

M**MORPHOLOGICAL AND ANATOMICAL ASPECTS OF THE LEAVES OF
RHIZOPHORA MANGLE L. (RHIZOPHORACEAE) UNDER DIFFERENT
LIGHTING CONDITIONS****CAMILLA REIS AUGUSTO DA SILVA**

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Abstract: The basic difference between sun and shade leaves arises because of the difference in their exposure to light intensities. To determine the adaptability of *Rhizophora mangle* L. leaves to different light conditions, we collected leaves from the upper peripheral region (sun leaves) and the internal lower part thereof (shade leaves) of six individuals. The following variables were analyzed: leaf thickness, palisade parenchyma, adaxial and abaxial epidermis, adaxial and abaxial cuticle, density, and stomatal index. The measurements were performed using microscopes equipped with an ocular micrometer. The sun leaves were found to be smaller, with more xeromorphic features such as thicker cuticle and adaxial and abaxial epidermis. The palisade parenchyma and mesophyll of the leaf blade in the sun leaves were found to be thicker than those in the shade leaves, although there was no significant difference in the cuticles of the abaxial face between both the leaf types. The frequency of stomata per square millimeter (average, 70/mm²) was also higher in the sun leaves than in the shade leaves (47 showed/mm²), and there were no differences in the width and length between them to the two types of leaves. Variations between sun and shade leaves indicated an adaptive ability of *R. mangle* to remain active under varying lighting conditions.

KEY WORDS: Ecological anatomy, Functional anatomy, Leaf plasticity, Luminosity, Mangrove.

ASPECTOS MORFOLÓGICOS E ANATÔMICOS DAS FOLHAS DE *RHIZOPHORA MANGLE* L. (RHIZOPHORACEAE) SOB DIFERENTES CONDIÇÕES DE LUZ

Resumo: A base da diferenciação entre folhas de sol e sombra está relacionada às diferentes intensidades luminosas a que está submetida. Visando entender a capacidade de adaptação das folhas de *Rhizophora mangle* L., associadas a diferentes condições de luminosidade, foram coletadas folhas da região superior, periférica de seis indivíduos (folhas de sol) e da região inferior, interna dos mesmos (folhas de sombra). As seguintes variáveis foram analisadas: espessura foliar, parênquima paliçádico, epiderme adaxial e abaxial, cutícula adaxial e abaxial, densidade e índice estomático. As medidas foram feitas em microscópio equipado com ocular micrométrica. As folhas de sol apresentaram-se menores e com características mais xeromórficas, como maior espessura da cutícula e da epiderme adaxial e abaxial. O parênquima paliçádico e o mesófilo da lâmina foliar nas folhas de sol apresentaram-se mais espessos que nas folhas de sombra, contudo, não havendo diferença significativa entre as cutículas da face abaxial para ambos os tipos de folhas. Foi observado também maior frequência de estômatos por mm², uma média de 70/mm² para as folhas de sol, enquanto que as folhas de sombra apresentaram 47/mm², não havendo diferenças entre largura e comprimento desses, para os dois tratamentos analisados. As variações observadas entre as folhas de sol e de sombra indicam uma capacidade adaptativa desta espécie de manter-se ativa a diferentes condições de luminosidade.

PALAVRAS-CHAVE: Anatomia ecológica, Anatomia funcional, Luminosidade, Manguezal, Plasticidade foliar.

INTRODUCTION

Brazil has 7,400 km of coastline and ranks third in area of mangroves, corresponding to 7% of the world's total sandy areas (Giri et al., 2011). These areas extend between latitudes 04°30'N and 28°30'S and share an environmental gradient variation, characterized by varying water level, salinity, and irradiance. For example, this wide range in which Brazilian mangroves are found is reflected in the differences in the spatial distribution of species, variations in the shape of trees, and spatial coverage of the region, as well as the architectural attributes and leaf structure (Farnsworth & Ellison, 1996; Schaeffer-Novelli et al., 1990).

