



Modifications in morphophysiological characteristics of *Garcinia brasiliensis* leaves in response to seasonal variations

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Abstract

The environment in which plants are inserted offers numerous stimuli that can influence plant ecophysiology and metabolism. *Garcinia brasiliensis* is a plant species of high interest for secondary metabolites extraction, however the comprehension about its behavior in response to environmental stimuli is still scarce. Thus, this study aimed to identify the influence of seasonality on the ecophysiology, biochemistry and anatomy of *G. brasiliensis*, aiming to contribute for the preservation and conservation of native populations and the propagation of this species. Evaluations were carried out under field conditions on six-year-old adult plants, in four replications over four months of the year (May, September, November and February). The evaluations were carried out in the middle third of the canopy, in fully expanded leaves. Gas exchange, chlorophyll “a” fluorescence, relative chlorophyll content, leaf water potential, carbohydrate, protein and amino acid content, and leaf anatomy were assessed. Gas exchange and production of carbohydrates and proteins were higher in November, when there was the highest rainfall rate. The maximum efficiency of the photochemical process in PSII was higher in February. Seasonality influences gas exchange, chlorophyll “a” fluorescence, production of primary metabolites and the nutritional status of *G. brasiliensis* plants. Anatomical changes are the least pronounced due to seasonal variations. Plants were more active metabolically in November, a month typical of spring in Brazilian tropical climate, which also corresponds to the growth season.

Keywords Chlorophyll fluorescence · *Clusiaceae* · Gas exchange · Leaf anatomy · Primary metabolism

Introduction

Environmental stimuli such as light, temperature, rainfall, nutrition, plant age and phenological stage can influence plant ecophysiology and metabolism (Morais 2009). Thereby, plants develop morpho-anatomical and physiological adjustments to cope with environmental variations aiming to survive and maximize the use of environmental

resources (Poorter and Nagel 2000; Li et al. 2020). For example, throughout the year, seasonal variations trigger changes in gas exchanges, stomatal conductance, mobilization of sugar storage, production of secondary metabolites, and anatomical characteristics in plants (Hernández et al. 2016; Mirsoleimani et al. 2018; Ribeiro et al. 2018; Chen et al. 2019).

Gas exchanges, biochemical and anatomical aspects of plant species are influenced by rainfall and temperature variations (Jassey and Signarbieux 2019). Thus, stomatal conductance and transpiration rates can vary in response to precipitation variability and influence plant photosynthetic rate and the capacity of carbon skeletons production (Ribeiro et al. 2018). Moreover, the quantum yield of PSII (Ribeiro et al. 2018), and the energy availability for photochemistry is also influenced by the precipitation rate (Ishida et al. 2006). Temperature variations are related to the variation in gas exchanges and plant primary production, regardless of

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the water conditions (Jassey and Signarbieux 2019), and the increase in temperature tends to reduce the potential quantum yield of photosystems (Ogaya et al. 2011). Nutritional status and developmental stage also influence plant photosynthetic performance (Tekaya et al. 2016; Amorim and De Melo Júnior 2017; Ramírez-Valiente et al. 2020), once higher photosynthetic rates are observed in well-nourished plants or in plants that are in a vegetative developmental stage.

Anatomical modifications such as number and density of stomata, thickness of vascular bundles, as well as thickness of cuticle, epidermis, leaf blade and parenchyma are related to the ability of plants to adapt to the climatic changes of the environment in which they are inserted (Lemos et al. 2018). The increase in incident radiation is related to lower thickness of the palisade and spongy parenchyma, and leaf epidermis (Amorim and De Melo Júnior 2017; Romanatti et al. 2019). In response to the increase in temperature, there may be a reduction in leaf thickness, and an increase in the density of stomata and trichomes (Shtein et al. 2011). In conditions of low temperatures characteristic of winter, there is an increase in mesophyll thickness and conductivity to adapt to unfavorable conditions (Ramírez-Valiente et al. 2020).

The seasonal changes also influence the production of nonstructural carbohydrates and the activity of saccharolytic enzymes, changing the carbon supply for plant growth, flowering or fructification (Ramírez-Briones et al. 2017; Tassoni et al. 2019). Since the production of secondary metabolites depends on the supply of carbon skeletons and energy from primary metabolism, the synthesis of secondary compounds can vary in response to seasonal variations (Hernández et al. 2016).

Garcinia brasiliensis Mart. belongs to *Clusiaceae* family which is cultivated throughout South America and other tropical regions of Asia, Africa, New Caledonia, Polynesia and America (Watermann 1986; Piccinelli et al. 2005; Zan et al. 2018; Fernandez et al. 2021). *G. brasiliensis* fruit peel and leaves have medicinal value (Martins et al. 2008; Kumar et al. 2013; Naves et al. 2019; Melo et al. 2021). Recently, it was verified the seasonal influence on the production of essential oil and phenolic compounds of interest in *Garcinia brasiliensis* leaves (Veroneze Junior et al. 2022). Once *G. brasiliensis* is an Amazonian species whose studies are focused on its medicinal application, the ecophysiological comprehension about this species is still scarce. In this way, studies related to the physiological and anatomical behavior of *G. brasiliensis* will also contribute to its management and cultivation aiming at the preservation of the species. Thus, we hypothesized that the physiological and anatomical traits of *G. brasiliensis* are influenced by seasonal variations. For this, this study aimed to identify the influence of seasonality

on the ecophysiological and morphoanatomical characteristics of *G. brasiliensis*.

Materials and methods

Experimental conditions: environment and plants characterization

The experiment was carried out on a population of *Garcinia brasiliensis* (*Clusiaceae*) under field conditions. The species was identified by taxonomic comparison with the material in the UALF herbarium of the Institute of Nature Sciences (ICN) of Universidade Federal de Alfenas (UNIFAL-MG), registered as 2566. The experiment was conducted in the rural area of Alfenas (21°20'03.0"S 45°58'19.5"W; climate CWA, according to Köppen and Geiger), when the plants were approximately six years old.

The experiment was conducted from May 2018 to February 2019, and the evaluations were performed in May, August, November and February. The months of evaluations corresponded to the middle of each season: autumn, winter, spring and summer, respectively. There were evaluated fully expanded leaves in the middle third of the canopy. Experiment was conducted in completely randomized design with four replicates. There was no fertilization or irrigation practices during the experimental time.

Environmental incident radiation was characterized using a Spectrum-radiometer (Luzchem, SPR-4002, Ottawa, Canada) and was measured using a portable digital solar energy meter (Instrutherm, MES-100, São Paulo, Brazil). Also, the climatological variables of maximum, minimum and average temperatures and rainfall in the months of evaluation were obtained from a climatological station near the experimental site.

