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Physiological characterization in varieties of the genus *Schizolobium* under water deficit

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***Abstract*— *The genus Schizolobium has a species divided into two varieties: Paricá belonging to the Amazon forest and Guapuruvu belonging to the Atlantic Forest, both of importance for ecology and forestry due to the quality of the wood. As they are values-adding varieties, information on the physiological mechanisms that regulate the metabolism of plants in a particular environment becomes important to establish their level of adaptation. Thus, the aim was to analyze the possible physiological and biometric differences between the two varieties regarding the tolerance to drought when submitted to the water deficit. The work was developed in nursery, in the experimental field of the State University of Bahia. The design was completely randomized – CRD with 2 treatments and 200 replicates. Statistical analyzes were performed by software R (R CORE TEAM, 2017). The results showed that the growth analysis through the physiological indexes was efficient to identify differences in the initial growth of Schizolobium plants and the physiological characteristics of gas exchanges were negatively affected by water stress in both varieties during the days of stress.*

I. INTRODUCTION

The genus *Schizolobium* is composed of trees characterized by the fast growth and good quotation of the wood in the market. The varieties of this genus are found from Central America to the South of Brazil, presenting a wide distribution Neotropical with two varieties of very similar characteristics and difficult to distinguish. They are divided in S. *parahyba* var. *parahyba* (guapuruvu) and S. *parahyba* var. *amazonicum* (paricá) (Turchetto Zolet et al., 2012).

The use of *Schizolobium* occurs mainly in the North and Northeast of the country. These regions are historically recognized as having a poor annual rainfall distribution. Thus, the newly installed seedlings in the field are subject to water deficit. According to Carvalho (2005), empirical observations have shown that in the periods of irregular rainfall the recently installed plants in the field undergo severe water deficiency, caused by high levels of incident solar radiation and the characteristics of their leaves. As they are bipinnated, they present lower values of resistance of the boundary layer and thus submitted to great loss of water.

According to Pimentel (2004), water is the fundamental substance to life. There are strong indications that there will be greater climatic variations with more frequent droughts that will cause a great effect in the relation of the plant with the environment. It becomes indispensable to obtain varieties more tolerant to the drought with high productivity, aiming the planting in adverse conditions with water economy. Some studies have been conducted with the objective of discovering the physiological bases that would explain the differences in photosynthetic yield among varieties (Verissimo, 2010).

The water balance of the plant is controlled by leaf transpiration and the absorption of water in the soil that under adverse conditions such as water deficiency decreases the relative water content and leaf turgor. The water potential of the leaf also reduces with the decrease of the availability of water in the soil, leading to the loss of turgescence and stomatal closure (Mansur & Barbosa, 2000). Therefore, leaf water content in conditions equal to or lower than 75% significantly diminishes the photosynthetic activity (Smit & Singels, 2006). It is known that there is great intra and interspecific variability regarding the adaptability of species to drought. Thus, it is important to evaluate the physiological behavior of different genetic materials against water deficit, in order to allow a subsequent recommendation of their cultivation.

As they are value-adding varieties, this information is useful for improvement of character selection and foment cultivation in arid and semi-arid regions. According to the above facts, the aim was to analyze the possible physiological and biometric differences between the two varieties regarding the tolerance to drought when submitted to water deficit.

II. MATERIAL AND METHODS

The experiment was carried out in the Experimental Field nursery of the Federal University of Recôncavo of Bahia. After obtaining lots of seeds of each variety, both from Penápolis - SP, 200 seeds of each variety were removed and they were submitted to the overcoming of dormancy by mechanical scarification (manual friction of the seed on both sides above the radicle in iron sandpaper nº 60). After scarification they were soaked in water for 24 hours (Carvalho, 2005)

The sowing occurred in a polyethylene bag with a size of 24 cm in height and 12 cm in diameter containing as a substrate a mixture of soil and manure (bovine manure) in a ratio of 2:1. Manual irrigation was performed once a day and when necessary, twice (morning / afternoon). The mean temperature and relative humidity of the air (RH) were recorded inside the nursery during the whole conduction of the experiment, recording a minimum temperature of 21.6 ° C and a maximum of 30.5 ° C with a mean RH of 79.5 in the morning.

