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Seasonal Variation of Chlorophyll and Carotenoids in Leaves of *Hancornia speciosa* in Three Central Areas of the Cerrado of the State of Tocantins, Brazil

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Abstract

This study aimed to evaluate the seasonal variation in concentrations of chlorophyll *a*, *b*, total chlorophyll and carotenoids in leaves of *Hancornia speciosa*, Gomes, during the periods of the year, relating them to the main phenological events, periods (rainy, dry and transitions) and populations evaluated. The survey was performed in three sites and the spatio-temporal analysis divided into four periods (rainy, rainy-drytransition, dry and dry-rainy transition), with 10 replicates (matrix plants). The data were collected in average intervals of 33 days from October 2014 to April 2017. The extraction and calculation of the chlorophyll and carotenoid contents of the leaves were expressed in mg/g DM, according to the equations of Arnon (1949) and Lichtenthaler (1987). There was a significant difference between the periods and sites analyzed for all pigments and their relationships. There was a greater amount of chlorophyll *a* than chlorophyll *b*; this difference was greater in the dry period. In the rainy period, we found a greater amount of total chlorophyll, carotenoids and total chlorophyll/carotenoid ratio. The behavior for the species follows that already observed for deciduous plants, closely related to water availability.

Keywords: mangabeira, photosynthetic pigments, rainfall

1. Introduction

The creation of a large database on functional characteristics is gaining priority in plant ecology research, with the purpose of contributing to the understanding and prediction of species distribution in present and future environments (Garnier et al., 2001). Foliar characters are often used as indicators of the relationship between the use of resources by plants, their biomass and the ecosystem functioning (Craine, Froehle, Tilman, Wedin, & Chapin, 2001). Studies have sought to establish relationships between environmental conditions and foliar properties, although leaf characteristics are usually measured at the peak of the growing season when environmental conditions favor carbon assimilation and there is an abundance of expanded and healthy leaves (Carvalho, Bustamante, Kozovits, & Asner, 2007), with scarce work conducted in savanna environments. In these ecosystems, low water availability, soil nutrient deficiencies and fire are the main causes of instability in final productivity.

In the Cerrado, soil water content decreases rapidly at the beginning of the dry season, followed by a prolonged drought period (Franco, 2002). The water deficit hinders the absorption of nitrogen, an essential component of chlorophylls, proteins, enzymes and nucleic acids, being indispensable for plant growth. Nitrogen deficiency reduces leaf area, accelerates leaf senescence, reduces the efficiency of photosynthesis, and consequently the photosynthetic activity of the plant (Uhart & Andrade, 1995). Plants native to this biome may present adaptations in response to water stress, such as decreased transpiration, leaf area and increased leaf senescence and abscission (Meir & Grace, 2005).

Chlorophylls are the most abundant natural pigments present in plants and occur predominantly in leaf chloroplasts, associated with carotenoids responsible for leaf color varieties. Chlorophyll is directly related to photosynthetic activity in plants. Thus, the nutritional status of plants is directly related to the quality and quantity of chlorophyll, which comprises a family of chemically similar substances called chlorophylls *a*, *b*, *c* and *d*; chlorophyll *a*, *b* and carotenoids are directly involved in the absorption of visible light for the transfer of electrons from water to the electron transport chain in the photosynthetic mechanism (Taiz & Zaiger, 2013). Thus, the knowledge of the foliar characteristics represents an interesting measure to compose the database on a species.

Mangabeira (*Hancornia speciosa*, Gomes) is a fruit tree belonging to the family Apocynaceae, which is more abundant in the areas of cerrado *strict sensu*, mainly in soils poor in organic matter, acidic, with low nutrient content, and is tolerant to water deficit, with better vegetative development under higher temperature and lower relative humidity (Vieira Neto, Cintra, A. L. da Silva, Silva Júnior, Costa, A. A. G. da Silva, & Cuenca, 2002). It presents a diversity of phenological events, and depending on the region, it has two flowering during the year, in the summer (rain) and winter (dry), in turn, fruiting differs between the harvests and fruit aspects, with higher and better production in the summer (Silva Júnior & Lêdo, 2006). In tropical savannas, temporal patterns of plant growth and reproduction are linked to climatic seasonality (Williams, Myers, Muller, Duff, & Eamus, 1997).

