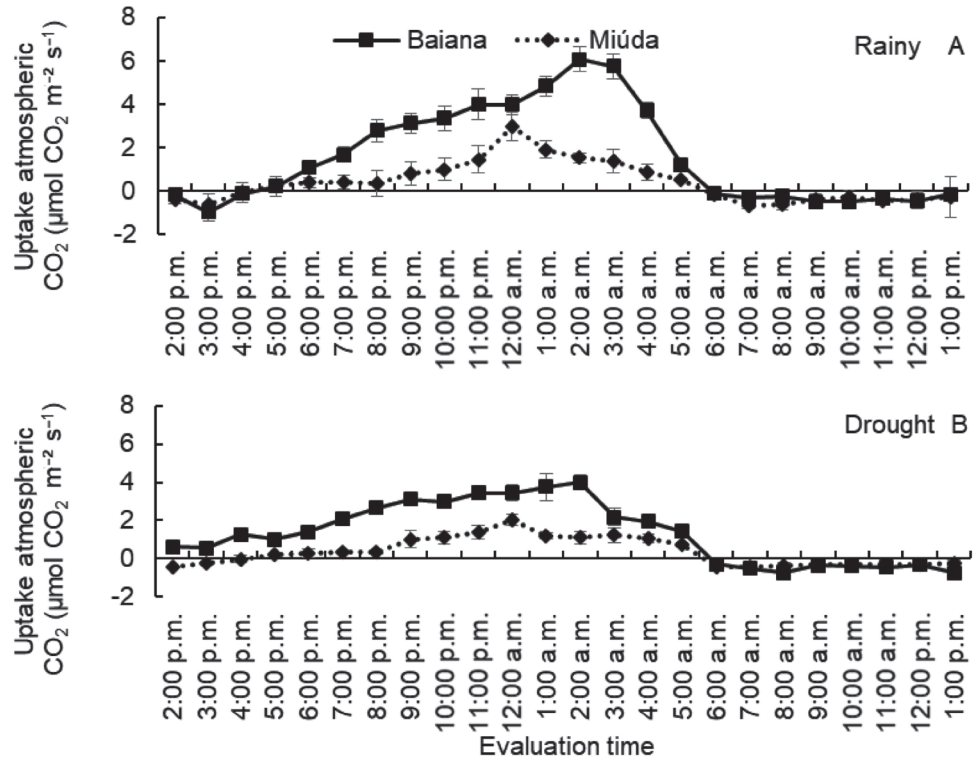

Fig. 4. Uptake atmospheric CO_2 by the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) and drought (B) seasons.

