Effects of Shading on Anatomical Aspects and Chlorophyll and Carotenoid Contents in Paricá

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Abstract

Paricá (*Schizolobium parahyba* variety amazonicum) is an important species for reforestation programs in the Amazon; nevertheless, the anatomical and physiological parameters of the seedlings of this species under shading are not yet fully understood. This study evaluated the chlorophyll and carotenoid contents, the stomatal structure, and the stomatal position in the leaf epidermis under shading of paricá seedlings. The experiment comprised a randomized block design in a subdivided plot scheme, with two sources (Belterra and Urupá) and different shading levels (full sun, 30%, 50%, and 70%). At 100 days after sowing, we performed the analyses of photosynthetic pigment concentration and leaf anatomy. We found that the leaf is hypoestomatic with paracitic stomata and unicellular filiform trichomes on both sides. Higher levels of shading increased the contents of chlorophyll a and b and reduced the stomata density and carotenoid contents in the leaves of young paricá, showing that paricá has phenotypic plasticity to shading.

Keywords: Schizolobium parahyba var. amazonicum, leaf anatomy, pigment

1. Introduction

Paricá (*Schizolobium parahyba* variety amazonicum (Huber × Ducke) Barneby) belongs to the Fabaceae family and is native to the Amazon region (Rosa et al., 2009; Epifanio et al., 2022). In Brazil, paricá is found in the states of Amazonas, Pará, Mato Grosso, and Rondônia. Paricá grows rapidly and can reach a height of about 30 m (Epifanio et al., 2022) with great potential use in reforestation and recovery of degraded areas (Binotti et al., 2019; Sousa et al., 2022).

The production of forest species seedling for the recovery of degraded areas and commercial plantations is expanding, requiring the development of different species to produce high-quality seedlings to ensure success of forest stands. Therefore, the effects of environmental factors, such as light, need to be evaluated to analyze the behavior of species of different successional groups.

Sunlight is an important meteorological element for plants with direct or indirect action on plant growth and development. Changes of light intensity in seedling nurseries influence the photosynthetic apparatus of plants, increasing the efficiency of energy absorption and the transfer to the photosynthetic processes (Taiz et al., 2017; Kerbauy, 2019). However, inadequate high levels of solar radiation compromise plant growth and development; thus, the contents of chloroplastid pigments, chlorophyll, and carotenoids can be used as important parameters of plant behavior under different environmental conditions.

In general, light intensity during plant growth and development influences their performance (Gondim et al., 2008) and leaf anatomy is one of the most affected aspects, since the leaf is a plastic organ and its internal

structure adapts to external environmental conditions (Schluter et al., 2003; Aragão et al., 2014). The morphological characteristics of the leaf surface established the amount of light absorbed or reflected. Studies have shown the positive effect of shading on the morphophysiological aspects in some forest species, such as *eucalyptus* and paricá (Sousa et al., 2022).

Studies have investigated the anatomical and ecophysiological aspects of forest species; however, these studies focused mostly on planted forest of *eucalyptus*, not on native Amazonian species. Therefore, this study investigated the chlorophyll and carotenoid contents, the stomatal structure, and the stomatal position in the leaf epidermis under shading in paricá seedlings.

2. Method

2.1 Study Site

The experiment was conducted in the greenhouse of Embrapa Amazônia Oriental, Belém, Pará State, Brazil (01°24'31.6637" S, 48°27'45.1786" W). The nursery phase was carried out from February 2011 to August 2011. The regional climate is type Af (Köppen classification) (Alvares et al., 2013), with an average annual temperature of 26 °C. Annual average of regional precipitation is around 3000 mm (Bastos et al., 2002).

The seeds used in the research were obtained from two natural tree populations in the Brazilian Amazon. One from the municipality of Belterra in the Mesoregion Baixo Amazonas and the Microregion Santarém, both in Pará State, with geographical coordinates 02°38'11" South and 54°56'14" West. The other from the Municipality of Urupá located in the micro-region and Ji-Paraná, central region of Rondônia State on parallel 13°07', with geographical coordinates 11°06'47.2" South and 62°17'38.3" West.