In estuarine environments, both genotypic aspects and phenotypic plasticity can be expressed through morphological and physiological changes that allow plants to survive under varying environmental conditions (Aranda et al., 2001). Morphological and anatomical studies on leaves exposed to environmental gradient variations such as soil types, altitude, light intensity, and water and salt availability (Biber, 2006; Falqueto et al., 2008; Farnsworth & Ellison, 1996) have shown that *Rhizophora mangle* L. is very well suited to the environment at and is the dominant species in neotropical regions.

Tree species in mangrove areas, mainly *R. mangle*, vary in their ability to respond to changes in light availability (Farnsworth & Ellison, 1996; Thompson et al., 1992) and, in these areas, for a species to survive in different environments with different light intensities, structural changes in the leaves are developed in response to physiological adaptations. Further, anatomical and sometimes morphological variations might occur across individuals as well as within the same individual (Gutschick, 1999).

The intensity of light received during the growth of plants might influence their growth and development, especially their leaf organs, resulting in differences between the sun and shade leaves: the sun leaves are thicker and have greater photosynthetic capacity and show changes in structure in response to different light levels, an attribute of species that have potential for acclimatization to varying light conditions (Larcher, 2000). According to Luttge (1997), these plants are genetically determined to grow under high or low light intensity condition, they can adapt ecophysiologicaly to low- or high-intensity light, indicating their degree of plasticity.

Studies on the leaves of mangrove plants often focus on the tolerance to light intensity and salinity (Biber, 2006; Falqueto et al., 2008). Falqueto et al. (2008) used *Laguncularia racemosa* Gaerth. and *Rhizophora mangle* L. as study species and showed that the increase in photochemical efficiency in both the species was not dependent on the dry or wet season, which causes increase or decrease of the water level and thus salinity, but instead was dependent on the luminosity during the day.

According to Mendes & Paviani (1997), leaves are highly responsive to environmental variations owing to their characteristic form and function and can be used to investigate the ecological anatomy.

This study aimed to compare the structural parameters of *R. mangle* leaves exposed to different light intensities in the mangroves of Belmonte, Bahia, Brazil, in order to determine whether sun or shade leaves of this specie have distinct patterns in response to variations in light intensity causing variation in their anatomy and morphology.

MATERIAL AND METHODS

The study material was collected from the mangrove of Belmonte (Lat.: 15°51'47"S and Long.: 38°52' 58"W), Bahia, at the mouth of Rio Jequitinhonha.

The sun leaves from the peripheral upper part and shade leaves from the inner lower region were collected from the third node of six *R. mangle* plants that were exposed to different light gradients. They were fixed with 70% formalin-acetic acid-ethanol (FAA). In the laboratory, the middle limb region of the leaves was cross-sectioned with the free-hand technique by using a razor blade; the sections were clarified using 50% commercial sodium hypochlorite and stained with astra blue and safranin (modified from the technique by Bukatsch, 1972 by Kraus & Arduin, 1997). The thickness of leaves, palisade parenchyma, and abaxial and adaxial epidermis was measured in the cross-sections. The thickness of the cuticle was measured using Sudan III dye (Foster, 1940, cited in Kraus & Arduin, 1997). The stomatal type, density, and index were determined by obtaining paradermic sections by using the epidermal dissociation technique. Briefly, the midline of the leaf lamina was cut into pieces and placed in Petri dishes containing hydrogen peroxide solution (30%) and glacial acetic acid (2:1) and left in an oven at 60°C for 48 h (Franklin, 1945, cited in Kraus & Arduin, 1997). Subsequently, the sections were washed in distilled water, and then stained with safranin and mounted on slides coated with 50% glycerol (Kraus & Arduin, 1997).

The stomatal density and stomatal index were measured using the ANATI QUANTI program (Aguar et al., 2007) from the images captured using Qimaging Go-3 cameras coupled to an Olympus BX41 optical light microscope; in all, 25 leaves were used for each treatment.

