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PHOTOSYNTHETIC PERFORMANCE OF MANGROVES *Rhizophora mangle* AND *Laguncularia racemosa* UNDER FIELD CONDITIONS[1]

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ABSTRACT – In mature mangrove plants *Rhizophora mangle* L. and *Laguncularia racemosa* Gaerth. growing under field conditions, photosystem 2 (PS2) photochemical efficiency, determined by the ratio of variable to maximum fluorescence (F_v/F_m), increased during the day in response to salinity in the rainy seasons. During the dry season, fluorescence values (*Fo*) were higher than those observed in rainy season. In addition, *Fo* decreased during the day in both season and species, except for *R. mangle* during the dry season. A positive correlation among F_v/F_m and salinity values was obtained for *R. mangle* and *L. Racemosa* during the dry and rainy seasons, showing that photosynthetic performance is maintained in both species under high salinities. Carotenoid content was higher in L. Racemosa in both seasons, which represents an additional mechanism against damage to the photosynthetic machinery. The chlorophyll content was not affected by salinity in either species.

Keywords: chlorophyll fluorescence, photosynthetic pigments, photosystem.

DESEMPENHO FOTOSSINTÉTICO DE Rhizophora mangle E Laguncularia racemosa EM CONDIÇÕES DE CAMPO

RESUMO – Em plantas adultas de Rhizophora mangle L. e Laguncularia racemosa Gaerth. crescendo em condições de campo, a eficiência fotoquímica do fotossistema 2 (FS2), determinada pela razão fluorescência variável : fluorescência máxima (F_v/F_m)*, aumentou durante o dia em resposta à salinidade, durante a estação chuvosa. Durante a estação seca, os valores de Fo foram superiores aos observados durante a estação chuvosa. Além disso, Fo reduziu-se ao longo do dia em ambas as estações e espécies, exceto para R. mangle durante a estação seca. Uma correlação positiva entre os valores de* F_v/F_m *e da salinidade foi obtida em R. mangle e L. racemosa durante as estações seca e chuvosa, mostrando que o desempenho fotossintético é mantido em ambas as espécies sob altas salinidades. O conteúdo de carotenóides foi maior em L. racemosa, em ambas as estações, representando um mecanismo adicional contra danos à maquinaria fotossintética. O conteúdo de clorofila não foi afetado pela salinidade das espécies estudadas.*

Palavras-chave: Fluorescência da clorofila, pigmentos fotossintéticos e fotossistema.

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1. INTRODUCTION

Rhizophora mangle L. and the *Laguncularia racemosa* Gaerth. mangroves are two major species in the district of Vitoria, Espírito Santo, Brazil and dominant in the American Atlantic coastlines. These species grow in environments with widely ranging salinity, from near freshwater to above seawater salinity (LOVELOCK and FELLER, 2003; SUARÉZ, 2003; SOBRADO, 2004; SOBRADO, 2005). Salinity constitutes the main stressor and regulator of the development and productivity of mangrove forests (MEDINA et al., 1990; SOBRADO and BALL, 1999). Mangrove species more tolerant to salinity may function with less efficient water transport, which may be related to more conservative water use (SOBRADO, 2004). Krauss and Allen (2003) suggested high salinity tends to favor the development, establishment and physiology of *R. mangle*. However, other studies show that L racemosa is more tolerant to the negative effects of salinity (SOBRADO, 2004). Evidence for less salt sensitivity in mangrove species includes biochemical adaptations by wich mangroves counter the high osmolarity of salt, like accumulation of compatible solutes or enhanced gland secretion (TAKEMURA et al., 2000; KRAUSS and ALLEN, 2003; SOBRADO, 2004). In contrast, responses of mangrove to salinity reflect alterations in photosynthesis, photosynthetic pigment content, trasnpiration rate, and enzyme activities. Generally, these negative effects also associated with other environmental factors, e.g., high light levels (TAKEMURA et al., 2000; KRAUSS and ALLEN, 2003).