At 15 days after the complete appearance of the two pairs of leaves the growth analysis evaluations were started, being again performed at 30, 45, 60, 75, and 90 days. We selected 05 plants of each variety and determined stem height (SH), neck diameter (ND), leaflet numbers (LN), leaf area (LA). The SH was obtained with a graduated ruler, in which the distance between the surface of the substrate and the apex of the plant was measured. The ND was measured with a pachymeter on the surface of the substrate. The LN was obtained by direct counting and the leaf area was obtained by scanning all leaflets (HP scanjet 8300®), in tiff format with 200 dpi and processed in ImageJ® software **(**Rasband, 2011). After measurements, all the material was placed in a forced circulation air oven at 65 ºC until its complete drying and then weighed in analytical balance (in the initial phase at 15 days) and precision scale (during the experiment) to obtain the Dry mass of the different organs (DM).

The experimental design was completely randomized to a factorial of 2 x 6. The Dickson Quality Index DQI = TDM / (PH / SD) + SDM / RDM) was used to evaluate the quality standard of the seedlings. Statistical analyzes were performed by software R (R Core Team, 2017). The Pearson correlation test was performed. The results were submitted to analysis of variance, where the effects of the varieties of the days of evaluation and the interaction between the factors varieties and days were analyzed by the F test at 5% of probability. The effect of the varieties according to the days of evaluation was verified through regression analysis. In the choice of model, the biological explanation and the significance of the coefficient of determination R^2 were taken into account.

At 60 days after emergence (DAE), the seedlings were transplanted to black vases of 35 cm height x 14 cm in diameter and capacity for 4.65 liters containing soil. Then foliar fertilization was done with N sol. 14% H2O (189 g / l), P2 O5 sol. 7% H 2 O (94.5 g / l), 3% as phosphite, K 2 O sol. 5% H2O (67.5g / l), Mg sol. 1.5% H2O (20.25 g / l), B1 sol. 0.1% H2 O (1.35 g / l), Mn sol. H 2 O 1.5 (20.25 g / l), M.S. 0.05% H2 O (0.675 g / l), Zn sol. H2O 2% (27g/l) and 20 days were expected for root accommodation.

The physiological characters were measured with the aid of an infrared portable gas analyzer (LcPro +, ADC, UK.). The plants of each variety were selected according to the uniformity in height and number of leaves. Liquid photosynthetic rate (A), transpiration (E), stomatal conductance (gs) and concentration of CO2 (Ci) were evaluated and measured in fully expanded leaflets of the most recent mature leaf for a period of 1-2 minutes to allow equilibrium of the photosynthetic rate. These evaluations were done in 5 replicates of each treatment.

For carrying out the curves of response to radiation and CO$_2$, the methodology of Long & Hallgren (1993) was used with modifications in the radiation sequence (monitoring time and integration of the 3 minutes readings, with temperature of the leaves kept at 25ºC and radiations of 0, 30, 80, 130, 250, 450, 650, 850, 1100 and 1500 □mol.m^2. s^{-1}) . For the CO$_2$ response sequence were used concentrations of 410, 200, 50, 350, 500, 750, 1000 and 1300 ppm of CO$_2$, with leaf temperature of 26ºC and radiation saturation of 750 □mol.m^2.s^{-1} with monitoring and integration time of 5 minutes.

Initially the original response data to the radiation and CO$_2$ were used to calculate some parameters and these parameters were used for the adjustment curve. For the radiation was used the model of Smith of 1936, because this model gives a correlation (R) of 99.73%. For the CO$_2$ adjustment curve, the model of Ethier & Livingston (2004) was used through the A/Ci Curve Fitting program 10.0 xls, version 11 December 2013 (available at www.landflux.org/tools.php).

Soil moisture was monitored volumetrically (m^3/m^3), using the Decagon system (Pullman, WA, USA) with a 5TE probe that monitors the dielectric characteristics of the soil. In order to measure tolerance to water deficit, daily evaluations of the changes were carried out, starting with the fully irrigated seedlings and finalized after photosynthesis reached close to zero. All evaluations were performed at 8:00 a.m., 12:00 a.m. and 17:00 p.m. and the readings recorded after A and gs stabilized and when the coefficient of variation, measured by LcPro +, was less than 1.0. The results were submitted to analysis of variance. The standard error of the samplings was used in the graphs.

III. RESULTS AND DISCUSSION

In the present work, the behavior of Paricá (*Schizolobium parahyba Var. Amazonicum*) and Guapuruvu *(Schizolobium parahyba var. Parahyba*) showed different by analyzing of parameters biometric. There were statistical differences between the varieties and throughout the growing days during the evaluation period for the following variables: leaflet number (LN), leaf area (LA), stem dry mass (SDM) and neck diameter (ND).