Soil chemical and physical characterizations were performed in composite soil samples collected from the layer between 0 and 20 cm in all the assessment months. Each composite sample was composed of samples collected in four positions, nearby the evaluated plants. The soil samples were air-dried, sieved using a 4-mm mesh, and the following chemical parameters were determined: Organic matter, pH in CaCl₂; H⁺; Al; P in ion exchange resin; Ca, Mg and K; Al; S-(SO₄)₂; B, Cu, Fe, Mn and Zn. The sum of bases (SB), cation exchange capacity (CEC) and the percentage of base saturation (V%) were also determined. For the physical attributes, the percentages of sand, silt and clay were evaluated. Soil analyses were performed according to Raij et al. (2001).

Leaf chemical characterization was performed in composite leaf samples collected in the assessment months. The composite samples were obtained from fully expanded

leaves collected from the middle third of the canopy of the four plants in each month of analyses. The leaf samples were sent to a Laboratory of Leaf Analyses, and the concentrations of N, P, K, Ca, Mg, S, B, Cu, Mn, Zn and Fe were determined according to Malavolta et al. (1997). For this, samples were ground in a rotor type mill (Pulverisette 14 Classic Line, Fritsch GmbH, Idar-Oberstein, Germany) at 16 000 rpm. Nitrogen (N) was determined by sulfur (S) digestion followed by the Kjeldahl distillation. The samples were subjected to nitro-perchloric digestions for nutrients determination. Calcium (Ca), magnesium (Mg), potassium (K), copper (Cu), iron (Fe), zinc (Zn), and manganese (Mn) were determined by high sensitivity atomic emission spectrometry (MIP-AES Agilent 4200, Santa Clara, CA, USA). Phosphorus (P), S, and boron (B) were determined by spectrophotometry (FEMTO 600 S, São Paulo, Brazil).

Ecophysiological evaluations

Ecophysiological evaluations were performed on fully expanded leaves in the middle third of the canopy, at the four cardinal points (North, South, East and West). The branches whose leaves were used for non-destructive ecophysiological evaluations (leaf gas exchange, chlorophyll “a” fluorescence and relative chlorophyll content) were marked. The same leaves were sampled throughout the whole experiment. Leaf water potential, a destructive evaluation, was carried out on different leaves throughout the experiment.

Leaf gas exchange

Net photosynthetic rate (A_n), leaf transpiration rate (E), stomatal conductance (g_s) and internal CO_2 concentration (C_i) were evaluated using an IRGA (Infrared Gas Analyzer, LI-6400 XT, Li-Cor, Lincoln, Nebraska, USA). Water use efficiency ($WUE = A_n / E$) and instant carboxylation efficiency ($k = A_n / C_i$) were also calculated. The measurements were carried out between 09.00 and 11.00 a.m., under artificial photosynthetically active radiation of $1200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at leaf level. After constructing a light curve, the radiation used was determined to define the luminous saturation point for *G. brasiliensis*. The chamber temperature was controlled at 29°C .

Chlorophyll “a” fluorescence

Chlorophyll fluorescence was measured by a modulated fluorimeter (Mini-PAM II, Heinz Walz, Effeltrich, Germany). After a 30-minute adaptation in the dark and controlled temperature (29°C), the minimum fluorescence (F_0) was measured with sufficiently low light, avoiding photochemical reactions. The maximum fluorescence (F_m) was

determined by applying a saturating light pulse of $7000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 0.8 s. In the samples adapted in the dark, the maximum quantum yield of the photosystem II (PSII; F_v/F_m), the maximum efficiency of the photochemical process in PSII (F_v/F_0), and the basal quantum production of non-photochemical processes in PSII (F_0/F_m) were estimated.

Relative chlorophyll content and leaf water potential

The relative chlorophyll content was determined based on chlorophyll meter readings (SPAD 502, Konica Minolta, Minolta, Japan). An average of ten readings was performed on each of the leaves for each orientation. The amount of chlorophyll was expressed in SPAD units.

Leaf water potential was determined with a Scholander pressure pump (SEC-3015G2, Soil Moisture Equipment Corp., Santa Barbara, CA, USA) at noon (Ψ_{md}), sampling two fully expanded leaves from the North and South directions of each of the plants.

Biochemical assessments

For biochemical assessments the material consisted of fully expanded leaves in the middle third of the canopy, at the four cardinal points (North, South, East and West). All collections were performed early in the morning, before 7:30 a.m. and in the absence of rain. The leaves were collected and then dried at 65°C in a forced-air oven until constant weight. Finally, the leaf samples were crushed in a Wiley mill.

Carbohydrates, amino acids and proteins were extracted using 200 mg of dry leaf matter, homogenized in 5 mL of MCW (3 mL methanol; 1.25 mL chloroform and 0.75 mL water). The mixture was left in contact for 24 h and then centrifuged at 1300 rpm for 30 min. The supernatant was used for the assessments of total soluble sugars, reducing sugars, amino acids and proteins. For starch extraction, the pellet from the previous extraction was resuspended with 1.5 mL of 30% perchloric acid (Rabêlo et al. 2019). After 24 h, the mixture was centrifuged at 1300 rpm for 30 min. The supernatant was used for assessing starch using the anthrone method, based on the glucose standard curve (Yemm and Willis 1954).

Total soluble sugars were determined using the anthrone method, based on the standard glucose curve (Yemm and Willis 1954). Reducing sugars were quantified using the dinitrosalicylic acid method (Miller 1959). The ninhydrin method was used for the quantification of amino acids based on the glycine standard curve (Yemm et al. 1955). The concentration of total soluble protein was determined in a

spectrophotometer at 595 nm, as Bradford (1976) described, using bovine serum albumin (BSA) as a standard.

Leaf anatomical assessments

Two fully expanded leaves were collected of each orientation (east and west) since they are the orientations at which the sun rises and sets, respectively, for representative results. Leaf orientation for anatomical analyses was determined based on previous tests. The collections were performed in the middle third of each plant. The fully expanded leaves were standardized for uniformity of size and color in all collections.

After collected, the leaf samples were fixed in 70% alcohol. The cross-sections were made freehand with a razor blade, and then washed five times: the first with 50% HCl for five minutes to lighten the cuts, the second with 1% acidified water for five minutes to remove excess HCl and three consecutive washes in distilled water for five minutes each to rehydrate the material. After washing, Astrablau (10% Safranin and 90% Astra Blue) was used for staining; 32 cuts were made for each assessment month, totaling 128 cuts. For each cut, in average three measurements were performed for each value observed.