The size and area of 25 leaves for each luminance gradient were measured according to the modified method described by Jordão et al. (1998).

For statistical analysis, Student's *t*-test was performed using the SPSS Statistic 8.0 program. The alpha of 0.05 was adjusted to 0.004 by using the Bonferroni method.

RESULTS AND DISCUSSION

R. mangle leaves are simple, opposite, petiole, elliptic to oblong, and are distributed almost exclusively at the ends of the branches. The sun and shade leaves are not easily differentiated by texture, but have distinct leaf area and medium length. According to March & Clark (2011) the so-called sun leaves are smaller than the shade leaves. However, in *R. mangle*, the average area of the sun leaves was larger than that of the shade leaves (Fig. 1a).

The greater area of shade leaves compared to that of sun leaves is related to the increased light-receiving surface, which provides a better adaptation for capturing solar light in shaded sites (Lee et al., 1996). The decrease of leaf size reduces the air layer adjacent to the leaf, allowing greater heat loss to the environment by convection, thereby requiring less transpiration to cool the leaf (Pooter, 1999).

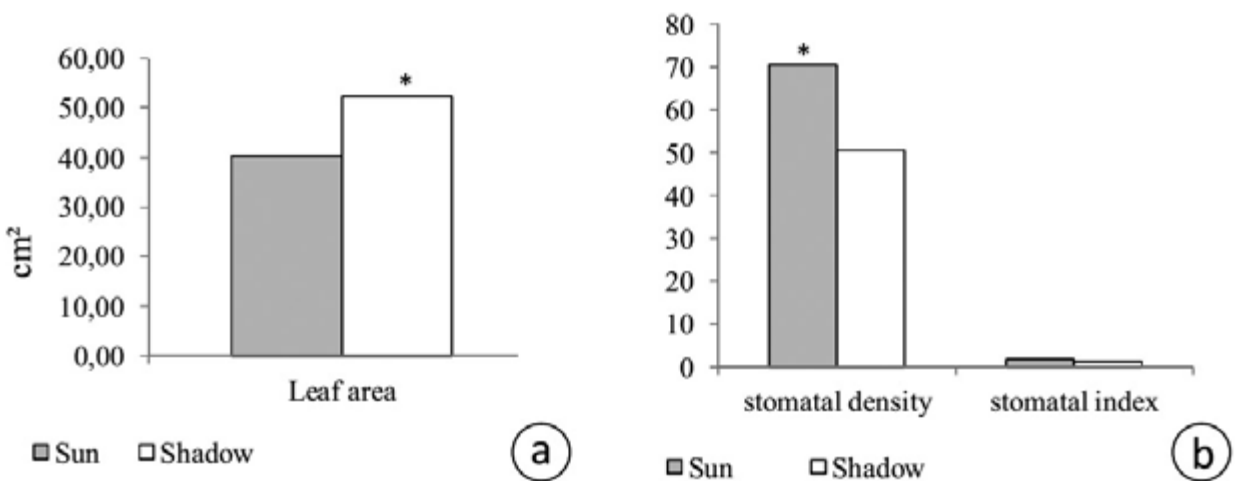


Figure 1 - Leaf area, stomatal density and stomatal index from the leaves of *Rhizophora mangle* L. a. leaf area (cm²) b. stomatal density (mm²) and stomatal index, in sun and shadow leaves of *R. mangle*. (*) Indicates significant difference, for statistical analysis Student's *t*-test, *p*-value less than 0.05.

The front view of the epidermis shows the adaxial cells with thick walls, having irregular size and shape (**Fig. 2a**). The abaxial surface has lenticels and cyclocytic-type stomata (**Fig. 2b**). The average number of stomata per square millimeter was higher in the sun leaves (**Fig. 1b**). According Milaneze-Gutierrez et al. (2003), the slight expansion of ordinary epidermal cells in the sun leaves

reflects higher stomatal density in these leaves, whereas the lowest stomatal density is found in the shade leaves; the authors attributed this difference to the difference in the light intensity existing between the two types of leaves. This finding is consistent with that for the sun and shade leaves of *R. mangle*.