Pilon, Udulutsch, and Durigan (2015) studied the phenological patterns of 111 cerrado species, indicating that the mangabeira is a semideciduous or deciduous species that loses leaves during the dry season of the year, agreeing with Lorenzi (2000), and A. V. Pereira, E. B. C. Pereira, and Silva Júnior (2010). According to Carvalho et al. (2007), who analyzed the concentration of pigments and nutrients in leaves of cerrado species with different phenological strategies, detected no differences in the behavior of deciduous and evergreen plants.

Despite the relevance of photosynthetic pigments to the leaf functioning, there are still no studies that relate the seasonal variations in their concentrations in cerrado species (Carvalho et al., 2007). Based on this premise, the goal of this study was to evaluate the contents of chlorophyll *a* and *b*, chlorophyll *a*/chlorophyll *b* ratio, total chlorophyll, carotenoid and total chlorophyll/carotenoid ratio, during the periods of the year, relating them to the main phenological events, periods (rainy, dry and transitions) and populations evaluated.

2. Material and Methods

2.1 Study Area

The study was conducted in the municipality of Porto Nacional (10°41′15.94″ S latitude, 48°22′55.60″ W longitude, 239.2 m altitude), central portion of the State of Tocantins, Brazil. The climate of the region is defined as tropical, its classification is Aw according to Köppen and Geiger. It has an average temperature of 26.1 °C (varying 3.0 °C during the year), with September being the warmest month (27.9 °C average) and July, the coldest month (24.9 °C). The average rainfall of 1,622 mm, with a difference of 258 mm rainfall comparing the driest month (June) with an average of 4 mm and the rainiest, March with 262 mm (Climate-data.org, 2017). It is characterized by two well-defined seasons: rainy (November to April) and dry (May to October). The mean annual rainfall of the ten-year period prior to the study (2004-2013) was 1643.75±276.85 mm and mean temperature was 27.5±0.4 °C. The study started in October 2014 to April 2017 (30 months). Rainfall data were recorded by the conventional meteorological station, located in the municipality of Porto Nacional, distanced approximately 10 km from each collection site, and data provided by the National Institute of Meteorology (Inmet & Bdmep, 2017).

For the purposes of analysis, the observations were divided into four collection periods (rainy, rainy-dry transition, dry and dry-rainy transition) defined according to the phytoclimatic model proposed by Walter and Lieth (1967), constructed for the region of National Harbor. The mean values of rainfall (mm) and temperature (°C) of the ten years prior to the survey of leaf characteristics (2004 to 2013) were used. For the Porto Nacional meteorological station, a Cartesian chart was drawn where the abscissa axis (X) represents the months of the analyzed years and the ordinate axis, the rainfall precipitation in mm (Y_1) and the average monthly air temperature, in °C (Y_2), in which the scale of the rainfall representation is twice that of the temperature. According to the Walter and Lieth phytoclimatic model, applied for forest situations, the year is divided into a super humid period (with monthly rainfall over 100 mm—adapted for this work—November to April), wet period (adapted in this work—transition from dry to rainy and vice versa—October and May months) and dry period (adapted in this work—June to September) (Figure 1).