The photosynthetic performance of *R. mangle* and *L. racemosa* mangrove species in the equatorial region of Brazil is little understood. It is known that integrity of membranes, activity of several enzymes, nutrient acquisition, photosynthetic pigment content, gas exchange and photosynthetic apparatus function are affected by high NaCl concentrations (ZHU, 2001). In halophytes, some studies have showed that salinity inhibits photosystem 2 (PS2) activity, while others indicate the opposite (TAKEMURA et al., 2000; LU et al., 2002; QIU-FANG et al., 2005). *Avicennia corniculatum and Bruguiera gymnorrhiza* showed a decrease in the photosynthesis, whereas *A. marina* was not affected by the salinity (TAKEMURA et al., 2000). Other studies showed that salt stress by itself has little effect on the PS2 photochemical efficiency and on photosynthetic pigment content under relatively low light levels. But, under high irradiance, it might cause damage in to PS2, as well as alterations in photosynthetic pigment composition (MISHRA et al. 1991; MORALES et al., 1992; BELKODJA et al., 1994; TUFFERS et al., 2001; NAIDOO et al., 2002). However, most of these studies were done in greenhouses, under controlled conditions, using mature mangroves propgules as a source of the plant material. Information about the physiological behavior of mature plants of *R. mangle* and *L. racemosa* under field conditions is scarce. Thus, the objective of the present study was to evaluate the photosynthetic performance of *R. mangle* and *L. racemosa* during the day in the dry and rainy seasons of the year and relate this performance to variations in interstitial water salinity.

2. MATERIAL AND METHODS

The study was conducted in the mangrove forest around of the Universidade Federal do Espírito Santo (20°15 '48"S and 40°18'17"N), Vitoria district, Espirito Santo, Brazil. Tidal amplitudes at the study area range between 0.3 and > 1 m. Mangrove forest is abundant on clay and loam areas situated above mean high water level. The relative air humidity at the study site was approximately 89% and annual average air temperature 26ºC. During the investigation period, annual precipitation was 1250 mm, distributed in a dry (April to September) and a rainy (October to March) season. Mangrove forest at the study site is composed of three tree species: *Rhizophora mangle* L. (Rhizophoraceae), *Laguncularia racemosa* Gaerth. (Combretaceae) and *Avicennia germinans* Stearn. (Avicenniaceae). *R. mangle* and *L. Racemosa* are the dominant trees at the study site, while A. Germinans is little represented. All data were collected from ten trees, which varied in height from 2-4 m. Interstitial water was collected in the morning (9 a. m.), midday (1 p. m.) and afternoon (5 p. m.) using 2.5 cm diameter PVC tube inserted at -0.2 m sediment depth, according to the method of Miller and Ullman (2004). Salinity measures of interstitial water were accomplished immediately after collecting the water in the tubes, using a portable condutometer *SCHOT Mod. LF1*.

Chlorophyll fluorescence parameters were determined *in situ* on fully expanded young leaves of *R. mangle* and *L. Racemosa*, in the morning, midday and afternoon using a portable Chlorophyll Fluorescence System (*Handy-PEA*, Hansatech, Norfolk, England) after 30 min of dark adaptation, using specially designed clips attached to the leaves. These same leaves were used for the determination of photosynthetic pigment contents (below). Each leaf was exposed to a saturation pulse of light (750 µmol m^{-2} s^{-1}; the value was obtained from a saturation curve) for 5 s. Initial fluorescence (*Fo*) and PS2 photochemical efficiency (F_v/F_m) were calculated automatically.

Chlorophyll and carotenoid contents were determined in the morning using the spectrophotometric method suggested by Arnon (1949). Chlorophyll a (*Chl a*), chlorophyll b (*Clh b*), chlorophyll a/b ratio and carotenoids (*Car*) were calculated using the following equations, suggested by Hendry Grime (1993): *Chl a* (mg/L) = 12,7 x A_{663} - 2,69 x A_{645}; *Chl b* (mg/l) = 22,9 x A_{645} - 4,68 x A_{663}; *Car* = [A_{480} + 0,114 x A_{663} - 0,638 x A_{645}) x V / 112,5 x Weight of the fresh matter of the leaf (*g*)], where V = volume of the acetone extract in cm^3 and A = absorbance at the specified wavelength (nm), measured with a 1 cm cuvette.