Fig. 1 shows the behavior of the number of leaflets per plant and the Leaf Area. A joint analysis of the two parameters shows that initially (up to 30 days) the plants behave very similar with no difference in leaf area or number of leaflets. However, after this period there are differences between these parameters with Paricá presenting a larger leaf area until the end of the study (90 days). As the number of leaflets is different from the 30 days, but similar to the 90 days, the difference in leaf area can only be explained due to Paricá presenting larger leaflet size. It is clearly observed a compensation between leaf area and number of leaflets between the two varieties.

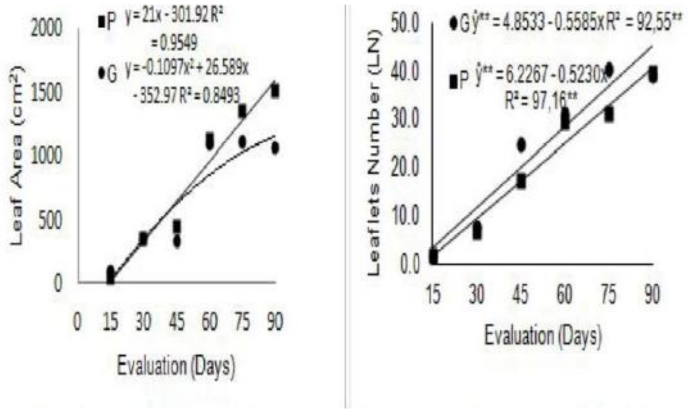

Fig.1 – Leaf area cm2 (AF) and leaflets number (LN) between paricá e guapuruvu varieties and throughout growing at 15, 30, 45, 60, 75 and 90 days. UFRB, Cruz das Almas, BA. 2020.

The relationship between leaf area and number of leaves is also known in several species, although it is complicated and difficult to understand due to the participation of other parameters of growth and response to environmental factors. By increasing the leaf area, the surface of absorption of total light radiation and the accumulation of photosynthetically active radiation (Caron, 2012) are increased. According to Barbieri Júnior *et al.*. (2007) when working with Jatobá, the high leaf area at the beginning of the development of the plants indicated that, there was investment in the development of the leaves for the capture of light radiation. Later, due to the maturation of the leaves and the direction of the photo-assimilates to other parts of the plant occurred the decrease of the photosynthetically active leaf area.

Fig. 2 shows the relationships between plant height and neck diameter for Paricá and Guapuruvu. There was no statistical difference for the height parameter among the varieties but for the parameter of neck diameter, there were observed significant differences between the varieties from 60 days with Guapuruvu having a larger neck diameter. The results of height and diameter are in agreement with those obtained by Caron (2010), evaluating *S. parahyba.* The neck diameter is considered a significant parameter to estimate the survival in the field of several forest species. The quality standard of the seedlings ready for planting correlates with this parameter, and this can be verified by significant increases in survival rates and plant growth in the field, according to a study by Binotto (2010) for eucalyptus.

Height and diameter showed a negative correlation by Pearson's correlation (Fig. 5), showing that smaller plants presented larger diameter. Similar results were found by Rego & Possamai (2006), evaluating the growth of the Jequitibá-Rosa. In Binotto's work (2010), he analyzes growth variables in forest species and shows that the height variable was only efficient to indicate the quality of seedlings when analyzed along with the diameter. According to Rosa (2009), the root collar diameter and the total dry matter are important morphological parameters together with the Dickson quality index (DQI) to evaluate the quality of Paricá seedlings produced under nursery conditions.

In order to avoid errors in selecting higher but weak seedlings, by discarding smaller ones but with greater vigor, the morphological parameters and the relationships used to evaluate the quality of seedlings should not be used in isolation. Thus, a good indicator of seedling quality is DQI, since in its calculation several important parameters used for quality evaluation are considered (Fonseca, 2002).

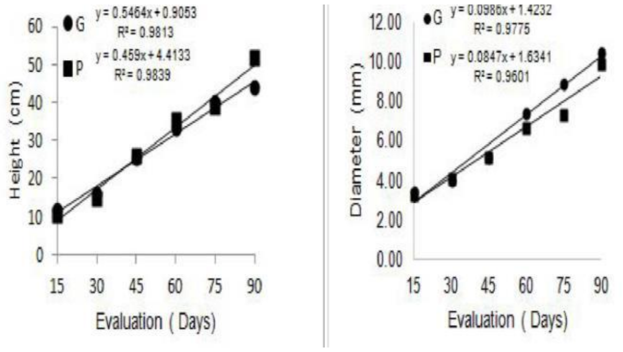

Fig.2: Height (cm) Neck diameter (mm) between Paricá e Guapuruvu varieties and throughout growth at 15, 30, 45, 60, 75 and 90 days. UFRB, Cruz das Almas, BA. 2020.