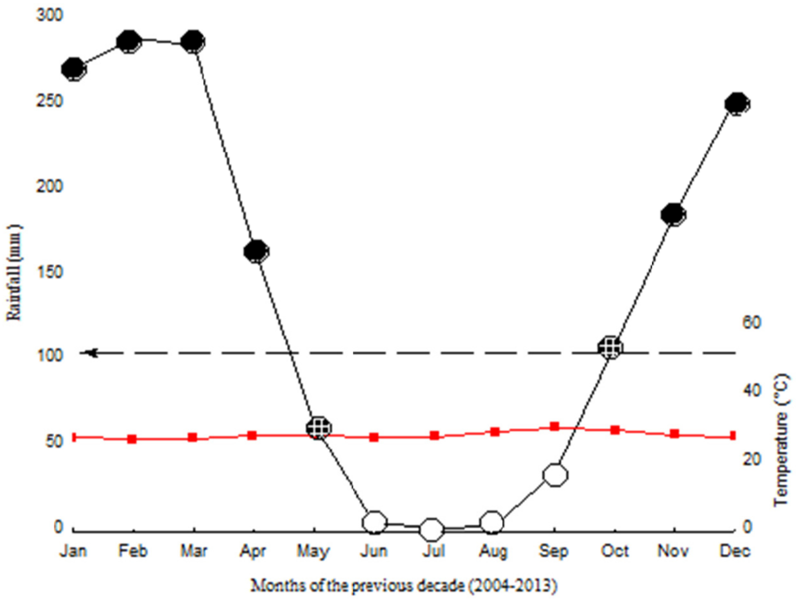

Figure 1. Climatic diagram for the region of Porto Nacional, State of Tocantins, Brazil, from 2004 to 2013, according to the phytoclimatic model of Walter and Lieth (1967)—adapted. The lower curve shows the average monthly temperature; the upper curve, the total rainfall of the month. The black circles represent the rainy period (November to April—with monthly rainfall over 100 mm; hachurized circles: transition period (May and October); and white circles: dry period (June to September) (adapted by R. J. de Oliveira)

2.2 Leaf Analysis

Three populations of typical cerrado with different densities of cover, spaced approximately 20 km apart, were selected. From the previous survey in each locality, 10 matrices were marked per site, at least 50 m away from each other, from which six completely expanded leaves were takenthroughout the outline of the plant. In the field, the leaves were wrapped in aluminum foil, identified according to the number of the matrix plant (Table 1), and packed in Styrofoam box with ice to protect from light and keep under refrigeration until laboratory processing carried out on the same day. In order to determine the contents of the photosynthetic pigments, 0.5 cm^2/leaf was collected in the laboratory, totaling an area of $3cm^2$ per plant, which were immersed in a volume (V) of 5mL Dimethylsulfoxide (DMSO) in capped glass vials covered with aluminum foil. The vials were left on the bench (25±2 °C) for 36 hours. Subsequently, the extracts were transferred to buckets and the absorbance values were read at 663 (A_{663}), 645 (A_{645}) and 470 nm (A_{470}) using a DMSO solution (blank) in a Biospectro SP22 spectrophotometer. To obtain the dry mass (DM), in grams, the same leaf area was dried for up to 48 hours in an oven at ±50 °C to constant weight, and weighed on an analytical balance with three decimal places of precision. Chlorophyll and carotenoid contents were expressed as mg/gDM, and calculated according to the Arnon (1949) and Lichtenthaler (1987) equations, as follows: *Chlorophyll a* = $(12.7 \cdot A_{663} - 2.69 \cdot A_{645}/1000 \cdot DM) \cdot V$; Chlorophyll *b* = $(22.9 \cdot A_{645} - 4.68 \cdot A_{663}/1000 \cdot DM) \cdot V$; *Total Chlorophyll* = $(20.2 \cdot A_{663} - 2.69 \cdot A_{645}/1000 \cdot DM) \cdot V$ and *Total Carotenoids* = $(1000 \cdot A_{470}) - (1.82 \cdot \text{Chloro.a}) - (85.02 \cdot \text{Chloro.b})/198 \cdot V$, where A_{470} = absorbance at 470 nm; A_{663} = absorbance at 663 nm; A_{645} = absorbance at 645 nm; DM = dry matter of sample (g); and V = sample volume (mL).

Table 1. Geographic coordinates of the matrices sampled in three natural populations of mangaba trees, located in the municipality of Porto Nacional, State of Tocantins, Brazil, 2014