The seeds were stored under refrigeration at 15 °C at the Laboratory of Forest Seed Analysis (Embrapa Amazônia Oriental, Belém, Pará State, Brazil) until their use in the experiment.

Seeds with tegumentary dormancy were scarified with sandpaper No. 100 and immersed in water at room temperature for 24 hours (Brasil, 2009). They were then sown directly into black polyethylene bags measuring 15×20 cm with lateral perforations using a substrate of black earth, tanned manure, and tanned sawdust at the ratio 3:2:1, respectively.

The experiment followed a randomized block design in subdivided plots 2×4 with factor A = origin: Belterra (PA) and Urupá (RO), factor B = shading levels with black "sombrite" cloth: 0%, 70%, 50%, and 30% shading with three blocks and 25 plants per plot. The reduction of solar radiation was confirmed by using a pyranometer.

At 100 days after planting, two plants were selected from each shading level. The leaflets were collected from the median region of the rachis, fixed in FAA 70 for 24 h, and stored in 70% alcohol for the anatomical analysis (Johansen, 1940).

2.2 Dissociation of the Epidermis

Sections of the median region of the leaflet were dissociated using commercial sodium hypochlorite at intervals of 4 to 5 h until complete separation of the epidermis. After complete separation, the adaxial and abaxial epidermises were washed in distilled water and then stained with 1% safranin stain.

2.3 Obtaining Histological Sections

To obtain cross sections, the median region of the leaflets underwent the usual microtomy processes: dehydration in ethyl series (butyl alcohol), infiltration in Leica synthetic resin (hydroxyethyl methacrylate), blocking, sectioning in a rotating microtome (7-5 μ m thick), and staining with 0.05% toluidine blue (O'brien et al., 1965).

2.4 Stomatal Density

Three leaflets were collected from each shading level of both plant varieties and eight random fields were counted from the apical, median, and basal regions of both sides, totaling 48 measurements per leaflet. The counting was performed with the aid of a millimeter eyepiece in a Zeiss Axiolab DRB-KP optical microscope. Each visual field had an area of 0.2 mm². The data obtained were expressed in number of stomas per square millimeter.

2.5 Obtaining Photomicrographs

The slides were observed under an optical microscope coupled to a digital camera and photomicrographs were captured at various magnifications with the aid of a Zeiss Axiolab HBO 50 microscope with a motican 2500 3.0 Mega Pixel digital camera (Plant Anatomy Laboratory-Embrapa Amazônia Oriental).

2.6 Determination of Chlorophyll and Carotenoid Contents

The contents of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were determined in one plant of each treatment and repetition. Two fully expanded leaves were removed, immediately packed in a Styrofoam box with ice and taken to the laboratory where four leaflets were removed from each leaf. The leaflets were macerated and eight sub-samples (0.30 mg/leaf) of fresh material were weighed and transferred to test tubes with 5 mL of DMSO (dimethylsulfoxide, 99% purity by volume).

The test tubes were closed with rubber caps and placed in water bath with preheated water at 70 $^{\circ}$ C and centrifuged (3,600 rpm) for 2 h for chlorophyll solubilization. The extraction process was considered complete when the sample leaves became transparent in the visual examination (Arnon, 1949). After, readings were taken in a spectrophotometer (Beckman, model 640 B) at wavelengths of 645 and 663 nm.

The extraction and quantification of total carotenoids were performed according to the methodology described by Duke and Kenyon (1986), using the molar absorptivity coefficients of Sandmann and Böger (1983).

2.7 Statistical Analysis

Averages of variables were evaluated using Shapiro-Wilk and Bartlet tests (p > 0.05) to verify normality and homoscedasticity. Data that did not achieve the assumptions of parametric tests were transformed using the Box-Cox method (Box & Cox, 1964). Next, the variables were evaluated in the variance analysis followed by Tukey test at 5% probability and the means were compared using the BioEstat 3.0 program (Ayres et al., 2003). Regression equations of the polynomial type were adjusted for the different variables analyzed as a function of the shading level.