Fig. 3 shows the behavior of the biometric parameter of Stem Dry Mass for the Paricá and Guapuruvu varieties. There were differences in the accumulation of dry mass of the stem (Fig. 3), which was higher in guapuruvu from the 30th day, which is probably justified because it also has a larger diameter from this period.

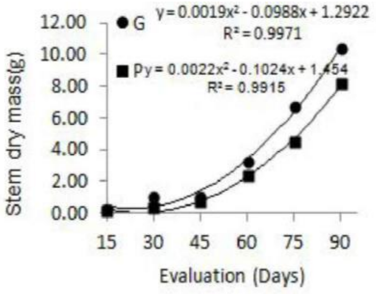

Fig.3: Stem dry mass (g) between Paricá and Guapuruvu varieties and throughout growth at 15, 30, 45, 60, 75 and 90 days. UFRB, Cruz das Almas, BA. 2020.

In the Dickson Quality Index (DQI) evaluation, no significant difference was observed between treatments, indicating that both varieties presented quality seedlings (Table 2). The higher the value of this index, the better the quality standard of the seedlings (Gomes & Paiva, 2012). For the parameter (H/D) R there was a significant difference between the treatments.

Table 2 – Mean seedling test of S. parahyba var. amazonicum and S. parahyba var. parahyba. UFRB, Cruz das Almas, BA. 2020.

Varieties	Parameters used to evaluate seedling quality		
	DQI	R (SDM/RDM)	R (H/D)
Paricá	1,59 a	5,39 a	5,20 a
Guapuruvu	1,74 a	6,80 a	4,20 b

Dickson quality index (DQI), shoot dry mass / root dry mass ratio (SDM / RDM) R, height / diameter ratio (H / D) R. Means followed by the same letter do not differ statistically from each other by the F test at 5% significance.

There was greater height/diameter ratio for Paricá 5,20 R (H/ D). According to Araújo (2017), the lower their value, the greater the ability of the seedlings to survive. Jose *et al.* (2009) consider that seedlings of high quality forest species should present H/ D indexes lower than 10, and when they meet these indexes, they present higher survival after planting. According to Araújo (2017), this method of non-destructive evaluation represents the balance of plant growth. The author, when evaluating the quality of Paricá seedlings, obtained H/ D ratio (5.47), (6.98), (6.29) and (6.35).

Rossa *et al.* (2013) evaluated the growth of Paricá seedlings for the same parameter and obtained results between (6, 31) to (7,18) and considered these values as a good development in height in detriment of the neck diameter. In this study, both varieties had a value lower than 10 with a lower average for Guapuruvu with 4.20. Garcia (2015) evaluating Guapuruvu seedlings, obtained a better H / D ratio of 3.23.

There was no statistical difference for (SDM /RDM) R. Caione (2012) obtained results ranging from 3.18 to 3.64. According to the author, there is no standard index, which is the most indicated for seedlings of *Schizolobium*. In this work, results of 5.39 were obtained for Paricá and 6.80 for Guapuruvu. Importance should be given to the variables of the root system of seedlings together with the study of their morphological parameters to ensure better performance in the field (Binotto, 2010).

According to Pearson's correlation analysis (Fig. 5), the dry matter variables are the most strongly correlated with the Dickson quality index (DQI), followed by the base diameter in agreement with the results obtained by Binotto (2010) in seedlings of eucalyptus. According to Fonseca *et al*. (2002), in order to obtain a quality parameter of the seedlings, it is necessary to take into account the evaluation of some variables of the morphological and physiological characteristics of the seedlings because they significantly influence in the quality standard.

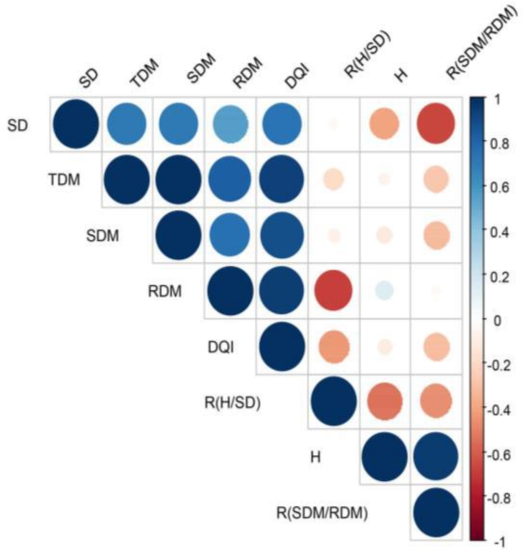

Fig.4: Correlogram based on Pearson's correlation in seedlings of S. parahyba var. amazonicum and S. parahyba var. for biometric variables: Stem Diameter (SD), Total Dry Mass (TDM), Shoot Dry Mass (SDM), Root Dry Mass (RDM) Dickson quality index (DQI), Height (H). UFRB, Cruz das Almas, BA. 2020.