Population	Matrics	Lat (South)	Long (West)	Alt (m)
Canaã	M01	10°40′25.21″	48°20′52.62″	278
	M02	10°40′14.94″	48°20′54.63″	284
	M03	10°40′15.27″	48°20′55.62″	282
	M04	10°40′19.48″	48°20′52.18″	280
	M05	10°40′20.65″	48°20′55.71″	274
	M06	10°40′26.43″	48°20′52.11″	275
	M07	10°40′27.64″	48°20′56.25″	275
	M08	10°40′27.48″	48°20′51.84″	273
	M09	10°40′26.13″	48°20′55.25″	282
	M10	10°40′24.48″	48°20′55.56″	282
Providência	P01	10°33′49.13″	48°24′38.92″	220
	P02	10°33′48.20″	48°24′37.80″	219
	P03	10°33′28.63″	48°24′45.37″	213
	P04	10°33′26.60″	48°24′46.33″	211
	P05	10°33′23.55″	48°24′46.64″	210
	P06	10°33′24.77″	48°24′44.51″	225
	P07	10°33′14.58″	48°24′41.32″	216
	P08	10°33′18.30″	48°24′39.00″	217
	P09	10°33′13.23″	48°24′37.33″	215
	P10	10°33′11.85″	48°24′43.62″	213
São Judas Tadeu	B01	10°48′07.65″	48°24′30.72″	238
	B02	10°48′11.02″	48°24′30.35″	239
	B03	10°48′05.12″	48°24′43.46″	277
	B04	10°48′03.50″	48°24′42.00″	274
	B05	10°48′04.80″	48°24′38.64″	257
	B06	10°48′09.39″	48°24′31.08″	250
	B07	10°48′04.92″	48°24′32.03″	239
	B08	10°48′10.49″	48°24′30.30″	238
	B09	10°48′03.56″	48°24′34.09″	239
	B10	10°48′07.31″	48°24′43.71″	278

2.3 Statistical Analysis

Each variable was tested by analysis of variance, and the differences in pigment contents and their ratios, compared by Tukey's post-hoc test, at a 5% probability level. The errors obtained for each analyzed variable were subjected to the Shapiro-Wilk normality test and the variances compared using the variance ratio test (Zar, 1999). Data were transformed into Box-Cox, in all cases, to correct normality and homogeneity. We used the softwares Past (Hammer, Harper, & Ryan, 2001) and Microsoft Excel® for data organization and analysis.

3. Results and Discussion

The mangabeiras present in areas of the Cerrado of Tocantins, Brazil, is a deciduous species lose their leaves during the dry season. In Figure 2 shows the concentrations of the pigments and their relationships over time (DAFR—days after the first reading). In September 2014, the 30 matrices were marked and the first leaves were collected for standardization of the methodology. Data collection started at 32 days after the first reading (DAFR). The month of October was considered a transition period between dry and rainy seasons (represented by the readings: 32, 404, 770 DAFR); the months from November to April—rainy season (readings 69, 95, 131, 161, 222, 432, 455, 493, 526, 559, 587, 803, 823, 860, 895, 925 and 956 DAFR); the month of May—transition period between rainy and dry seasons (readings: 250 and 620 DAFR); the months from June to September—dry period (readings: 277, 315, 371, 647, 680, 711 and 744 DAFR).

Mangaba tree has arboreal habit and seed dispersal by zoochory, flowering (February, May, September to November), fruiting (May, June to August, October to December), new shoots (January to March, May, September, October and December), fall of leaves (August, September) (Pilon et al., 2015).

The chlorophyll content is an important indicator of leafsenescence (Carvalho, Casali, Souza, & Cecon, 2003) which is accelerated by water deficit and nitrogen deficiency (Machado, Durães, Rodrigues, Magalhães, & Cantão, 2004). In the analyzed region, leaf fall is higher in July and August (215, 680 and 711 DAFR), coinciding with the lower values of chlorophyll *a*, chlorophyll *b* and total chlorophyll (Figures 2A, 2B and 2C). The highest values for chlorophyll *a* were observed in September (371 and 744 DAFR), period of young leaves, and October (32, 404 and 770 DAFR), end of the dry period and transition to rainy, coinciding with flowering, contributing to the observation of Franco, Bustamante, Caldas, Goldstein, Meinzer, Kozovits, Rundel, and Coradin (2005) in which the deciduous species present greater efficiency in the use of water at the end of the dry season with the emergence of new leaves and with a lower "construction cost"—a smaller specific leaf area.

It can be observed an increase in the chlorophyll content and a permanence in the levels of carotenoids for the months of September (371 and 744 DAFR) and October (32, 404 and 770 DAFR) (Figures 2A, 2B, 2C and 2E). Carvalho et al. (2007) also observed that, in the rainy season, pigment concentrations tend to increase, being significantly higher than at the end of the dry season for chlorophyll *a* and *b* and chlorophyll/carotenoid ratio. This increase in the concentration of chlorophylls in the rainy season is related to the presence of leaves of a new cohort, as also observed in the present work, period with a greater number of young leaves and beginning of flowering.

