Chloroplastid pigment contents and chlorophyll a fluorescence in amazonian tropical three species

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CHLOROPLASTID PIGMENT CONTENTS AND CHLOROPHYLL a FLUORESCENCE IN AMAZONIAN TROPICAL THREE SPECIES

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ABSTRACT – Plants react to changes in light and hydrological conditions in terms of quantity and composition of chloroplastic pigments, which affects the photosynthetic properties and consequently the accumulation of plant biomass. Thus, the chloroplastic pigment concentration and chlorophyll a fluorescence of three Amazonian species (Bertholletia excelsa, Carapa guianensis e Dipteryx odorata) were investigated in sun and shade leaves form the tree crown collected during two distinct periods of precipitation (dry and rainy seasons). Pigment contents were determined by spectrophotometry and fluorescence variables were determined using a portable fluorometer. The results demonstrated that the species showed high concentrations of Chl a, Chl b e Chl total during the wet season in relation to the dry season, especially in shade leaves. A higher concentration of carotenoids was found in B. excelsa, when compared with leaves of C. guianensis and D. odorata. In leaves of B. excelsa and D. odorata no significant difference was found in relation to the photochemistry of photosystem II (F v/F m) between the wet and dry seasons. In conclusion, the three species react differently to variations in the light and precipitation conditions regarding light capture, aspects that might be considered in the management of forest plantations.

Keywords: Bertholletia excelsa, Carapa guianensis and Dipteryx odorata.
1. INTRODUCTION

Due to human impact, site conditions in plantations are different from those in primary forests, affecting the growth of the planted trees even when species were used (BAUCH et al., 1999). Comparative studies carried out in primary forests and forest plantations in the Amazon showed significant alterations of light conditions as well as water and nutrient supply (SZOTT and PALM, 1996; NARDOTO and BUSTAMANTE, 2003). Information on growth and demands for light, nutrients, and water of tree species selected for reforestation programs is therefore needed for sustainable timber production (BAUCH et al., 1999). Until the present day, most of the native tree species of the Amazon selected from the primary forest lack information on their physiology and field performance. Photosynthesis is strongly influenced by light intensity and water supply for the plant (DUNISCH and MORAIS, 2002; GONÇALVES et al., 2005). In particular, in plantations established in sites with seasonal climate, a strong spatial and temporal variation in light and hydrological conditions can be expected, which to a certain point explain the differences in plant growth and biomass accumulation between trees grown in plantations and trees grown on natural sites (SCHROTH et al., 2000).

Plants respond to changing light conditions in terms of quantity and composition of pigments in the chloroplasts. At low light intensities, shade leaves have a higher content and a lower ratio of Chlorophyll a/b, which is considered to stabilize the light absorbance between photosystem I and II (ALVARENGA et al., 2003; CARVALHO et al., 2006). On the other hand, due to the relationship of chlorophyll a to b in the reaction centre and in the light harvesting complexes (WALTERS, 2005), the ratio of chlorophyll a to chlorophyll b increases at high irradiance (increase of ratio PSII / Chl\text{total}).

In addition to light conditions, the water supply modify the electron transport rate and efficiency of light transfer in the antenna during the photosynthesis, which also might have a significant influence on the productivity and biomass allocation in plants (WANG et al., 2006).

The excess of irradiance can drastically decrease the photosynthetic capacity in the plant, contributing to the occurrence of photoinhibition (KITAO et al., 2000). The photoinhibitive process can increase when the excess of irradiance is associated with other factors like water deficiency and high temperatures (LEMOS FILHO, 2000; THOMAS and TURNER, 2001). The photoinhibitive damage is mainly related to changes in the physical-chemical properties of the thylacoidal membranes and electron transport inactivity. These factors cause a reduction in the photochemical quantum yield of PSII, an increase in the dissipation of excess non-photochemical energy, and a decrease in the carboxylation efficiency, which is most likely related to the formation of reactive oxygen species (GILMORE and GOVINDJEE, 1999).

The analysis of chlorophyll a fluorescence is a powerful tool in order to study the conversion, and the transfer of energy in the photosystem II (GONÇALVES et al., 2001; GONÇALVES and SANTOS JUNIOR, 2005; RIBEIRO et al., 2004).