An Axio Scope A1 photomicroscope photographed the cuts, and the cuts were measured using the AxioVision SE64 Rel. 4.8 software. There were measured the thickness of the adaxial cuticle (AD Cuticle), abaxial cuticle (AB Cuticle), palisade parenchyma (PPT) and spongy parenchyma (SPT), and mesophyll (MT). The value of each measurement was

obtained by the average of three measurements in each cut. There was also calculated the ratio between spongy parenchyma/palisade parenchyma.

Data analysis

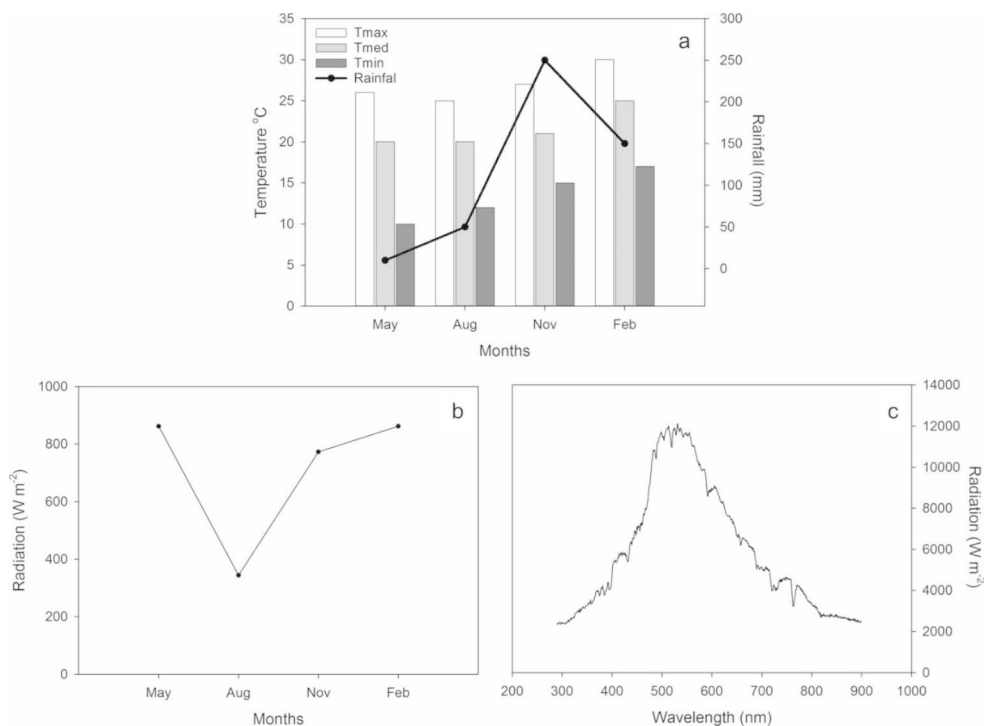
The means and standard errors of the means were calculated for ecophysiological and biochemical assessments ($n=64$, four positions of the canopy in four repetitions), and for water potential and anatomical assessments ($n=32$, two positions of the canopy in four repetitions). Data was subjected to analysis of variance and the Scott Knott test ($p \leq 0.05$), using the SISVAR 5.6 software (Universidade Federal de Lavras – UFLA). The relationships between morphophysiological parameters and months of the year were obtained through principal component analysis (PCA) (Jolliffe and Cadima 2016). The coefficients for each component were set using a correlation matrix.

Results

Climatic characterization of the environment

Taking an overview of the climatic variables over the months of analysis, the lowest minimum temperature (T_{min}) and rainfall were observed in May (Fig. 1A, B). The lowest incident radiation was observed in August (Fig. 1B). In November, the greatest rainfall occurred (Fig. 1A). Medium temperature (T_{med}) was maintained from May

Fig. 1 Minimum, medium, and maximum temperature and rainfall (A), incident radiation (B), and characterization of incident radiation (C) at the experimental site in May (May), August (Aug), November (Nov), and February (Feb) in *Garcinia brasiliensis*



to November. In February, there was a higher mean T °C (Fig. 1A) and higher incident radiation (Fig. 1B). Concerning the incident radiation spectrum, incidence peaks were observed between 480 and 600 nm (Fig. 1C).

The chemical and physical characterization of the soil are shown in Tables 1 and 2, respectively. The chemical composition of the soil was very similar throughout the experiment. In November, there was a reduction in soil cation exchange capacity and in the sum of bases and a decrease in K, Ca and Mg concentrations in relation to the other months of analysis (Table 1). There is a typical clay soil (Table 2).

Nutritional characterization of plants

Concerning the nutritional analysis of the plants, the highest variations were observed in November (Table 3). Comparing the nutrient content over the assessment months, the lowest Zn and Cu levels were observed in August; in November, there was a decrease in K, Ca, B and Fe. The other nutrients did not show high variations over the months.

Principal components analysis

Principal component analysis taking the physiological and morphoanatomical traits investigated showed that the two principal components accounted for 42.74% of the variation in the analyzed data (Fig. 2). The separation of May, November and February is very clear by PC1 and PC2, while August did not show such an obvious distinction.

In May, the basal quantum production of non-photochemical processes in PSII (F_0/F_m), the SPAD Index, starch content (Starch) and palisade parenchyma thickness (PPT) were the most related variables. In August, the thickness of the cuticle (CT), the thickness of spongy (SPT) parenchyma and the diameter of secretory channels (SCD) were the parameters with the greatest influence. In November, some gas exchange parameters such as net photosynthesis (A_n), transpiration (E), stomatal conductance (g_s) and internal CO₂ concentration (C_i) were the variables that showed the greatest influence as well as the biochemical parameters such as contents of reducing sugars (RS), total soluble sugars (TSS), total protein (PTN) and aminoacids (AA). In February, the maximum quantum yield of the PS II (F_v/F_m), the maximum efficiency of the photochemical process in PSII (F_v/F_0), water use efficiency (WUE), instant carboxylation efficiency (k), thickness of the mesophyll (MT), and the number of the secretory channels (SCN) were the most correlated parameters (Fig. 2).