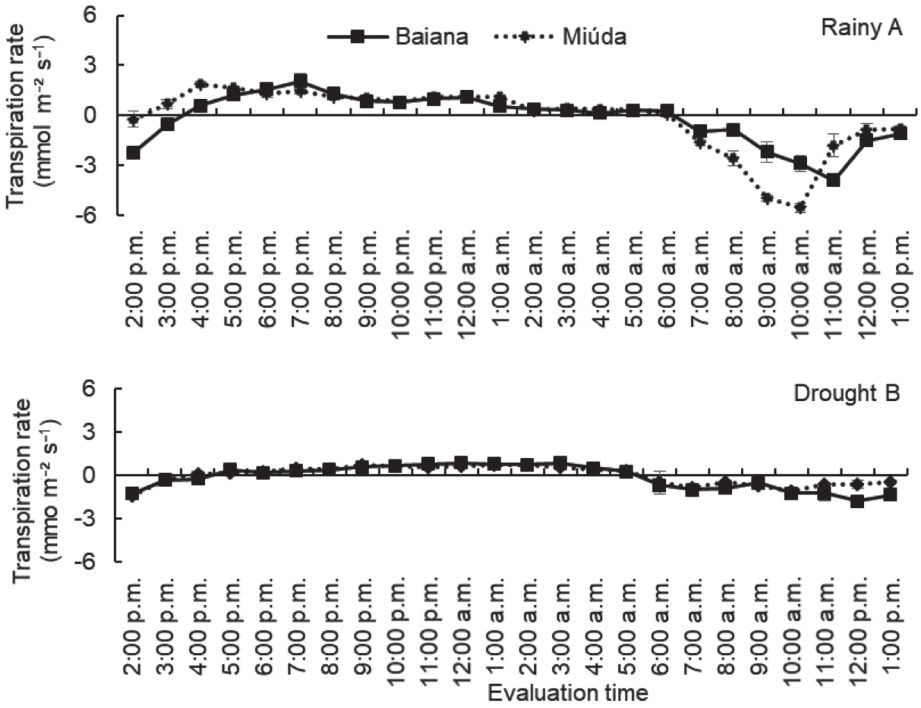

Fig. 5. Transpiration of the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) drought (B) seasons.

With respect to the remaining variables, the instantaneous water-use efficiency (WUE) also varied with the forage cactus varieties Baiana and Miúda. In the rainy season, the forage cactus Baiana reached its peak at 4:00 a.m. (24.3 μmol CO_2 m^{-2} s^{-1} uptake for each mmol H_2O m^{-2} s^{-1} lost by transpiration), In this period, the Baiana variety from 01:00 to 04:00 was statistically superior to the Miúda palm. However, in the dry season this peak was at 12:00 a.m., with 3.43

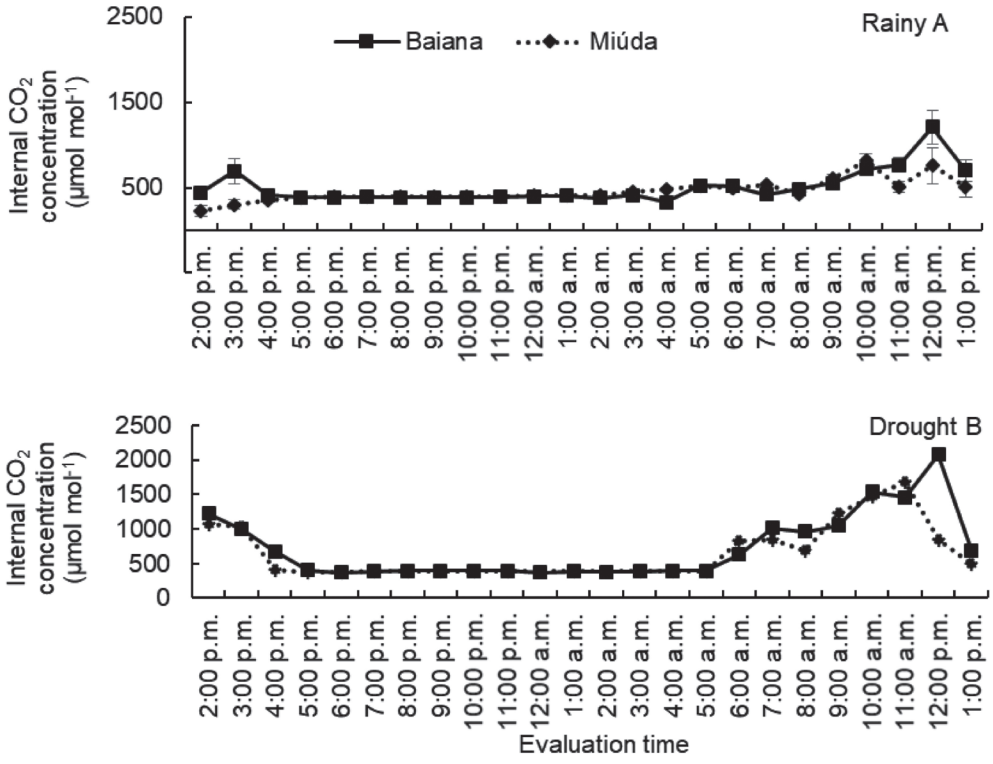

Fig. 6. Internal CO_2 concentration of the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) and drought (B) seasons.