Observing the parameters of gas exchanges (fig. 6), there was interaction between the varieties and the evaluation schedules for the gas exchanges A (Photosynthesis), gs (Stomatal Conductivity) and Ci (Internal CO_2 Concentration) with greater assimilation of CO_2 and greater stomatal conductivity in the morning for the two varieties. The stomatal conductivity drops at noon for both varieties, but there is a more significant decrease in gs in Paricá.

According to Taiz *et al.* (2017), the stomatal activity is an important variable in the balance between the gain of photosynthesis and the loss of transpiration. The loss of turgidity causes the closure of the stomata and this is one of the first adjustments developed by the plants to avoid the continuous loss of water.

Fig. 6 shows the behavior of photosynthesis and transpiration for both varieties. We can observe that already at 8 o'clock in the morning, both varieties present high photosynthetic and transpiratory rates. In the interpretation of these data, it can be noted that Paricá maintains the highest photosynthetic rates of Guapuruvu during the morning, but this changes at midday (Fig. 6). Such behavior of photosynthesis between the two varieties may have been possibly due to the reduction in stomatal conductance (Fig. 7), which was significantly lower in Paricá at noon, limiting the assimilation of CO_2.

Radiation intensity may also cause differences in the photosynthetic rate between varieties. However, in this case the two varieties have the same availability and luminous intensity as can be evidenced in Figure 8. It can be seen that the varieties received almost the same amounts of photosynthetically active radiation for all data monitoring schedules of gas exchanges. Data on the photosynthetic behavior of the genus *Schizolobium* practically do not exist in the available scientific literature.

However, Marenco *et al.* (2014) working with species of the Amazon forest, observed that the photosynthesis of these species remains high in the morning until reaching a peak, which occurs before noon, steadily declining in the afternoon. According to the authors, this occurs because at the leaf level the photosynthesis increases with the irradiance until reaching saturation point (SP). From this point, increases in irradiance do not cause increases in photosynthesis. Santos Lopes *et al*. (2015a and 2015b) while studying the behavior of S. *parahyba* under different shading intensity, obtained photosynthetic rates lower than those found in this work for plants of similar ages. Cordeiro (2006) found oscillation of 8.85 mmol CO $m^{2}s^{1}$ at 10.45 mmol CO $m^{2}s^{1}$, evaluating physiological characteristics of paricá seedlings from different sources.

Considering the behavior of the transpiration (Fig. 6), it can be noted that there was no difference between the varieties during the evaluation periods, being at 12 a.m. the period of greatest loss of water by transpiration for both varieties as well as of the highest luminous intensity (Fig. 8). Such behavior is discussed in the relevant literature and is a reflection of the increase in the water demand of the plant for the 12 o'clock time, where the plant undergoes a heat load and the increase of evaporative demand. Interesting to observe the reduction of stomatal conductivity between the two varieties, which affected the photosynthetic rate of Paricá, does not seem to have affected the transpiration of the varieties. In this case, the plants were in good availability of water because this is the period where they were being irrigated, which makes us understand that the absorption of water from the soil was sufficient to maintain the rates of transpiration. The action of stomata seems to be of great importance for the Paricá variety in the maintenance of transpiration rates. According to Kramer (1983) the stomatal conductivity can affect transpiration and photosynthesis differently. The influence of conductivity on transpiration is mainly stomatal, but for photosynthesis, it may be both stomatal and non-stomatal.