Each data collection consisted of ten repetitions, in a randomized block design. For salinity, *Fo* and F_v/F_m, statistical comparisons were made between dry and rainy seasons and between times during the day. Chlorophyll and carotenoid content values were compared between species and between seasons. Salinity and F_v/F_m values were correlated in both species and seasons. Results were examined by one-way analysis of variance (ANOVA) and differences between means were assessed using Tukey test at 5% significance level (STATSOFT, 1995).

3. RESULTS AND DISCUSSION

Significant increase in salinity was observed in the rainy season at 1 p. m. and 5 p. m. in relation to that at 9 a. m. (Table 1). Furthermore, the salinity values obtained in this season were higher than those in the dry season. During the rainy season, PS2 photochemical efficiency (F_v/F_m) of dark-adapted leaves sampled at 9 a. m. were 0.73 and 0.69 for *R. mangle* and *L. Racemosa*, respectively (Table 1). These values were significantly lower than those obtained at 1 and 5 p. m. and were inversely related to initial fluorescence values (*Fo*).

During the dry season, *Fo* mean values were higher than those observed in the rainy season for both mangrove species. In the dry season, *Fo* values decreased significantly in *L. racemosa* during the day, from 1027 in the morning, to 875 and 675 in the midday and afternoon, respectively (Table 1). In contrast, F_v/F_m values increased significantly during the day for *L. racemosa*: 0.66, 0.74 and 0.80 at 9 a. m., 1 and 5 p. m., respectively. For *R. mangle*, the F_v/F_m values were 0.68 at 9 a. m. and 0.77 at 1 and 5 p. m. (Table 1). Carotenoid content was higher to *L. racemosa* in both seasons. There were no significant differences for chlorophyll content between species and between seasons.

F_v/F_m reflects the PS2 potential efficiency and it is used as indicators of plant photosynthetic performance (KAO and TSAI, 1999; SHIRKE and PATHRE, 2003; RIBEIRO et al., 2004). In this study, the increase observed in F_v/F_m during the day for *R. mangle* and *L. racemosa*, in both seasons, can be attributed to a decrease in *Fo*, although the *Fo* values did not present a statistically reduction in *R. mangle* during the dry season. *Fo* originates exclusively from the PS2 light harvest complex (LHC2) after the application of low red light on leaves adapted to the dark (LICHTENTHALER et al., 2005) and its values are, generally, strongly related to photoinhibition phenomena. In addition, during the rainy season, F_v/F_m of dark-adapted leaves increased similarly during the day in response to increases in salinity for both species. These results strongly suggest that *R. mangle* and *L. racemosa* increase their photosynthetic efficiency in response to increases in salinity levels.

Several studies have shown that high salinity causes a decrease of photosynthetic efficiency in mangrove species grown in greenhouses under different concentrations of NaCl. However, the effects of salinity on photosynthetic parameters of mangrove species under field conditions are contradictory. The main reason for this contradiction is the large variation of field environmental conditions. Thus, results obtained in a specific mangrove are cannot be extrapolated to mangroves situated in other regions.

In this study, a positive correlation (r^2 = 0.99) (not shown) among F_v/F_m and salinity values was obtained for both species and seasons, indicating that *R. mangle* and *L. racemosa* presented high tolerance and adaptability at high salinity. Halophytic plants are tolerant to salinity because they are able to take up water through the accumulation of inorganic ions or other compatible solutes such as betaine and proline. Consequently, the osmotic potential decreases, which in turn attracts water into the cell and enables to maintain pressure potential (MOGHAIEB et al., 2004).

Table 1 - Photosynthetic efficiency (F_v/F_m), initial fluorescence (*Fo*), chlorophyll content (Chl a, Chl b, Chl a/b ratio) and carotenoid content (Car) in leaves of *Rhizophora mangle* L. and *Laguncularia racemosa* Gaerth, subjected to different salinity levels under field conditions. (n = 10)

***Tabela 1** - Eficiência fotossintética (F_v/F_m), fluorescência inicial (Fo), conteúdo de clorofila (Chl a, Chl b, Chl a/b ratio) e conteúdo de carotenóides (Car) em folhas de Rhizophora mangle L. e Laguncularia racemosa Gaerth. sujeitas a diferenças da salinidade em condições de campo (n = 10)*