Table 1 Chemical analysis of the cultivation soil for *Garcinia brasiliensis* plants. The samples were collected in May, August, November and February

Months	pH	OM		Total C	P		K		Ca	Mg	H ⁺ Al		Al	H	S. B	CEC	V%
		g dm ⁻³	dm ⁻³		mg dm ⁻³	mg dm ⁻³	mmol dm ⁻³	mmol dm ⁻³			%						
May	5.7	17	10	2	2.7	23	6	20	0	20	31.7	51.7	61				
Aug	5.4	18	10	4	3.4	25	7	20	0	20	35.4	55.4	64				
Nov	5.8	17	10	4	0.7	5	1	14	0	14	6.7	20.7	32				
Feb	5.8	15	9	9	2.8	22	6	12	2	10	30.8	42.8	62				
Months	B	Cu	Fe	Mn	Zn	S	K	Ca	Mg	Al	Ca/Mg	Ca/K	Mg/K				
		mg dm ⁻³		CEC (%)													
May	0.4	1.9	8	2.1	0.8	4	5.2	44.5	11.6	0	3.8	8.5	2.2				
Aug	0.3	3.6	11	4.1	1.1	3	6.1	45.1	2.6	0	3.6	7.4	2.1				
Nov	0.2	4.7	12	3	1.2	7	3.4	24.2	4.8	0	5.0	7.1	1.4				
Feb	0.2	2.9	10	3.6	1.1	15	6.5	51.4	14.0	4.7	3.7	7.9	2.1				

* The values correspond to the mean of four soil collection points in each month of analysis. OM: organic matter; Total C: total carbon; SB: sum of bases; CEC: cation exchange capacity; V%: percentage of base saturation.

Table 2 Physical soil analysis. The samples were collected in May, August, November and February, forming a composite sample where the mean is described below

Clay	Silt	Total Sand	Coarse Sand	Fine Sand	Classification
-----g kg ⁻¹ -----					
246	257	497	285	212	Clay loam soil

Ecophysiological and anatomical evaluations

The plants showed the highest photosynthetic rate (A_n) (Fig. 3A), transpiration (E) (Fig. 3B) and stomatal conductance (g_s) (Fig. 3D) in November. Intercellular CO_2 concentration (C_i) (Fig. 3C) was higher in August and November, with statistically similar values. Instant carboxylation efficiency (k) (Fig. 3E) was higher in May and November than

Table 3 Nutritional analysis of *Garcinia brasiliensis* leaves in May, August, November and February

Months	N	P	K	Ca	Mg	S	B	Cu	Mn	Zn	Fe
-----g kg ⁻¹ -----											
May	11.0	0.7	10.1	13.4	1.0	1.8	111.1	10.5	598.1	47.9	136.92
Aug.	13.0	0.6	9.2	8.9	0.9	1.6	94.0	3.8	635.2	28.0	128.20
Nov.	13.0	0.8	7.9	4.3	1.5	1.7	42.8	5.9	453.6	32.3	21.93
Feb.	13.0	1.0	10.3	8.7	1.4	1.7	65.1	4.9	450.0	47.9	72.98

* The values correspond to the reading of a composite sample consisting of fully expanded leaves of the middle third in each of the four points of the canopy.

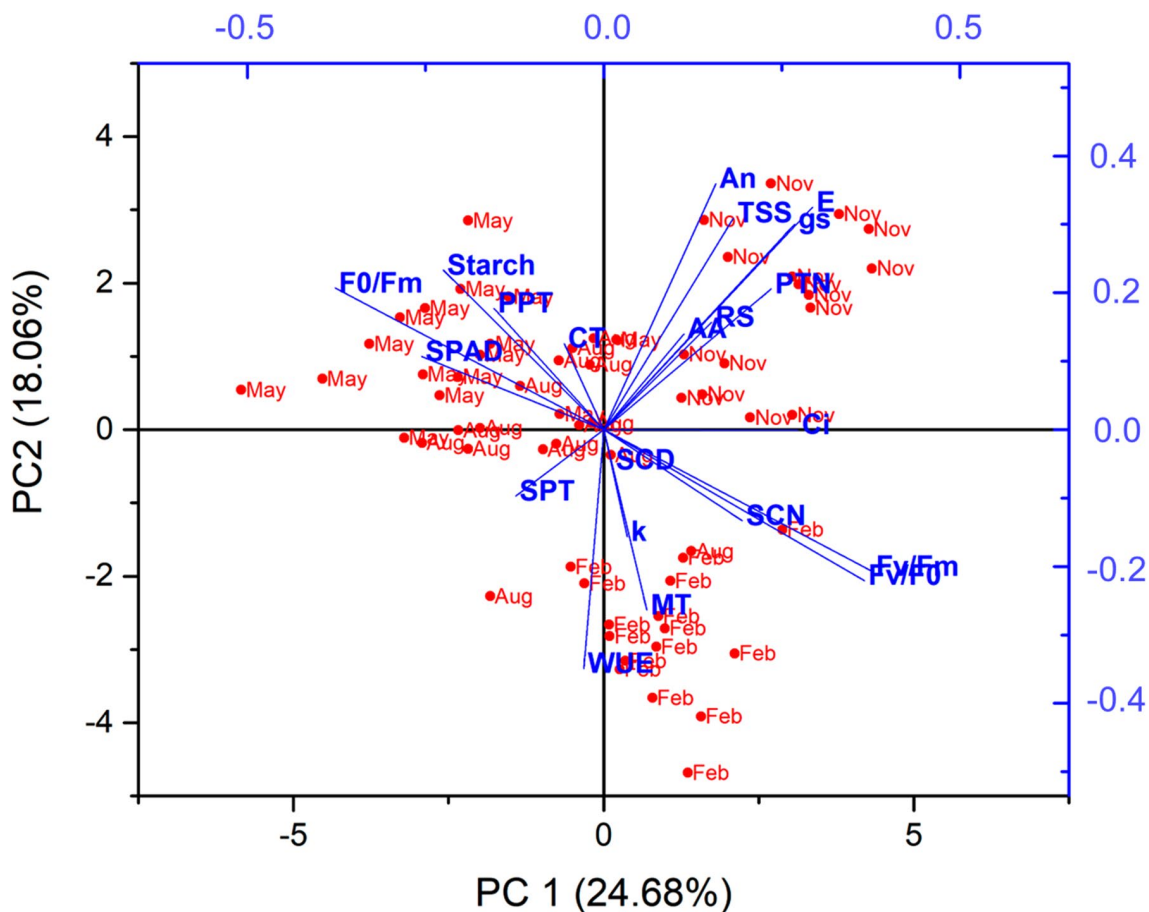
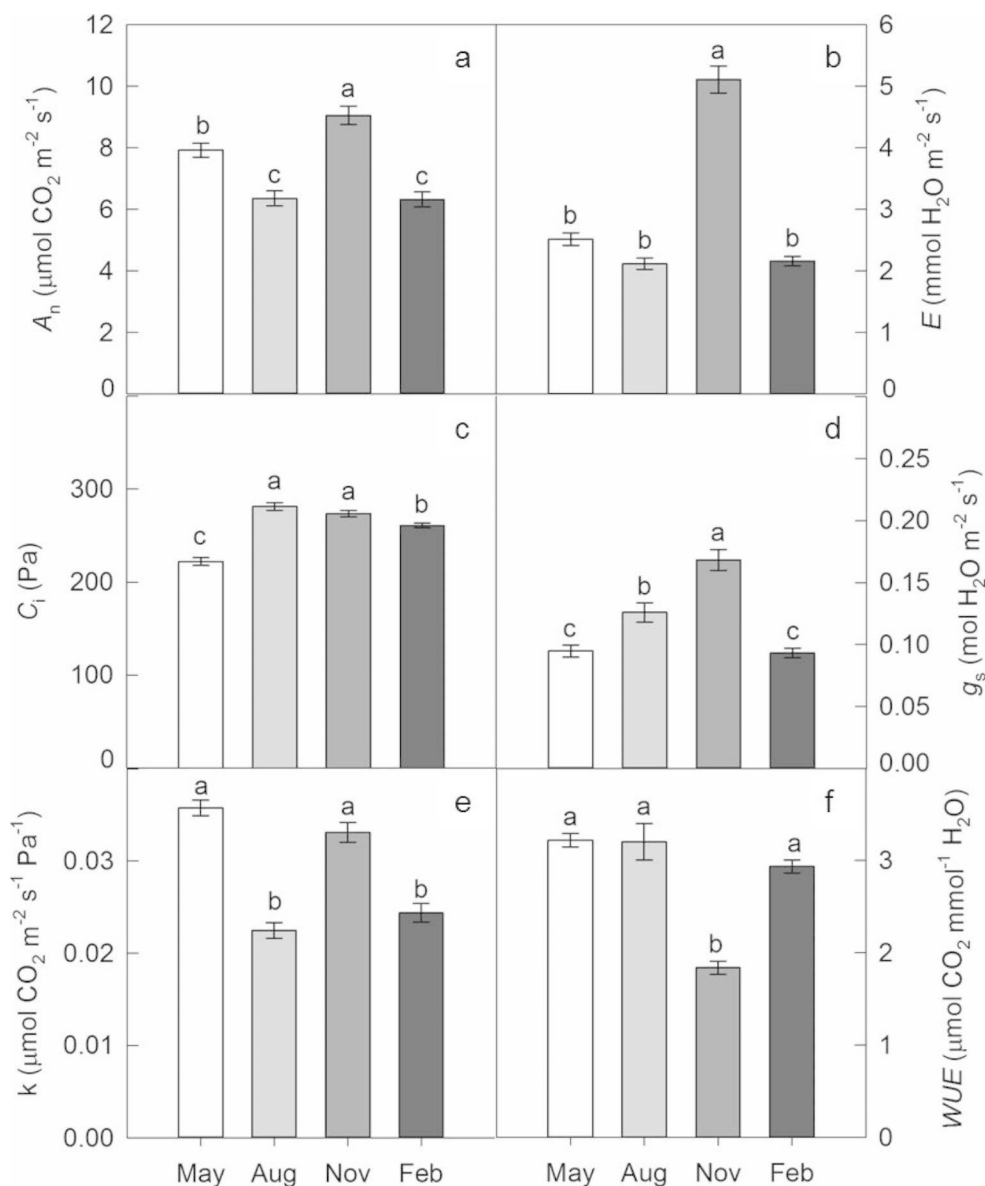