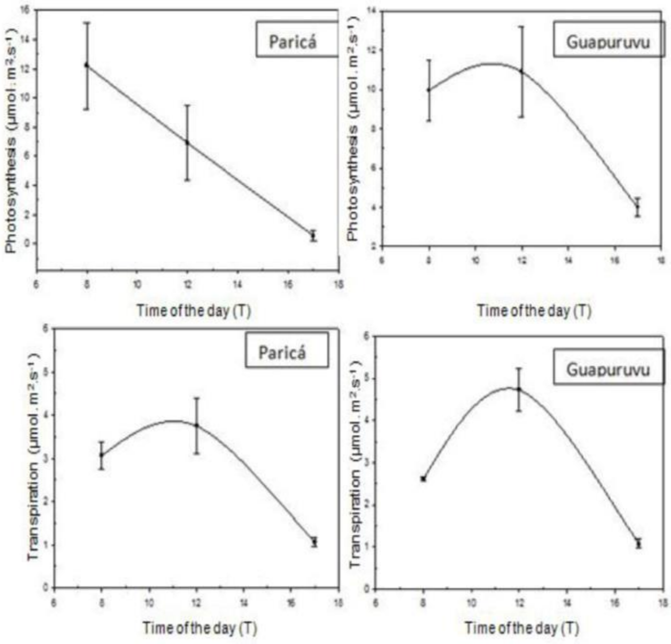

Fig.6: Behavior of photosynthesis and transpiration at 08:00 a.m., 12:00 a.m and 17:00 p.m. in Paricá and Guapuruvu seedlings, respectively. UFRB, Cruz das Almas, BA. 2020.

Figure 7 shows the behavior of the internal CO_2 concentration (Ci) in the leaf stomatal chambers of the two varieties. It can be noted that Guapuruvu has a higher internal concentration of CO_2 than Paricá. This is because Paricá has always presented lower stomatal conductivity, except 5 p.m. when the two varieties are practically equals. Thus, the higher rate of photosynthesis at noon in Guapuruvu is linked to a considerable amount of internal CO_2 concentration now that the plants were with greater stomatal conductance.

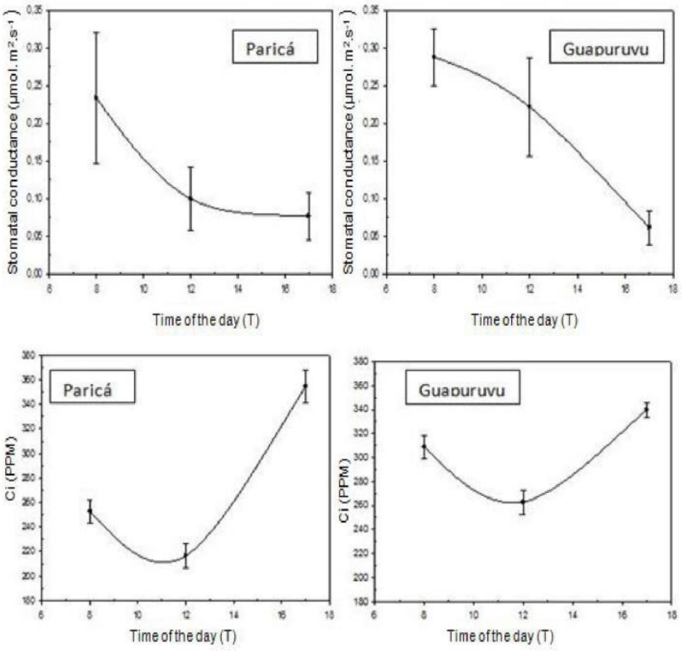

Fig.7: Stomatal conductivity and internal CO_2 concentration (Ci) at 08:00 a,m,, 12:00 a,m, and 17:00 p.m. in Paricá and Guapuruvu seedlings, respectively. UFRB, Cruz das Almas, BA.2020.

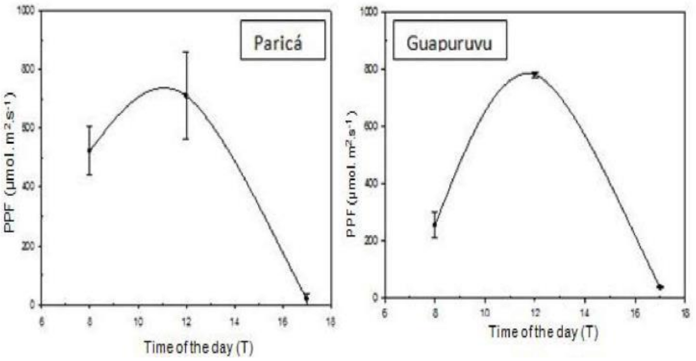

Fig.8: Photosynthetic photon flux (PPF) at 8:00 a.m., 12:00 p.m. and 5:00 p.m. in Paricá and Guapuruvu seedlings, respectively. UFRB, Cruz das Almas, BA. 2020.

The soil moisture condition during the study that started with wet soil with values of 0.150 m3 / m3, which indicates the field capacity (with a tension of 0.1 atm). After 5 days of water deficit, it presented values near zero with a water content of 0.050 m3 /m3, reaching the permanent wilting point (PWP) (with a tension of 15 atm). The scientific literature suggests that in the permanent wilting point the amount of water that is in the soil can no longer be absorbed by the plant (TAIZ *et al*., 2017).