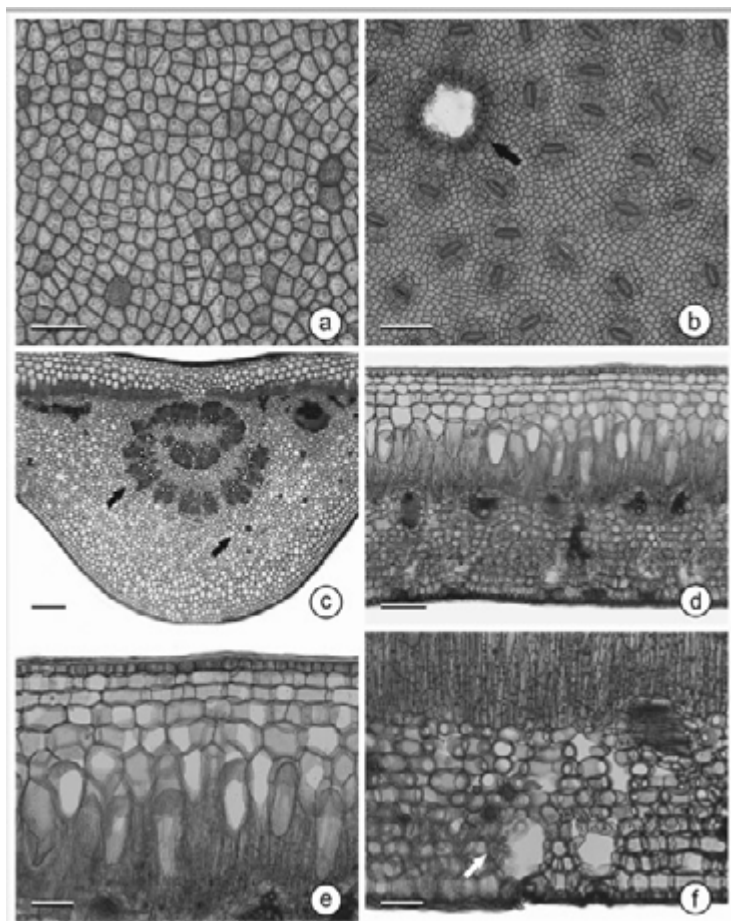


Figure 2 - Paradermic and transverse sections of the leaf of *Rhizophora mangle* L. (Rhizophoraceae) - a. adaxial epidermis b. abaxial epidermis showing the presence of lenticels (arrow), c. overview of the midrib showing the presence of sclereids (black arrow), d. overview of the mesophyll, e. detail of the last layer of hypodermic cells more elongated; f. detail of the substomatal chambers (white arrow). Bars = 50 μ m (a, b), 200 μ m (c) and 100 μ m (d), 50 μ m (e, f).

The distance between the stoma directly influences transpiration; thus, with a higher stomatal density, transpiration decreases, which is caused by the increased moisture content around the stoma. With increased stomatal density, precipitation can increase or decrease, depending on the strategy used by the plants (Larcher, 2000). In the case of *R. mangle*, the leaves exposed to the greatest light intensity were acclimated to the increase of light, suggesting a strategy for survival in environments with high light conditions.

The stomatal index refers to the number of stomata divided by the number of epidermal cells in a leaf; to compensate for the effects of leaf expansion (Beerling, 1999) and according to Cutter (1986), the stomatal index is mainly affected by humidity in the air. However, no significant difference was noted in the stomatal index between the two treatments analyzed (**Fig. 1b**).