3. Results and Discussion

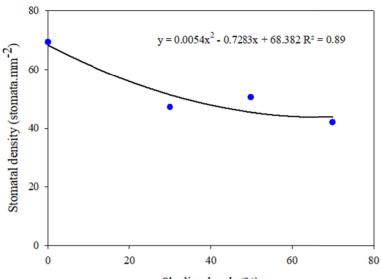
We found significant effects (p < 0.05) only for the shading factor on the variables stomatal density (DE), chlorophyll a (ca), chlorophyll b (cb) and total chlorphyll (ct). On the other hand, there was origin × shading interaction for the carotenoid contents (carot).

Table 1. Mean squares and significance level for stomatal density (DE), chlorophyll a (ca), chlorophyll b (cb), total chlorphyll (ct) and carotenoid contents (carot.) in leaflets of *Schizolobium parahyba* var. amazonicum in different origins and shading levels at 100 days

Source of variation	Df	DE	Ca	cb	ct	carot
Origin	1	10.01 ^{ns}	1.93 ^{ns}	3.99 ^{ns}	11.49 ^{ns}	19.38 *
Blocks	2	45.84 ^{ns}	6.18 ^{ns}	4.67 ^{ns}	19.59 ^{ns}	3.92 ^{ns}
Residual A	4	8.93	4.39	3.39	14.80	2.90
Shading	3	855.17 **	37.76**	18.62**	107.49**	57.60**
Origin × Shading	3	3.66 ^{ns}	1.49 ^{ns}	2.42 ^{ns}	7.30 ^{ns}	20.79**
Residual B	12	19.71	6.05	2.57	14.15	2.62
Total	23	2932.55	447.77	231.73	1191.13	418.38

Note. df = degree of freedom; * Significant at the 0.05 level of error probability, ** Significant at the 0.01 probability level of the error and ns: not significant at the 0.05 level of probability by the F test.

The paradermal sections in leaf blades showed that the highest values of stomatal density were observed in the full sun treatment (69.35 mm²). In the shades of 30, 50, and 70%, the number of stomas was lower than in full sun, with a reduction of 68%, 72.9%, and 61% respectively (Figure 1).



Shading levels (%)

Figure 1. Mean of number of stomata in plants of Schizolobium parahyba amazonicum under shading levels

The larger number of stomata in plants under full sun may be related to the successional group of the species, classified as pioneer (Schwartz et al., 2017; Smychniuk et al., 2020), thus with adaptability to conditions of high solar irradiance. Dalmolin et al. (2015) observed no statistical difference in the stomatal density of young plants of *Curatella americana* L. grown in full sun and shade (76% shade).

The positions and the number of stomas in the leaf show a classification as hypo-stomatic, with the occurrence of stomas only on the abaxial side, and as the paracitic type with rare occurrences of anomocytic stomas (Figures 2 and 3). Besides the stomas, we observed the presence of unicellular filiform tector trichomes in both sides in *S. parahyba* v. amazonicum in the samples Urupá and Belterra, mostly on the abaxial side (Figures 2A and 2E). These structures are probably related to the reduction of transpiration in pioneer species, which are subjected to intense light.