In this study the relationship between the light intensity, precipitation, the concentration of chlorophyll pigments, and the fluorescence of chlorophyll a in the leaves of tree native high value tree species (Bertholletia excelsa, Carapa guianensis, Dipteryx odorata) of the Central Amazon was investigated in order to contribute to a better understanding of physiological aspects of tropical tree species and to the establishment of sustainable production in plantations.
2. MATERIALS AND METHODS

2.1. Study area and plant species

The present study was carried out at the experimental site of Embrapa Amazonia Ocidental (Manaus, Amazonas, Brazil), 3°8'S, 59°52'W, in a polyculture plantation system of approximately 10 years of age. The soil is classified as a poor “Yellow Latosol”, developed by tertiary sediments with a reduced capacity for cation exchange. The area is well-drained and a fraction of the clay in the soil is dominated by caolinita. This type of soil is commonly found across large areas of central and eastern Amazon (SCHROTH et al., 2000). According to the Köppen classification, the climate of the region is an Am type, typically tropical with annual average precipitation, temperature and atmospheric humidity of 2500 mm, 26 °C and 85%, respectively. The period of greatest precipitation is found between February and April and the period of least precipitation between July to September (Figure 1). For the determination of the physiological parameters the species Bertholletia excelsa, Carapa guianensis and Dipteryx odorata grown in environmental conditions of the plantations were selected according to their high potential for reforestation programs and timber quality. Leaves fully expanded and showing good health were collected during two distinct precipitation regimes (dry and wet seasons).

2.2. Pigment analysis

To determine chloroplastid pigments, six trees per species were randomly chosen and three fully expanded leaves were collected per plant. The data were collected in sun and shade leaves in the months of February 2000 (rainy season) and September 2000 (dry season). We consider as sun leaves, leaves growing in direct sun light, and shade leaves as leaves shaded by others during all day. Leaves were placed between layers of ice in a thermal insulated box and transported to the Plant Physiology and Biochemistry Laboratory (National Institute of Amazon Research), where they were analyzed immediately. Then, samples with 0.1 g of leaves (fresh material) were grounded in 10 ml of 80% acetone and 0.5% (W/V) magnesium carbonate (MgCO₃) and immediately afterwards 10 ml of 100% acetone was added. The suspension was filtered and the absorbance was determined using a spectrophotometer (Jenway 6105 UV/VIS spectrophotometer) with wavelengths at 663 nm (chlorophyll \(a\) – Chl \(a\)), 645 nm (chlorophyll \(b\) – Chl \(b\)) and 480 nm (carotenoids – \(C_x+c\)) (ARNON, 1949). Then the values for Chl \(a/b\) and Chl \(_{total}\) / \(C_x+c\) were evaluated. The calculations for both the mass (\(\mu\)mol g\(^{-1}\)) and leaf area (\(\mu\)mol m\(^{-2}\)) of the chloroplastid pigments were obtained using the equations of Hendry and Price (1993).

![Figure 1](image-url)
2.3. Chlorophyll a fluorescence measurements

The determination of chlorophyll a fluorescence was conducted using a Plant Efficiency Analyzer-MK2–9600 – Hansatech, Norfolk, UK) in completely expanded leaves of appropriate phytosanitary state according to Gonçalves et al. (2001). The data were collected in sun and shade leaves as described for pigment analysis, in the months of February 2000 (rainy season) and September 2000 (dry season) between 9:00 am and 11:00, three trees per species were randomly chosen and three fully expanded leaves per plant were collected. The selected leaves were subjected to a 30 minutes period of adaptation to darkness, time enough for complete oxidation of the reaction centres. Immediately, the leaves were exposed to a pulse of saturated light at an intensity of 2250 µmol m$^{-2}$ s$^{-1}$ (75% of the equipment capacity) and a wavelength of 650 nm. After the induction of fluorescence in fast kinetic the initial fluorescence ($F_0$), the maximum fluorescence ($F_m$), the variable fluorescence ($F_v = F_m - F_0$) and the photosynthetic efficiency of PSII ($F_v / F_m$) were determined.