In the cross-section of both sun and shade leaves, a collateral vascular bundle and other smaller associated bundles were noted in the midrib. The epidermis is uniseriate with thick cuticle, followed by several layers of angular-type collenchyma. Sclereids were noted in the spongy parenchyma and around the central bundle (**Fig. 2c**). The central rib of the sun leaves had greater height and width (**Fig. 3a**). Castro et al. (2005) showed that, in *Mikania glomerata* Sprengel, higher exposure to light causes changes in the size and organization of the vascular bundle; further, according to Seth et al. (1999), in environments where the photosynthetic rates are higher, further increase in the midrib size can be noted. These findings might explain the observed variation in midrib size between the two types of leaves investigated.

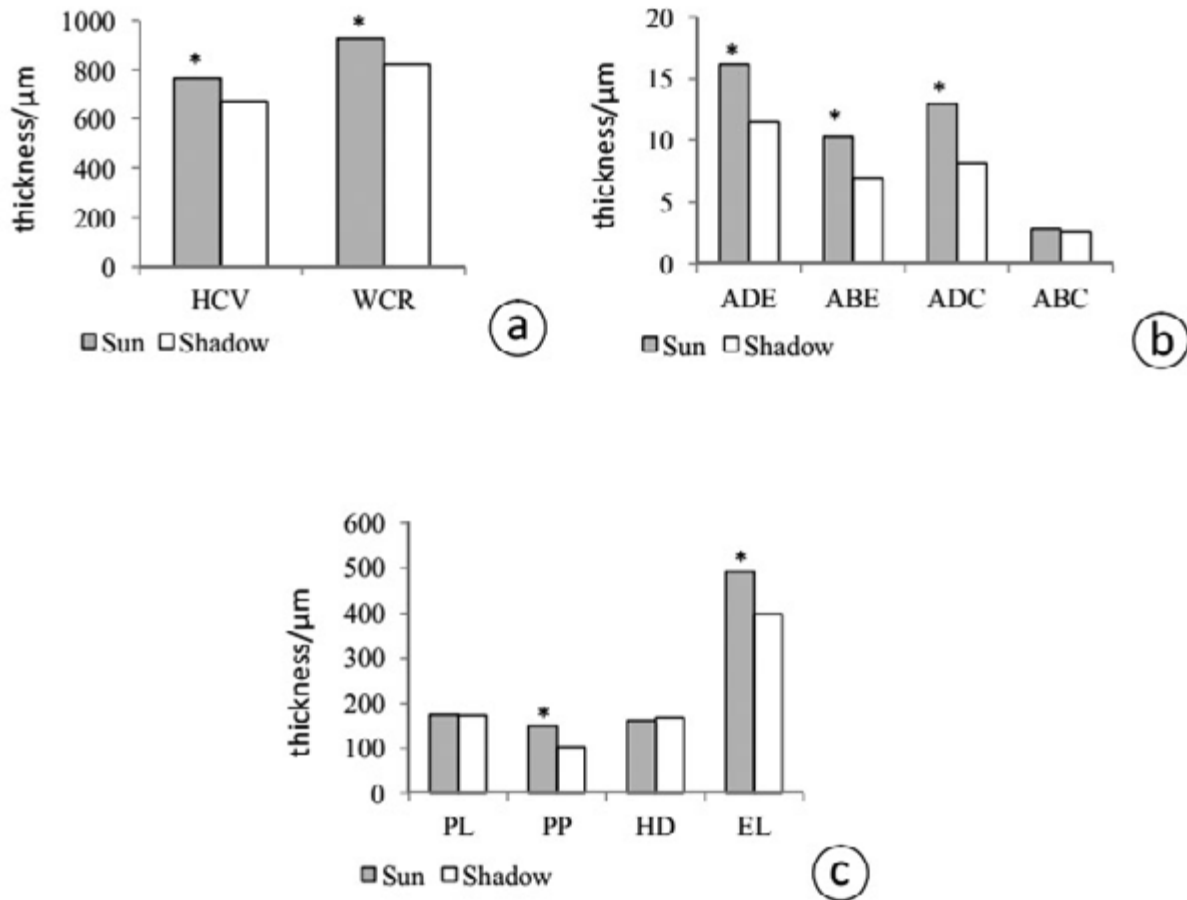


Figure 3 - Quantitative data from the leaf anatomy of *Rhizophora mangle* L. a. leaf central vein size; HCV: height of leaf central vein; WCR: width of leaf central vein; b-c. mesophyll measures; ADE: adaxial epidermis; ABE: abaxial epidermis; ADC: adaxial cuticle; ABC: abaxial cuticle; PL: spongy parenchyma; PP: palisade parenchyma; HD: hypodermic; EL: width of leaf lamina. (*) Indicates significant difference, for statistical analysis Student's t-test, p-value less than 0.05.