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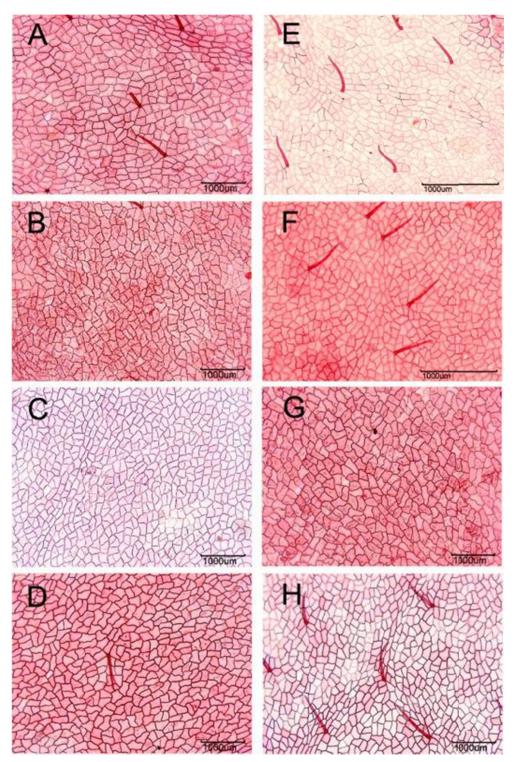


Figure 2. Frontal view of the adaxial epidermis of paricá leaves from two sources submitted to four shading levels. Origin from Belterra: A-D: A-30%; B: 30%; C: 70%; and D: 0% shade, respectively. Origin from Urupá E-H: E: 30%; F: 50%; G: 70%; H: 0% shade, respectively

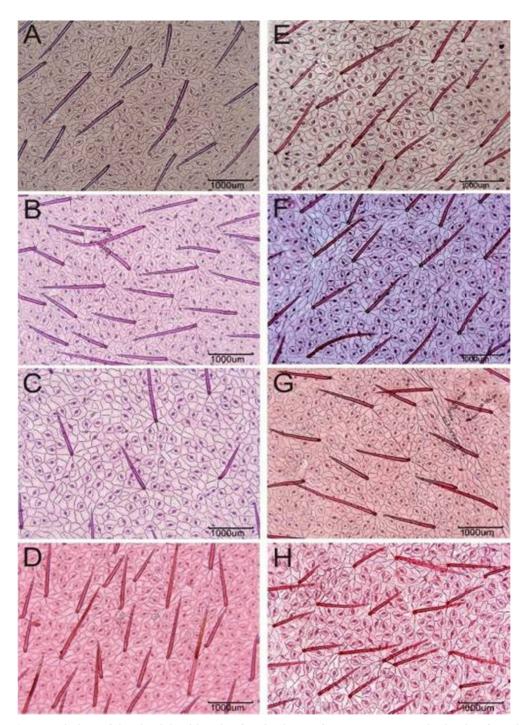


Figure 3. Frontal view of the abaxial epidermis of paricá leaves from two sources submitted to four shading levels. Origin from Belterra: A-D: A-30%; B: 50%; C: 70%; and D: 0% shade, respectively. Origin from Urupá E-H: E: 30%; F: 50%; G: 70%; H: 0% shade, respectively

The highest contents of chlorophyll a, chlorophyll b, and total chlorophyll were observed at 30% shade (8.46, 5.53, and 13.99 mg g⁻¹, respectively), while the lowest contents were found at 0% shade (4.85, 3, 19, and 3.99 mg g⁻¹, respectively) (Figure 4). In plants under full sun, the contents of chlorophyll a, chlorophyll b, and total chlorophyll reduced compared to plants under shading (Figure 4). Li et al. (2019) reported increased the chlorophyll contents in Vernicia fordii under shading and Wang et al. (2021) also found the same behavior in *Pinus*.

Albuquerque et al. (2015) observed higher contents of chlorophyll a, chlorophyll b, and total chlorophyl in seedlings of Brazil nut grown under 75% shading and in full sun. On the other hand, Sousa et al. (2022) found no significant difference for the contents of chlorophyll a, b, and total chlorophyll in *Eucalyptus urograndis* and *Schizolobium parahyba* var. amazonicum under different shading levels (0%, 50%, plastic, 50% + pet wool).

Epifanio et al. (2022) analyzed the effect of shading levels on morphophysiological characteristics in two varieties of *Schizolobium parahyba* and observed a significant effect of shading on photosynthetic pigments, with lower concentrations at 70% shading.