2.4. Experimental Design and Statistical Analysis

The experiment was arranged in completely randomized block with three repetitions in factorial scheme 3x2x2 with three tree species ($B. excelsa$, $C. guianensis$ and $D. odorata$), two precipitation seasons (dry and wet) and two light environments (sunlight and shade). The results were examined by analysis of variance (ANOVA) followed by the Tukey test ($P = 0.05$), using the software for statistical analyses SAEG (1993).

3. RESULTS AND DISCUSSION

During the wet season, a higher chlorophyll contents on a fresh weight basis was found in the leaves (in particular in shade leaves) of all species compared to the drier season (content of Chl $a$, Chl $b$ and Chl$_{total}$ 19-23, 14-25, and 19-24% higher during the wet season than during the dry season, Figure 2). The chlorophyll content in sun leaves increased Chl $a$, Chl $b$ and Chl$_{total}$ by 54, 31, and 48 % during the wet season compared with the dry season in leaves of $D. odorata$ (Figure 2), whereas no significant differences were found in chlorophyll content between the wet and the dry season for $Bertholletia$ and $Carapa$. However, these results indicate a disorder in the thylakoids membrane under conditions of reduced water availability to the plant (TUBA et al., 1993)

![Figure 2](image-url)
Chloroplastid pigment contents and chlorophyll $a$ ...

In a similar way, we found higher contents of carotenoids and higher ratios of Chl $a/b$ and of Chl$_{total}$/C$_{x+c}$ in leaves harvested during the wetter season compared with leaves harvested during the drier season (Figure 3).

Higher contents of Chl $a$, Chl $b$, and Chl$_{total}$ were found in shade leaves compared with sun leaves (Figure 2). Because chlorophyll is constantly synthesized and destroyed in the presence of light, but under high light intensity, the rate of decomposition is greater, a balance is established at a lower concentration. For this reason, shaded leaves exhibit higher concentrations of chlorophyll on fresh weight basis than leaves under direct sunlight (TAN et al., 2000). In other words, the chlorophyll content decreased when the levels of irradiance increased (MENDES et al., 2001). Significant differences in the content of C$_{x+c}$ between leaves harvested in the wet season and leaves sampled in the dry season were found in $C. guianensis$ and in $D. odorata$ (Figure 3).

Beside the strong influence of the water supply on the chlorophyll content of the leaves, we found a significant impact of the light availability on the content of leaves chlorophyll and carotenoids.

Concerning the content of chromatophoric groups, it was recorded in all species a higher ratio of Chl $a/b$ in sun leaves compared with shade leaves during the rainy season (Figure 3). According to Engel and Poggiani (1991), a greater relative proportion of Chl $b$ in shaded plants is significant because it allows the capture of energy at different wavelengths as well as the transfer of a specific Chl $a$ molecule, which takes part in the photochemical reactions of photosynthesis. Since adaptation to low irradiance can involve an increase in light-harvesting complexes of photosystem II, a decrease of Chl $a/b$ ratio was expected in shade leaves in comparison with sun leaves. (MENDES et al., 2001).

Unexpectedly, during the drier season, it was observed a higher ratio of Chl $a/b$ in shade leaves of $D. odorata$ compared with sun leaves (Figure 3). Due to the little protection of the photosynthetic mechanism, the value of $a/b$ can decrease in leaves exposed to direct sunlight because chlorophyll $a$ has the tendency to degrade faster in conditions of high irradiance than chlorophyll $b$ (ENGEL and POGGIANI, 1991).

Figure 3 – Concentration of carotenoids, chlorophyll $a/b$ ratio and chlorophyll total/carotenoids ratio on leaf mass basis, in different periods of precipitation (Dry and Wet), under different exposures to light, sun leaves (○) and shade (■) factorial 3x2x2. Means followed by the same small letters on the left and right, for different exposures to light and different periods of precipitation, respectively, and by the same capital letter for species, in the same precipitation period, are not significantly different by the Tukey test to 5%, (n=18).

Figura 3 – Concentração de carotenóides, razão clorofila $a/b$ e razão clorofila total/carotenóides na base de massa foliar, em diferentes períodos de precipitação (seco e chuvoso), sob diferentes exposições de luminosidade, folhas de sol (○) e folha de sombra (■) fatorial 3x2x2. Médias seguidas pela mesma letra minúscula à esquerda e à direita, para diferentes exposições à luz e diferentes períodos de precipitação, respectivamente, e pela mesma letra maiúscula para espécies, no mesmo período de precipitação, não diferem entre si, pelo teste de Tukey a 5% (n=18).
Changes in the ratios of Chl \( a/b \) can be considered as an adaptation to changing light conditions. An increasing ratio of Chl \( a/b \) at high irradiance favors the increase of PSII units and the efficiency of energy conversion (WALTERS, 2005).