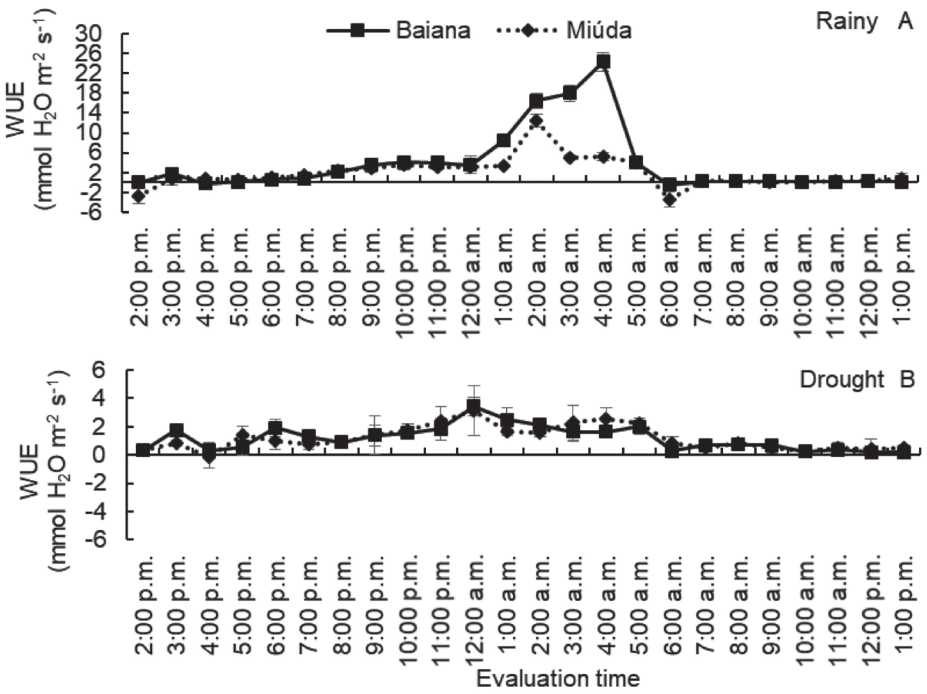

Fig. 7. Instantaneous water-use efficiency of the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) and drought (B) seasons.

μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1} (Fig. 7A, B). As for the forage cactus variety Miúda, the highest values were obtained at 2:00 a.m. and 12:00 a.m., with 12.45 and 3.13 μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}, respectively, in the rainy and in the dry season (Fig. 7A, B).

The intrinsic water-use efficiency (iWUE) reached the highest mean values in the rainy season, with peaks of 61.2 and 46.5 mol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1}at 2:00 a.m. for the varieties Baiana and Miúda (Fig. 8A), respectively. However, it is observed that, for the first variety, this apex lasted for