In this study, we observed that the concentration of total chlorophyll was higher under 30% of shade (Figure 4). Studies relating the pigment content of sun and shaded leaves show that the total chlorophyll contents are higher in shaded leaves when compared to leaves under full sun (Carvalho et al., 2007; Chaves et al., 2008).

Paricá plants grown at 0% shade had lower total chlorophyll content; however, this reduction is due to the lower irradiance in the treatments under shade. These results reveal that Paricá plants have different strategies in the accumulation and use of photosynthetic pigments in environments with variations in light availability and spectral composition.

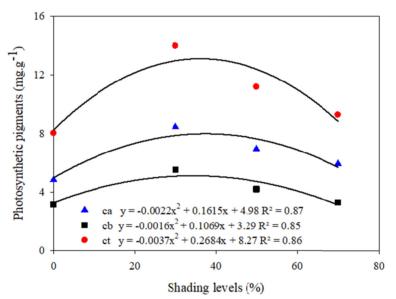


Figure 4. Contents of chlorophyll a (ca), chlorophyll b (cb), and total chlorophyll (ct) in plants of *Schizolobium* parahyba variety amazonicum from different sources and shade levels

This difference in the production of the photosynthetic pigments is due to a physiological adaptation of plants under shading, which amplifies the efficiency of light capture (Azevedo, 2014; Figueiredo, 2019). According to Dymova and Golovko (2007) and Lichtenthaler and Babani (2007), sun-exposed leaves respond to high irradiance and reduce the portion of chlorophyll that composes the antenna complex; however, we did not observe a difference in total chlorophyll contents in the treatments in our study.

The results found for the chlorophyll b contents show significant differences for the shading factor with the highest value observed at 30% shade (5.53 mg g⁻¹) (Figure 4). Studies indicate that the higher chlorophyll b concentration in plants exposed to low light intensity refers to a mechanism of acclimation to low light (Rego & Possamai, 2006; Dias & Marenco, 2007). Plants use chlorophyll b to capture energy at other wavelengths previously absorbed by the canopy leaves, transferring the absorbed energy to chlorophyll a of the reaction center (P680) of photosystem II, which initiates the photochemical process of photosynthesis (Taiz et al., 2017).

The contents of carotenoids form the Belterra source presented the highest values in full sun (75.02 mg g⁻¹); however, the contents reduced sharply with the increase of shading levels 30%, 50%, and 70% (35.8, 37.18, 14.40 mg g⁻¹). The Urupá source showed the same behavior; nevertheless, the carotenoid content was slightly higher than the Belterra source at 70% of shading (Figure 5). Other studies have found this same behavior in forest species (Epifanio et al., 2022).

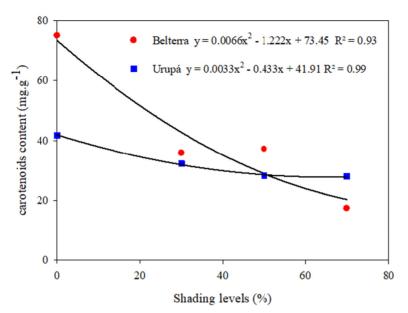


Figure 5. Carotenoid content in *Schizolobium parahyba* variety amazonicum plants from different sources and shade levels

The high carotenoid contents in full sun are linked to its function in the plant, as carotenoid is an accessory pigment to capture energy as well as a photoprotective agent of plants, preventing photooxidative damage to chlorophyll molecules when the plant is subjected to high solar irradiance (Taiz et al., 2017). Excess light energy can lead to the production of toxic compounds such as peroxides, superoxides and singlet oxygen, which can generate damage to the photosynthetic apparatus (Kerbauy, 2019). Due to their role as antioxidants, these pigments interact with the toxic compounds, preventing and/or reducing the occurrence of harmful processes (Taiz et al., 2017).

4. Conclusions

Higher levels of shading increased the contents of chlorophyll a and b and reduced the stomata density and carotenoid contents in the leaves of young paricá, showing that paricá has phenotypic plasticity to shading.

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