Fig. 2 Principal component analysis of parametric variables related to biochemical, ecophysiological and anatomical parameters of *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). PC, Principal components; A_n , Net photosynthetic rate; E , transpiration rate; C_i , internal CO_2 concentration; g_s , stomatal conductance; k , instant carboxylation efficiency; WUE , water use efficiency; F_v/F_m , maximum quantum yield of the PS II; F_v/F_0 , the maximum efficiency of the photochemical process

in PSII; F_0/F_m , basal quantum production of non-photochemical processes in PSII; SPAD, SPAD index; RS, reducing sugars content; TSS, total soluble sugars content; Starch, starch content; PTN, total protein content; AA, amino acids content; CT, cuticle thickness; PPT, palisade parenchyma thickness; SPT, spongy parenchyma thickness; MT, mesophyll thickness; SCD, secretory channels diameter; SCN, secretory channels number

Fig. 3 Net photosynthetic rate – A_n (A), transpiration – E (B), intercellular CO_2 concentration – C_i (C), stomatal conductance – g_s (D), instant carboxylation efficiency – k (E) and water use efficiency – WUE (F) in *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean ($n = 16$)



in the other months. In November, *G. brasiliensis* plants had the lowest water use efficiency (WUE) (Fig. 3F).

The maximum quantum yield of PSII – F_v/F_m (Fig. 4A) and the maximum efficiency of the photochemical process in PSII – F_v/F_0 (Fig. 4B) had the highest values in February. The basal quantum production of non-photochemical processes in PSII – F_0/F_m (Fig. 4C) showed the highest average in May.

The relative chlorophyll content was lower (Fig. 5A) and leaf water potential (Fig. 5B) was higher in November. In the other assessment months, they were higher and did not differ.

The content of reducing sugars (Fig. 6A) and total soluble sugars (Fig. 6B) was higher in November and did not differ between May, August and February. Starch content (Fig. 6C) was higher in May and gradually decreased over

the months, with the lowest value in February. Total proteins content (Fig. 6D) was higher in August and November. Amino acids (Fig. 6E) did not show statistical differences in their content over the months.

The thickness of the adaxial cuticle showed no statistical difference in the months of analysis, while the abaxial cuticle was thinner in November than in the other months (Fig. 7). The palisade parenchyma showed greater thickness in August and February. The spongy parenchyma had a greater thickness in May and August, followed by November and February. The thickness of the mesophyll did not vary over the months analyzed. The ratio spongy parenchyma/palisade parenchyma did not vary from May to November but was lower in February (Fig. 7A). Regarding the higher caliber of secretory channels found in the central leaf vein, a smaller diameter was found in August, with no

Fig. 4 Maximum quantum yield of PSII – F_v/F_m (A), maximum efficiency of the photochemical process in PSII – F_v/F_0 (B), basal quantum production of non-photochemical processes in PSII – F_0/F_m (C) in leaves of *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean ($n = 16$)