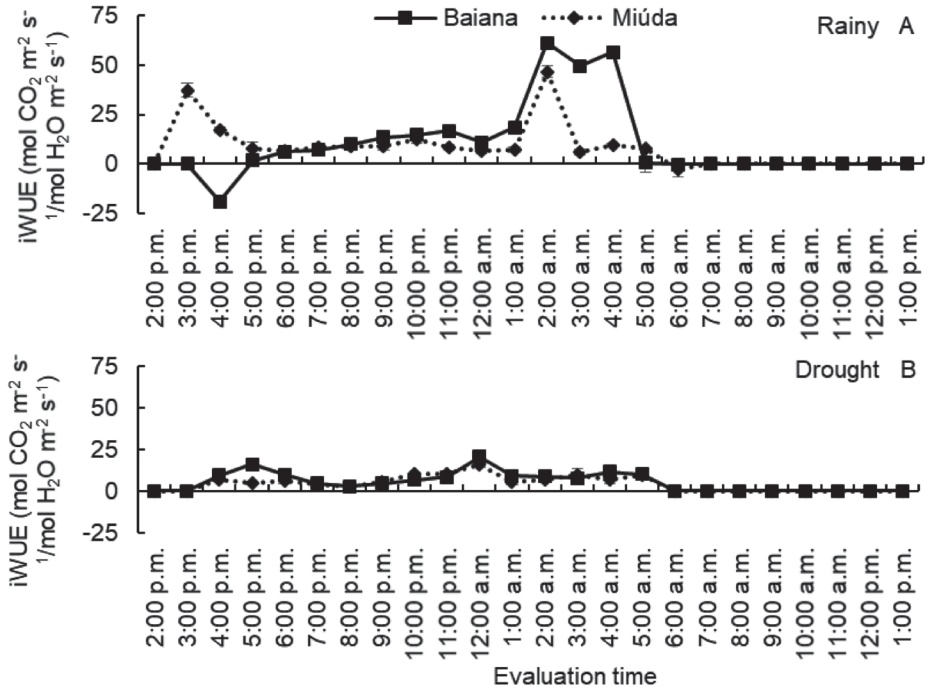

Fig. 8. Intrinsic water-use efficiency of the forage cactus varieties Baiana and Miúda in different evaluation times in the rainy (A) and drought (B) seasons.

three hours, with no significant statistical difference in this period; for the latter, however, another peak occurred at 3:00 p.m. (37.2 mol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1}), which is possibly a consequence of the high CO_2 uptake to the detriment of stomatal conductance (Figs. 3, 4). In the dry season, the highest iWUE values occur at 12:00 a.m. for both varieties, with 20.5 and 15.6 mol CO_2 m^{-2} s^{-1}/mol H_2O m^{-2} s^{-1} for the varieties Miúda and Baiana, respectively (Fig. 8B). When analyzing the iWUE in the different seasons, it is noted that the forage cactus variety Baiana surpasses the variety Miúda by 31.5%. This increment is verified in most of the variables studied here, although the latter presents an earlier CO_2 uptake.

Discussion

The remarkable adaptability of forage cactus in environments with rainfall restrictions, associated with crassulaceous acid metabolism, characterized by nocturnal stomatal opening, enables this plant to become the most cultivated xerophilic species in Brazil.

It is observed that, in the rainless season, this cactus maintains its physiological functions active even if at a lower rate, which is possibly the consequence of the water reserve accumulated in the parenchyma during the rainy period, allowing the active maintenance of the physiological functions in the dry period. Even in this period, the gs for both varieties was above the value considered as a severe water deficit (0.1 mol m^{-2} s^{-1}) (Fig. 3), demonstrating that although the dry period may extend to three quarters of the year, this succulent xerophilous succulent maintains its gs active (Flexas et al. 2014).

Although belonging to the same species (*Nopalea cochenillifera*), the variety Miúda, even obtaining a higher gs, still demonstrates that it is less efficient in CO_2 uptake than the variety Baiana, with a reduction of 34.3% (Fig. 4). This detail is of great importance when choosing the appropriate variety to be cultivated by producers since the varieties of the subgenus *Nopalea*, although less rustic than the *Opuntia* varieties, are highlighted by the absence of spines, easy management, higher dry matter content, and high palatability.

Nobel (2009) points out that CAM plants, such as agaves and cacti, present a maximum photosynthetic rate of 7.6 μmol CO_2 m^{-2} s^{-1}, but this rate is normally 2.5. These low photosynthetic rates cause the plant to present a very low growth, although it is seen that, in the rainy season, the values keep close to this maximum for some hours, especially from 12:00 a.m.to 2:00 a.m. (Fig. 4). A change was verified in the peak time of CO_2 uptake in the dry season, which possibly occurred so that the plants could make better use of the atmospheric conditions late at night given the presence of dew, which is considered an ideal condition for the cultivation of forage cactus (Souza et al. 2020). This shows that the forage cactus, although being a CAM plant, can be characterized as a facultative C_3 since once presenting favorable edaphic moisture conditions, it begins the uptake of CO_2 in the afternoon, when there is still sunlight, finishing in the morning. This versatility allows this cactus to be one of the most efficient plant species in the world, reaching fresh matter yields up to 800 mg ha $year^{-1}$.