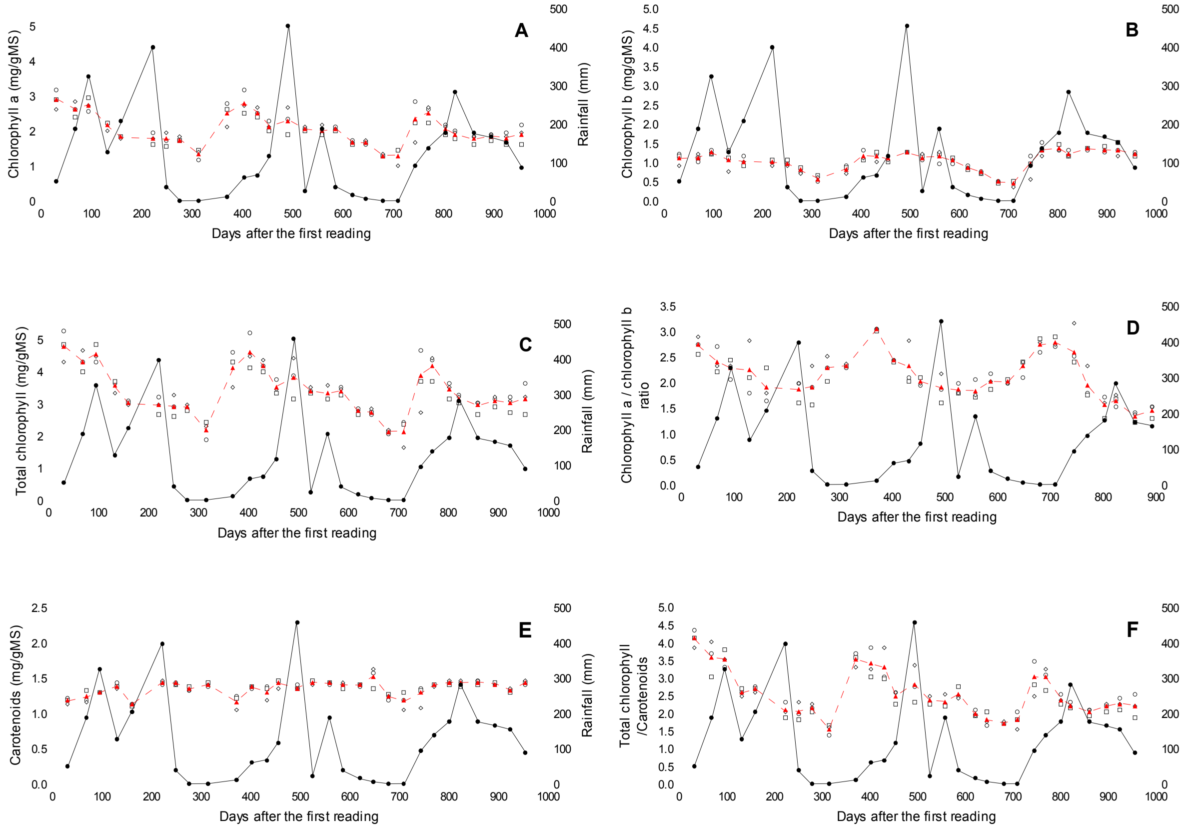

Figure 2. Contents of pigments: (A) Chlorophyll *a*; (B) Chlorophyll *b*; (C) Total chlorophyll; (D) Chlorophyll *a*/chlorophyll *b* ratio; (E) Carotenoids; (F) Total chlorophyll/carotenoids ratio, in the localities: (○) Canaã; (□) Providência; (◊) São Judas Tadeu; (▲) Average of sites (dotted line); (●) Precipitation (mm) (line)

One way to predict the nutritional level of nitrogen (N) in plants is to measure the chlorophyll content in the leaf, because there is a positive correlation between the content of this pigment and the nitrogen content in the plant (Piekielek & Fox, 1992; Smeal & Zhang, 1994; Booij, Valenzuella, & Aguilera, 2000). The variation in pigment concentration and its ratios were significantly influenced by the period (rainy, dry and transitions) and by the site (Canaã, Providência and São Judas Tadeu), except for the carotenoid content that did not differ between sites (Table 2).