Parameters	*Dry season*			*mean*	*Rainy season*			*mean*
	9 a.m.	1 p.m.	5 p.m.		9 a.m.	1 p.m.	5 p.m.	
Rhizophora mangle L.								
Salinity	10.5±3.4	12.04±1.9	11.7±1.4	11.4±0.81 B	13.4±0.3 b	19.8±3.6 a	20.94±0.8 a	18.04±4.0 A
Fv/Fm	0.68±0.02 b	0.77±0.03 a	0.77±0.03 a	0.74±0.05	0.73±0.02 b	0.8±0.003 a	0.82±0.001 a	0.78±0.04
Fo	986±267	886±192	844±200	905±72.94 A	840±133 a	581±0.0 b	546±11.8 b	655±160.5 B
Chl *a*	0.96±0.2	-	-	-	1.10±0.13	-	-	-
Chl *b*	0.64±0.1	-	-	-	0.77±0.14	-	-	-
Chl *a/b* ratio	1.47±0.12	-	-	-	1.4±0.0	-	-	-
Car	0.22±0.03 *	-	-	-	0.26±0.03 *	-	-	-
Laguncularia racemosa Gaerth.								
Salinity	10.5±3.4	12.04±1.9	11.7±1.4	11.4±0.81 B	13.4±0.3 b	19.8±3.6 a	20.94±0.8 a	18.04±4,0 A
Fv/Fm	0.66±0.02 b	0.74±0.001 a	0.8±0.004 a	0.73±0.07	0.69±0.02 b	0.8±0.01a	0.83±0.0 a	0.77±0.07
Fo	1027±38.5 a	875±33.5 b	675±24 c	859±176.5 A	893±17.5 a	653±51 b	530±10.8 c	692±184.6 B
Chl *a*	1.03±0.2	-	-	-	1.13±0.16	-	-	-
Chl *b*	0.59±0.19	-	-	-	0.82±0.13	-	-	-
Chl *a/b* ratio	1.5±0.0	-	-	-	1.47±0.06	-	-	-
Car	0.3±0.04 *	-	-	-	0.34±0.03 *	-	-	-

Small letters indicate statistical difference along the day within of each season, while capital letters indicate statistical difference between seasons.
Significant differences in carotenoid content between species in each season are represented by an asterisk (*) (Tukey test at 5% probability level)

Leaf chlorophyll content (Chl a, Chl b and *a/b* ratio) on a fresh mass basis showed no significant difference for both (daily and seasonal) periods (Table 1). Lu et al. (2002) observed that salt stress had no effects on the photosynthetic pigment composition in halophyte *Suaeda salsa*, grown outdoors in plastic pots, subjected to salt concentrations up to 400 mM. Thus, chlorophyll content in R. mangle and*L. racemosa* seems to be insensitive to salinity variations during the day and in both dry and rainy seasons.

Rhizophora mangle and *L. racemosa* differed in carotenoid content (Table 1). Higher carotenoid content was observed in *L. racemosa* in both seasons. Carotenoids play important roles in plants: they act as pigment antenna, enhancing the reception of luminous energy for the reaction centers, and protect chlorophyll against photoxidative damage (GONÇALVES et al., 2001; BEHERA and CHOUDHURY, 2002). During the protective action, they are degraded. It is possible that*L. racemosa* is adapted to maintain higher carotenoid levels in its cells. On the other hand, low carotenoid contents can be a consequence of β-carotene degradation and subsequent zeaxantin synthesis (through de-epoxidation of violaxanthin to antheraxanthin and to zeaxanthin), which are also involved in protection against photoinhibition. This is a photoprotective mechanism and allows excess energy to be dissipated.Conversion of violaxanthin to zeaxanthin has been observed in other mangrove species under high salinity (SOBRADO and BALL, 1999).

It is probable that the increase in both synthesis and accumulation of antioxidative pigments (carotenoids) in *L. racemosa* during both dry and rainy seasons represents an additional mechanism against damage to the photosynthetic machinery. Since F_v/F_m ratios of 0.75 - 0.85 have been considered normal for unstressed plants (HUNT, 2003; OLIVEIRA et al., 2006), it can be suggested that *R. mangle* and *L. racemosa* were able to maintain photosynthetic activity in response to salinity increases because of their protection mechanisms.

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