Furthermore, it is worth highlighting the greater flexibilization of transpiration that occurs in the rainy period, which also demonstrates that, in this cactus, the highest CO_2 uptake rates are not always accompanied by high transpiration: instead, this rate stabilizes at low levels, especially at times of higher CO_2 uptake. This increases the water-use efficiency by the crop, also indicating that, even in favorable conditions of higher edaphic and atmospheric moisture, transpiration remains at reduced levels (Fig. 5). A greater stability of this variable is noted in the dry period, suggesting that both varieties manage to keep their physiological functions as a consequence of their succulence and high water storage, especially in their parenchyma, which maintains the chlorenchyma in activity (Taiz et al. 2017, Souza et al. 2020).

It was also noted that, in the dry season, the internal CO_2 concentration was greater in both varieties than in the rainy season (Fig. 6). This trend to higher values is possibly linked to the effects of drought, such as the absence of soil moisture, increase in the atmospheric temperature and the temperature on the surface of the cladodes (Fig. 2B, C, D), causing a reduction in stomatal conductance (Fig. 3) and in CO_2 uptake (Fig. 4) since increased internal CO_2 rates affect stomatal opening, by which most stomata remain closed (Luttge 2002). Nevertheless, it is verified that this higher concentration in the dry season did not prevent the forage cactus varieties from performing their gas exchanges, even if at lower rates.

The forage cactus is one of the most efficient species regarding WUE compared to C_3 and C_4 plants, as evidenced by our results. It is still possible to verify that, in the rainy season, the highest WUE occurs at different times from the peak of CO_2 uptake (Fig. 7) due to the reduction in the transpiration rate late at night to the detriment of the maintenance of CO_2 uptake for a longer time, favored by environmental conditions such as the presence of edaphic moisture and lower temperatures. Lopes et al. (2020), fertilizing Guinea grass with 1,200 kg N ha^{-1}, obtained a maximum WUE of 6.31 μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}, while Oliveira et al. (2017), evaluating bean crops under different irrigation water salinities, did not verify WUE values above 2.0 μmol CO_2 m^{-2} s^{-1}/mmol H_2O m^{-2} s^{-1}. Compared to the results of the present study, it is verified that the forage cactus can surpass both C_3 and C_4 plants, regarding WUE, by up to 12 and 4 times, respectively. Therefore, it is seen that CAM plants present higher WUE, which may be directly related to the environment in which they live. Therefore, water availability is often limited, leading plants to withstand drought without severe metabolism damages since they compensate for it in other ways: for example, it is known that a CAM plant fixes much more carbon (10-40 g of carbon for each 1000 g of transpired water) than a C_3 or

C_4 plant (1-3 and 2-5 g, respectively) for the same amount of transpired water (Nobel 2009). Therefore, these differences suggest that the forage cactus (*Opuntia* spp. or *Nopalea* spp.) night be one of the productive solutions for the dry Northeast, not only for forage production but also for human feeding through cactus sprouts and fruit farming, especially in small dry land farming areas.

Studies of this nature, evaluating different varieties/cultivars/genotypes belonging to the same species, and even more to the same subgenus (in this case, *Nopalea*) are especially essential in genetic improvement programs that aim at selecting materials with higher gas exchange intensity, which may provide higher yields.

Conclusion

In both dry and rainy seasons, the forage cactus variety Baiana presents higher gas exchange intensity than the variety Miúda; In the rainy season, there was an increase in the gas exchange rates for both the evaluated varieties (Baiana and Miúda). In the rainy period, the peak of CO_2 uptake is from 1:00 a.m. to 3:00 a.m. for the variety Baiana and from 11:00 p.m. to 2:00 a.m. for the variety Miúda, while in the dry season, this peak is from 11:00 p.m. to 2:00 a.m. for both varieties, these being ideal intervals for measuring gas exchanges in the field.

Acknowledgements

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