In general, chlorophyll *a* and *b* occur in the ratio of approximately 3, and growth conditions and environmental factors may affect this ratio (Carvalho et al., 2007). Mangaba trees evaluated in this work, in the three sites and throughout the collection period, presented annual mean values of 2.09±0.60 (mean±standard deviation), for chlorophyll *a*/*b* ratio (Figure 2D), which according to Lichtenthaler (1987) classify them in shade plants, although the analyzed environments are considered open environments. The *a*/*b* ratio differed statistically from the collection site ($F_{2, 838} = 12.837$, $p < 0.0001$), and the highest ratio was found in the São Judas Tadeu Farm (2.20±0.60), statistically different of Canaã (2.08±0.51) and Providência (2.01±0.66), which did not differ from each other. The highest values of the chlorophyll *a/b* ratio were verified in periods of lower water availability, reaching 3.0 in September 2015 (371 DAFR). These results corroborate with Lichtenthaler (1987), who states that sun plants or exposed to conditions of high luminosity present ahigher chlorophyll *a*/*b* ratio, situation found in São Judas Tadeu. At this site, the chlorophyll *b* content was significantly lower (1.00±0.36) than the other two sites (Canaã: 1.08±0.38 and Providência: 1.06±0.36) that did not differ from each other. Significant difference was detected for the effect of periods (rainy, rainy/dry transition, dry and dry/rainy transition) for all pigments analyzed and their ratios (Table 2).

Table 2. Values of mean square (F) and probability (p) for the Box-Cox transformed data for the contents of pigments: Chlorophyll *a*; Chlorophyll *b*; Total chlorophyll; Carotenoids; Chlorophyll *a*/*b*; Total Chlorophyll/Carotenoids ratio between total chlorophyll and carotenoids. Values p < 0.05 are considered significant

Source of variation	Df	Chlophyll a		Chlophyll b		Total Chlophyll		Carotenoids		Chlophyll a/b		Chloph/Caroten	
		F	p-value	F	p-value	F	p-value	F	p-value	F	p-value	F	p-value
Periods	3	86.0181	0.0000	146.3657	0.0000	88.0599	0.0000	107.4058	0.0000	12.5763	0.0000	73.3751	0.0000
Site	2	6.0797	0.0024	7.0830	0.0009	5.9701	0.0027	12.8374	0.0000	0.5938	0.5524	3.5020	0.0306
Error	838												

No significant difference was detected between the rainy/dry transition and dry periods for chlorophyll *a* (Figure 3A), total chlorophyll (Figure 3C) and total chlorophyll/carotenoids ratio (Figure 3F). For these comparisons, the dry period always had a significantly lower effect. It should be noted that the averages presented in the Figure 3 are for the data transformed by *Box-Cox*, and cannot be used directly to express the levels of chlorophyll and carotenoids in each period.