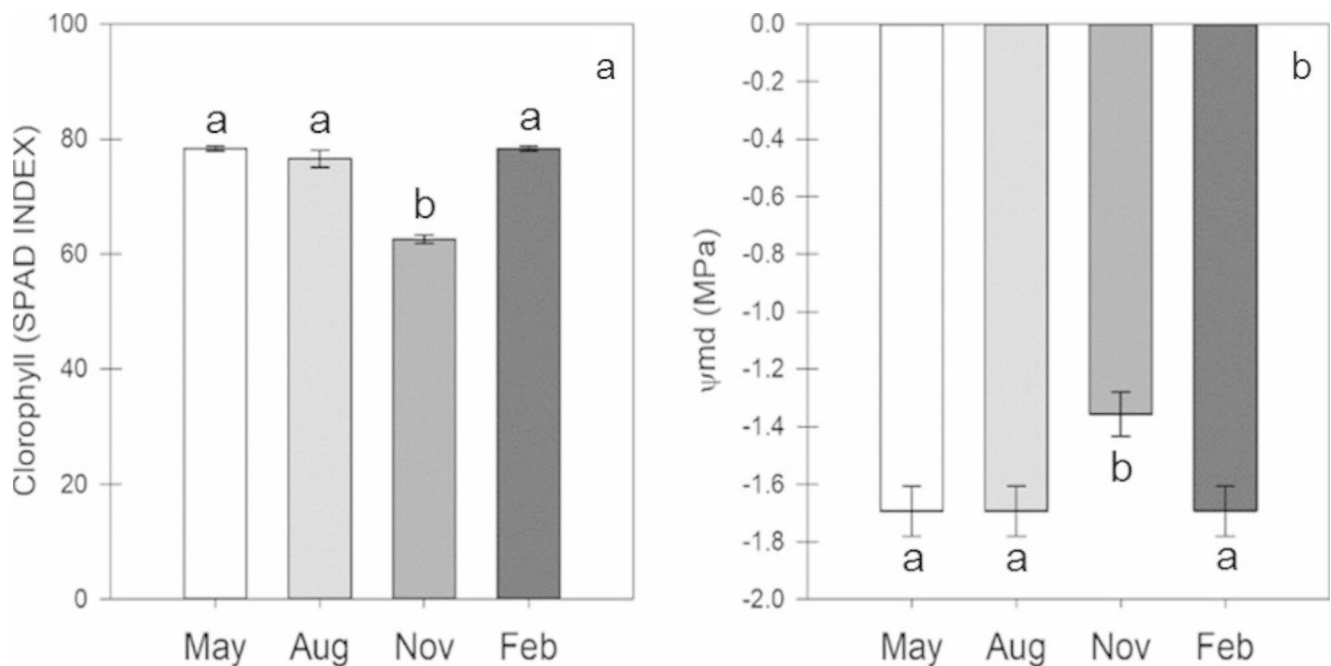
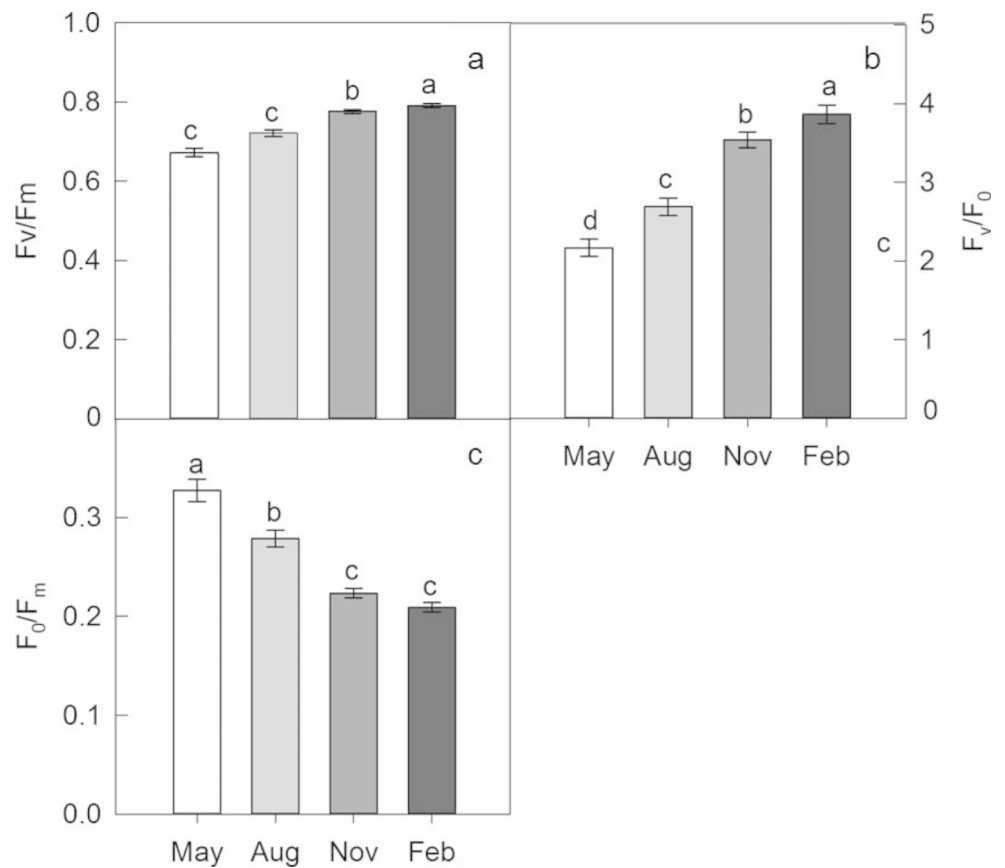
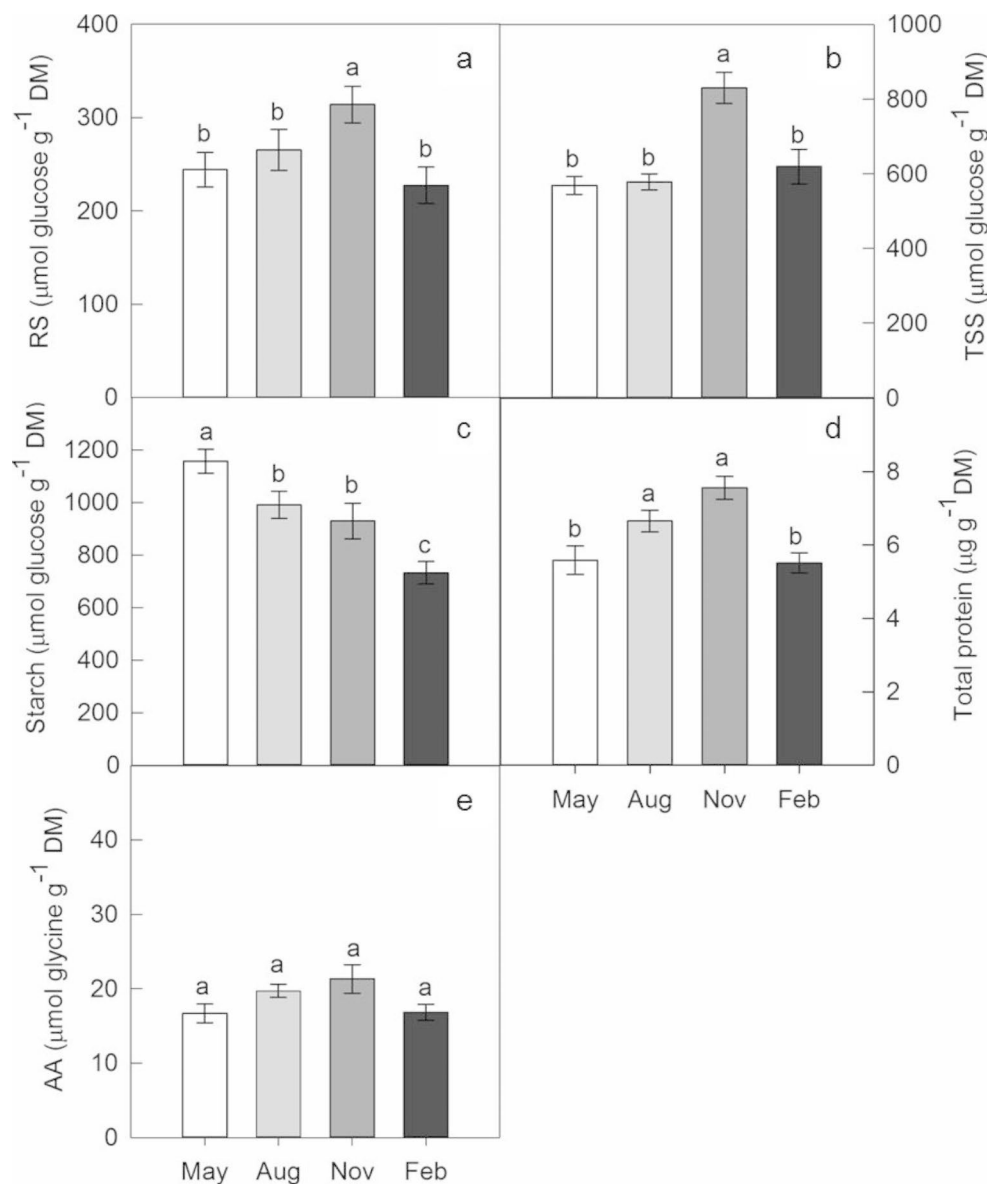