When submitted to the water deficit, it was possible to observe that, for the evaluated parameters of gas exchanges, the varieties presented similar behavior, reaching values of photosynthetic rate close to zero during the afternoon with five (5) days of water deficiency. The values of A and E presented by the varieties decrease as the water deficit progresses, reaching values close to zero when the water restriction is more severe.

The interactions of varieties x evaluations (hour) were not significant for any of the varieties tested during the evaluation days. Thus, regardless of the varieties, the period that gave the greatest CO_2 assimilation was at 8 a.m. and the highest transpiration rate was at 12 a.m., differing statistically from the other periods evaluated. For stomatal conductance (gs), the highest value was observed in the period of 8 a.m. and at 5 p.m. These two periods did not differ statistically. The lowest value for Ci was in the 12-hour period due to the decrease in stomatal conductivity.

At 5 days of stress the net assimilation rate of CO_2 reached values close to zero and the seedlings showed partial fall of the leaflets. According to Rocha (2017), when evaluating the development of Paricá seedlings under the effect of water stress, it was observed that from 7 days there was a decrease in the number of leaflets. This shows that when subjected to periods of drought the plant first reduces its individual leaves to stabilize its metabolic process, ensuring their survival before aborting the other vegetative parts.

According to Duarte (2016) and Rocha (2017), paricá seedlings present low tolerance to water deficit ar because of stress, reduce leaf numbers in order to maintai water. Chaves *et al*., (2004) points out the reduction of le area as one of the first forms of defense of plants again water deficiency in order to decrease tl photosynthetically active area and thus reduc transpiration.

There were no statistical differences in the chlorophy content of the varieties studied for Chlorophill A and I The determination of the chlorophyll content in the leav is important to estimate the photosynthetic potential of tl plants by their direct connection with the absorption ar transfer of light energy that will reflect in the grow (Rego & Possamai, 2006).

The curves in response to photosynthetically active radiation are shown in Fig. 9 and 10. These are important because they show various aspects of photosynthetic apparatus and how they react to the radiation absorption condition. In Fig. 9 and 10 it can be seen that the Paricá reached a higher photosynthetic rate when the saturation of the radiation of photosynthesis occurs. However, practically the two varieties had this point reached at 200 mol.m^2.s^{-1}.

These varieties saturate with a much lower radiation availability than most tropical forest species, as stated by Marenco (2001) and Taiz *et al*., (2017) most plants saturate at irradiances of 500 to 1000 µmol m^2 s$^{-1,}$. According to Kerbauy (2008), when the luminous intensity exceeds 200 µmol m^2 s^{-1}, (10% of full solar radiation), the increase in luminous intensity does not entail anymore a proportional increment up to about 500 to 1000 µmol m^2 s^{-1}. However, we must admit that species with the potential for rapid growth take advantage of presenting such response mechanisms because they are constantly in competition with other species by radiation and are able to take advantage of low incidences.

Another important aspect is that, according to Long & Hallgren (1993), the slope of the exponential part of the curve response indicates the carboxylation rate of Rubisco. For this reason, Paricá presents a slightly higher carboxylation rate than Guapuruvu and, therefore, may have a higher respiration rate as evidenced in Figure 16, because the rubisco reacts with both CO_2 and O_2.

The study of the A/Ci response curve (Fig. 11 and 12) shows that these varieties suffer a reduction of photosynthesis caused by a resistance effect of CO_2 diffusion in the mesophile, which reduces its photosynthesis rate by approximately 1 µmol. m^2.s^{-1} (compare the value indicated by the arrow and the value of the origin of the slope of the curve). Study according to Farquhar & Sharkey (1982).

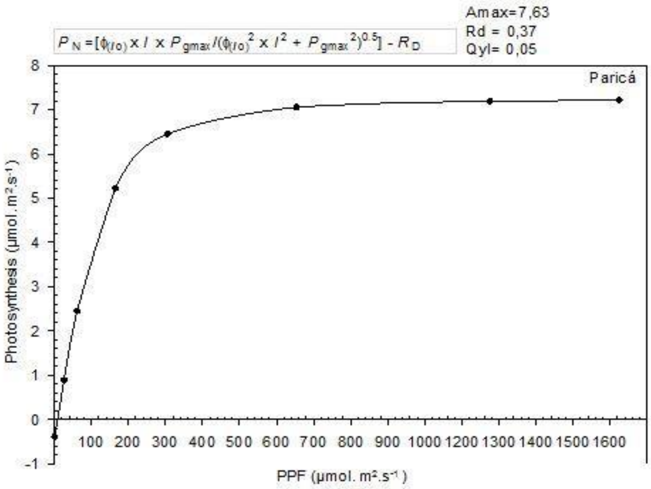

Fig.9: Response of paricá to the variation of the photosynthetically active radiation. Curve adjusted according to the formula in the graph. UFRB, Cruz das Almas, BA. 2020.