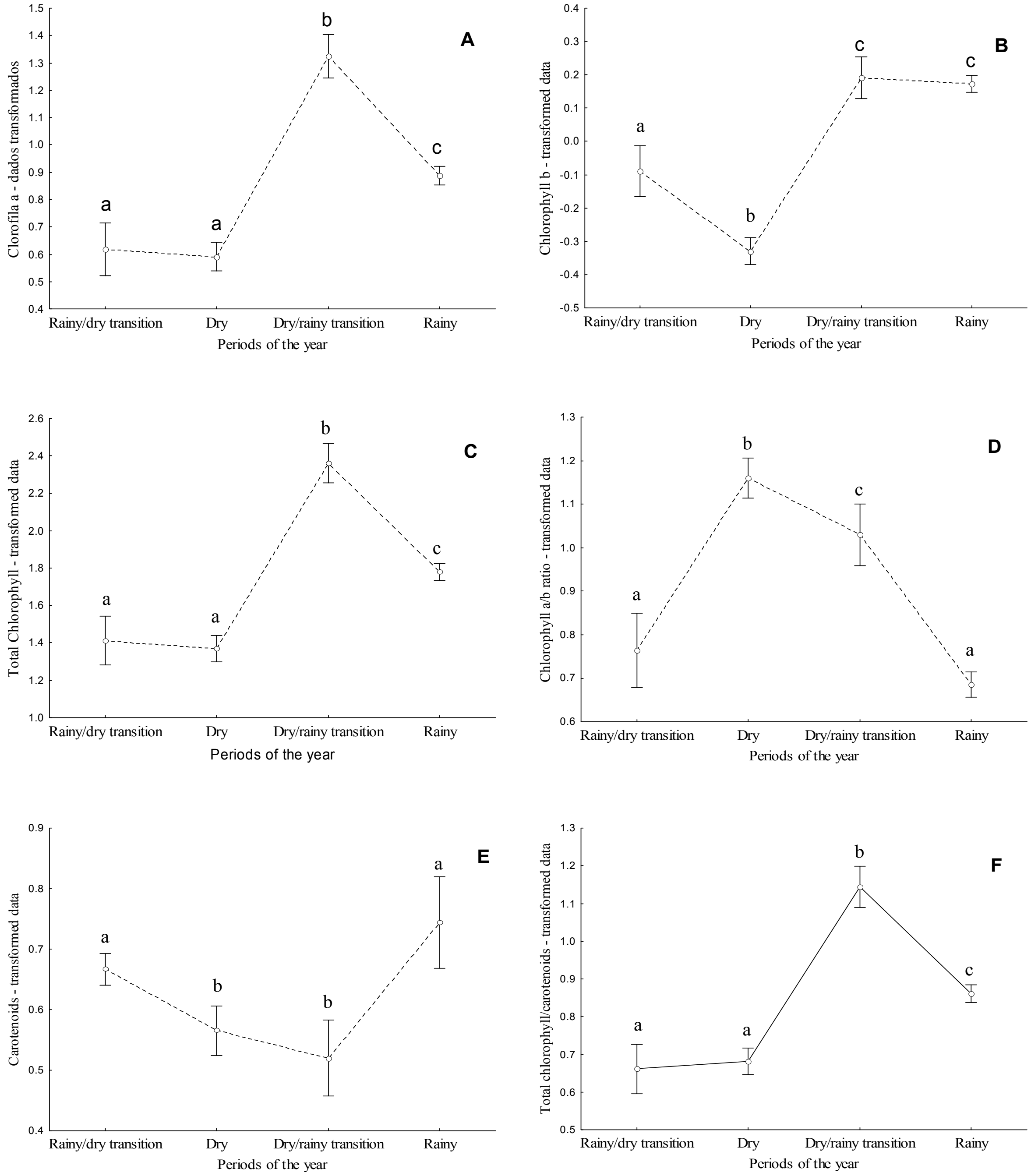

Figure 3. Contents of pigments: (A) Chlorophyll *a*; (B) Chlorophyll *b*; (C) Total chlorophyll; (D) Chlorophyll *a*/chlorophyll *b* ratio; (E) Carotenoids; (F) Total chlorophyll/carotenoids ratio, per period of the year (rainy/dry transition, dry, dry/rainy transition and rainy)

Note. The ordinates of the graphs represent the means and the 95% confidence interval for the data transformed by Box-Cox; different letters on the comparisons indicate that the means are significantly different by the Tukey's test at the 5% probability level.

4. Conclusion

(1) The seasonal variation of the chlorophyll *a*, chlorophyll *b* and total chlorophyll in mangaba tree (*Hancornia speciosa*) follows the same observed for the deciduous species, that is, is closely associated with the availability

of water in the soil, and the highest values were found shortly after the fall of leaves and the beginning of a new cohort.

(2) The mean chlorophyll *a* content was 2.0 times higher than the chlorophyll *b*, and was 1.8 times in the rainy period (November to April) and 2.5 times in the dry period (June to September). The maximum value found reached the 3/1 ratio recommended in the literature, indicating that this ratio is associated with the moment of data collection.

(3) There was a significant difference between the analyzed periods and collection sites for all pigments and their ratios;

(4) The total chlorophyllcontent was 21.8% higher in the rainy period, compared to the dry period, and its highest value occurred in the dry/rainy transition due to the higher number of young leaves in this period, whereas for carotenoids. it was 5.4% higher in the rainy period, although with little difference between the months of the year.

(5) The total chlorophyll/carotenoids ratio was 16.3% higher in the rainy period when compared to the dry period, and the highest value was observed in the dry/rainy transition period.

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