Further, in a cross-section of the mesophyll, unistratified adaxial epidermis covered by a thick cuticle was noted (**Fig. 2d,e,f**). Both the epidermis and cuticle of the sun leaves were different from those of the shade leaves in terms of thickness (**Fig. 3b**). Sun leaves have a thicker adaxial cuticle probably because they act as a barrier against high irradiation and reflection, as well as water loss. The thickness of the adaxial cuticle can vary since it is influenced by environmental conditions (Rossatto & Kolb, 2010); however, no significant differences were observed between the two leaf types with regard to the cuticle on the abaxial surface of the leaf blade (**Fig. 3b**).

With regard to luminance, leaves exposed to the greatest amount of light developed thicker epidermis on both adaxial and abaxial surfaces, confirming that the epidermal tissue can vary with respect to light intensity (Hlwatika & Bhat, 2002), and the epidermis is more thicker on the adaxial surface since it is most directly exposed to light. Dickinson (2000) indicated that the maintenance of optimal levels of temperature for physiologi-

cal processes is influenced by the increase in the thickness of the epidermis on the adaxial surface of the cuticle under high light conditions.

The dorsiventral-type mesophyll consists of five layers of hypodermis: the first layer is thinner, and the most inner layer has cells invaginated with palisade parenchyma (**Fig. 2d, e**). This mesophyll facing the adaxial face has two to three layers of very elongated cells, with the first layer next to the adaxial surface having more elongated cells and the inner layer having smaller cells, followed by 9–10 layers of spongy parenchyma and sclereids (**Fig. 2e**).

Extensive substomatic layers were observed (**Fig. 2f**); according to Fahne and Cutler (1992), these layers are important for the establishment of a long diffusion gradient between the chlorenchyma and the environment. These substomatic layers might have developed to ensure that air spaces in the leaves are maintained for improved gas exchange efficiency (Scatena & Rocha, 1995).

The palisade parenchyma was thicker in the sun leaves than in the shade leaves (**Fig. 3c**).

The light gradient changes the palisade parenchyma, that is, the higher the light level, the more elongated their cells and/or the greater the number of stratum (Taiz & Zeiger, 2004). The palisade parenchyma facilitates the penetration of light into the spongy parenchyma and exhibits high capacity to respond to light stimulation; therefore, it influences the leaf thickness (Castro et al., 2005). Thus, exposure of the leaf to increased light intensity can increase the thickness of the leaf blade, as was observed in the leaves of *R. mangle* (Fig. 3c). According to Dickison (2000), depending on the species, the increase in light intensity might increase the leaf thickness, epidermis and parenchyma development, as well as the total number of leaf cells.

Analysis of the morphology and anatomy of *R. mangle* suggested that the leaves respond to light either directly or in an inversely proportional manner. Despite having relevant characteristics and resistance to saline environment in which they grow, *R. mangle* also develops anatomical plasticity when subjected to different lighting conditions, which might explain the lack of significant differences between mangrove plant leaves in the dry and rainy seasons; difference in plant leaves were noted even over a day, as reported by Falqueto et al. (2008).

Analyses of the sun and shade leaves of *R. mangle* indicated that they have high degree of tolerance and adaptation to high salt concentration noted in the mangrove environment; further, the ability of the sun leaves to withstand the stress to which these plants are exposed was considerably greater than that of the shade leaves, regardless of the level of salt concentration.

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