Fig. 5 Relative chlorophyll content (A) and leaf water potential ψ_{md} (B) in leaves of *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). Means

followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean ($n = 16$)

Fig. 6 Contents of reducing sugars – RS (A), total soluble sugars – TSS (B), starch (C), total proteins (D) and amino acids – AA (E) in leaves of *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean ($n = 16$)



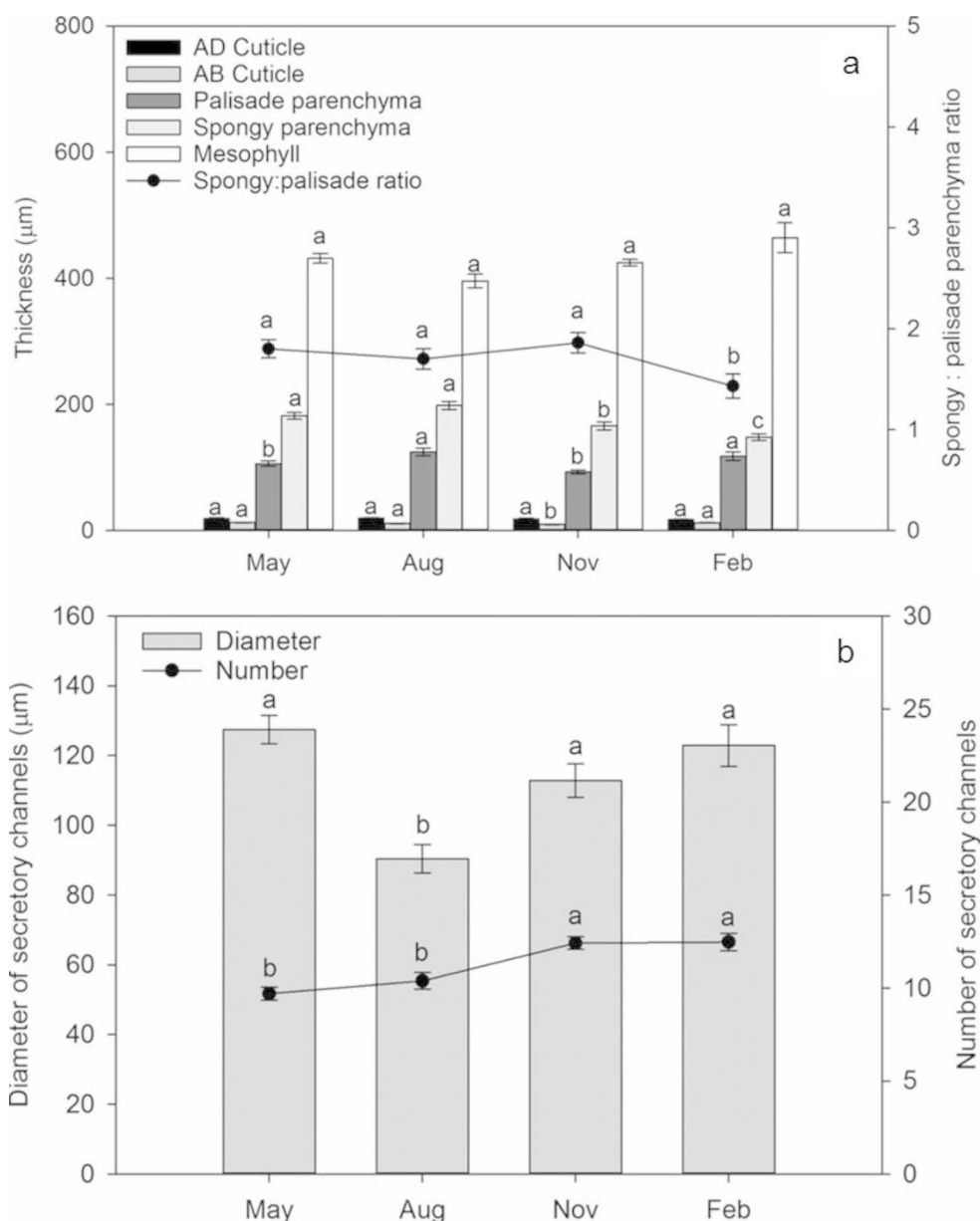
differences between the other months. In addition, the highest total number of secretory channels in the central vein was found in November and February (Fig. 7B).

Discussion

Climatic variations over the months of analysis influenced the ecophysiological responses of *Garcinia brasiliensis*, which reflected in metabolic changes. The studied plants showed greater metabolic activity in November, as well as high gas exchange and in the contents of reducing sugars, total soluble sugars and total proteins. In February, when there was a reduction in rainfall and an increase in temperature and incident radiation, a reduction in photosynthetic activity and starch content was observed.