During the rainy season a higher ratio of Chl total/\( C_x+c \) was found in sun leaves of \( B.\ excelsa \) and \( D.\ odorata \) compared with shade leaves (Figure 3). This result indicates that in these two species a mechanism for photoprotection is activated (HENDRY and PRICE, 1993).

Significant differences in chlorophyll contents were found between species only in sun leaves. During the drier season the highest contents of Chl \( a \), Chl \( b \), and Chl total were found in \( B.\ excelsa \), whereas the lowest contents were found in leaves of \( D.\ odorata \). During the rainy season lowest contents were found in the leaves of \( C.\ guianensis \).

The highest content of carotenoids was found in leaves of \( B.\ excelsa \), indicating a high capacity for photoprotection in this species. The Chl \( a/b \) ratio in leaves of \( D.\ odorata \) was 10 to 18% higher compared to leaves of \( B.\ excelsa \) and \( C.\ guianensis \) (shade leaves, drier period, Figure 2). In addition, \( D.\ odorata \) showed the highest ratio of Chl total/\( C_x+c \) when compared with the other two species (Figure 3).

The analysis of chlorophyll fluorescence in the leaves showed significant differences of the initial fluorescence \( F_o \) and the photochemical efficiency PSII \( F_v/F_m \) among the three species (Figure 4). The stable relationship of \( F_v/F_m \) over time observed in \( B.\ excelsa \) and \( D.\ odorata \) gives evidence for the assumption that drier periods had no significant impact on the efficiency of the PSII (BAKER and ROSENQVIST, 2004). Therefore it can be concluded that different assimilation rates observed in these species during the wet and the dry seasons (MORAIS unpublished data) are due to stomata closure and reduced uptake of \( CO_2 \) during the drier season (BAKER and ROSENQVIST, 2004).

![Figure 4](image_url)

**Figure 4** – Fluorescence Variables of chlorophyll a, \( F_o \) (initial Fluorescence), \( F_m \) (maximum Fluorescence), \( F_v \) (variable Fluorescence) and \( F_v/F_m \) (Efficiency of the PS II) of tree species, in different periods of precipitation (Dry and Wet), under different exposures to light, sun leaves (■) and shade (▲) in the area of Manaus, factorial 3x2x2. Means followed by the same small letters on the left and right, for different exposures to light and different periods of precipitation, respectively, and by the same capital letter for species, in the same precipitation period, are not significantly different by the Tukey test to 5%. (n=9).
Except for C. guianensis, no significant differences in chlorophyll fluorescence $F_o$ were observed between sun and shade leaves, but highest values of $F_v$, $F_m$ and $F_v/F_m$ were found in shade leaves (Figure 4).

The reduction in the $F_v/F_m$ ratio was predominantly due to the reduction of the $F_m$ value, indicating a reduced efficiency at the transfer of energy, whereas the activity pool of the PSII seems unaffected (KITAO et al., 2000; OLIVEIRA et al., 2002). In addition, the low values for $F_m$ in the sun leaves could be associated with the dissipation of energy like heat, which is a strategy that could be used by D. odorata when exhibiting low values for $F_m$ (GONÇALVES et al., 2001). The very low differences of the $F_v/F_m$ values (4-8 %) measured under changing light and hydrological conditions indicate a good physiological adaptation of the three species to a reduced light availability and water supply.

4. CONCLUSION

In spite of the seasonal variation in the content of photoactive pigments in the leaves of these species, the results indicate that the environmental conditions of the plantations did not significantly compromise the performance of the photosynthetic mechanism, even in the period of low precipitation in the region, thus confirming that the species studied were not subjected to stress. The species considered in this study had different strategies to capture light energy, both incidental and diffuse. Therefore, the present work concludes that these ecophysiological differences among species might be considered for the establishment of productive, sustainable and management of forest plantation systems.

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