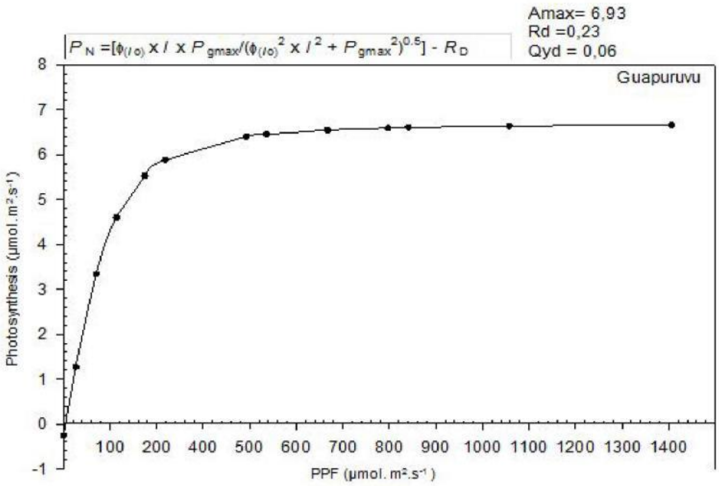

Fig.10: Response of guapuruvu to the variation of the photosynthetically active radiation. Curve adjusted according to the formula in the graph. UFRB, Cruz das Almas, BA. 2020.

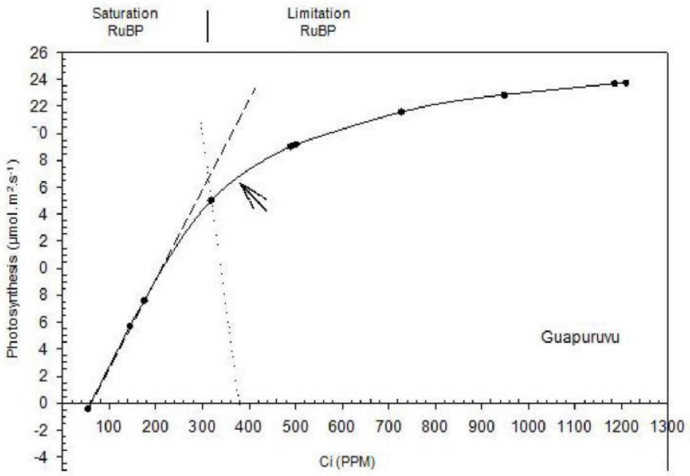

Fig.11: Figure 16 - A / Ci response curve Guapuruvu seedlings. UFRB, Cruz das Almas, BA. 2020.

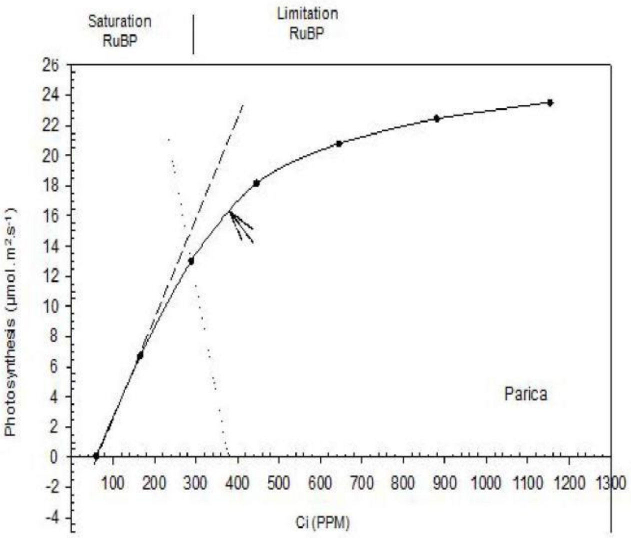

Fig.12: A / Ci response curve for Paricá seedlings. UFRB, Cruz das Almas, BA. 2020.

IV. CONCLUSION

Growth analysis through physiological indexes was efficient to identify differences in the initial growth of *Schizolobium* plants.

The physiological characteristics of gas exchanges were negatively affected by water stress in both varieties throughout stress days.

Although the curves of radiation response and CO2 concentration can serve to identify genetic differences between varieties, the *Schizolobium* varieties used in this study had similar behavior and did not serve as an evaluative parameter.

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