The parameters related to gas exchanges and the contents of primary compounds contributed the most for data variation in November. Under high water availability for the plants there are fewer stomatal (Ribeiro et al. 2018) and biochemical limitations, so that the greater gas exchange and the functioning of the biochemical step yielded a higher net photosynthetic rate in plants. In this case, the higher transpiratory rate may also have contributed to the reduction of leaf temperature, maintaining the photosynthetic process (Kauwe et al. 2019). The good functioning of the photochemical stage in November was proven by the absence of photoinhibition. The efficiency of the photosynthetic apparatus in November was not affected, even by the reduction in the amount of chlorophyll observed in the leaves; thus, the plant must have had some compensatory effect to overcome the smaller amount of photosynthetic pigments

Fig. 7 Thickness of the adaxial (AD Cuticle) and abaxial (AB Cuticle) cuticle of the palisade and spongy parenchyma and mesophyll in *Garcinia brasiliensis* plants over the year, in May (May), August (Aug), November (Nov) and February (Feb). Means followed by the same letter do not differ according to the Scott-Knott test ($p \leq 0.05$). The bars correspond to the standard error of the mean ($n = 16$)



without compromising photosynthesis (Cai et al. 2020). In this context, low concentrations of Fe and Zn may have contributed to the reduction in photosynthetic pigments, since Fe is a cofactor for enzymes of chlorophyll synthesis, participating in the electron transport of ferrous enzymes. Zn is related to the electron transport process in the photochemical stage of photosynthesis (Roosta et al. 2018). This may be due the fact that when the plant is expanding, growing and producing other organs, with the dilution of nutrients to produce new drains, the nutrients are divided for the structuring of new organs.

The greater photosynthetic activity of *G. brasiliensis* in November probably enabled a more effective metabolism performance. This is reinforced by the fact that it is a typical spring month, when plants are in accelerated metabolism

(Lambers et al. 2008). In this context, all primary metabolic production was probably focused on maintaining the active drains of the plant, regrowth and the beginning of the reproductive process. Besides the intensive plant growth phase and preparation for flowering and fructification, the primary metabolites may also be related to the better carbon supply for the secondary metabolism (Hernández et al. 2016; Veroneze-Junior et al. 2022). On the other hand, in drier months (May and August) the plants accumulated more starch, indicating that carbon investments are limited by the environment and not by acquisition methods in these periods (Liu et al. 2018).

In May, when high incident radiation and low rainfall were observed, the stomatal limitation seems to be related to a higher leaf temperature, since the transpiration rate

was reduced due to low water availability. Furthermore, there was a photochemical limitation represented by the low F_v/F_m (<0.7). The basal quantum production of non-photochemical processes in PSII (F_0/F_m) is used to indicate damage to photosystems caused by stress, suggesting normal values or patterns between 0.14 and 0.20 (Roháček 2002). Thus, it was found that greater damage occurred in the months of May and August than in the other months. A similar response was found with *Rhododendron ponticum* plants, suggesting that plants under stress can adapt their photosynthetic apparatus according to the increased irradiance in the environment (Osório et al. 2010). Thus, the plant still had high biochemical efficiency, which allowed the use of available C_i in the mesophyll (Stojnić et al. 2019). In addition, in May the plants showed greater photosynthesis; despite the less favorable environmental conditions, the plants were well-nourished, with higher contents of Ca, B, Cu and Zn than the other months. The good nutrition resulting from fertilization with organic compost in olive trees was related to the better photosynthetic performance of these plants compared to non-fertilized plants (Tekaya et al. 2016).

In August, there was a greater biochemical limitation of photosynthesis. In this case, in addition to the reduction in CO_2 input into the mesophyll, the enzymes of the Calvin Cycle probably had lower activation due to the lower incident radiation. Allied to this, the limitation in the photochemical stage was demonstrated by the occurrence of photoinhibition (Sharma et al. 2020).

In February, the high incident radiation and temperature was probably responsible for stomatal and biochemical limitations in photosynthesis. Therefore, the plant had a lower transpiratory flow to cope with water availability, which probably reduced CO_2 input into the mesophyll. Moreover, the increase in leaf temperature, among other metabolic processes, can alter the activity of Calvin Cycle enzymes and decrease A_n (Sharma et al. 2020). Also, despite the F_0/F_m values being greater than 0.20, the damages were not enough to affect F_v/F_m (greater than 0.7) not even the photosynthetic rate. Also, the potential photosynthetic activity (F_v/F_0) increased progressively from May to February, when it reached values very close to 4. Values for F_v/F_0 between 4 and 6 are considered normal, indicating no damage to the efficiency of the photochemical process (Zanandrea et al. 2006). Nevertheless, the increase in the production of phenolics in February may have contributed to the protection of the photosynthetic apparatus in *G. brasiliensis* (Veroneze Junior et al. 2022).

Considering the anatomical measurements, the reduction in the thickness of the spongy parenchyma and the increase in the thickness of the palisade parenchyma led to a reduction in the proportion of spongy parenchyma/palisade

parenchyma in February. This anatomical modification in mesophyll in summer is typical of leaves of sunny places, in which there is a greater chlorophyll synthesis (Xu et al. 2001). According to Romanatti et al. (2019), under excessive UV light cause damages to palisade parenchyma, which has morphological modifications in its structure, including the reduced number of chloroplasts. Regarding the leaf mesophyll, variations in its thickness are common as a way of adapting to environmental variations (Bosabalidis and Kofidis 2002; Marchiori et al. 2019). In general, the species tend to have thickening in the mesophyll during under high photosynthetic active radiation (Marchiori et al. 2019). There are invasive species whose increase in mesophyll thickness is related to the better physiological performance of these plants over the seasons, resulting in growth and competitiveness in relation to other species (Oliveira et al. 2017). In the case of *G. brasiliensis*, there was a change in parenchyma thickness in the summer, but it was not enough to change mesophyll thickness over the seasons. In addition, changes in cuticle thickness were observed only on the leaf abaxial face in November. It is important to emphasize the greater thickness of the adaxial cuticle in relation to the abaxial, a typical pattern of leaves that receive greater solar radiation (Bahamonde et al. 2018).

Conclusions

Seasonality influences gas exchange, the fluorescence of chlorophyll “a”, production of primary metabolites and the nutritional status of *G. brasiliensis* plants. Anatomical changes are the least pronounced due to seasonality. Plants were more active metabolically in November, a month typical of spring in Brazilian tropical climate, which also corresponds to the growth season.

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Declarations

Competing interests The authors have no competing interests to declare that are relevant to the content of this article.

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