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RESPONSE OF A WOODY SPECIES FROM ATLANTIC RAIN FOREST, *Hedyosmum brasiliense* MART. EX MIQ. (CHLORANTHACEAE), SUBMITTED TO WATER STRESS

RESPOSTA DE UMA ESPÉCIE LENHOSA DA MATA ATLÂNTICA, *Hedyosmum brasiliense* MART. EX MIQ. (CHLORANTHACEAE), SUBMETIDA A ESTRESSE HÍDRICO

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ABSTRACT

The studies about water status for woody plants of the Atlantic rain forest are negligence, despite its altitudinal gradient which reflect in five topographic formations with different water availability and the poor conservation status of this biome and high level of habitat fragmentation that demands a deeper scientific knowledge regarding species response to environmental heterogeneity. Seedlings of *Hedyosmum brasiliense* Mart. ex Miq., present at all altitudinal gradient of Atlantic rain forest were submitted to water stress and the results indicated that the species presents strategy that provides delay of tissue dissection through the adoption of mechanisms that can promote higher water absorption and retention with possibly aids its altitudinal distribution in Atlantic rain forest . These mechanisms were the increase of the root length, stomatal density, epidermis and palisade parenchyma thickness, root xylem arches; the higher distribution of carbon to root related to the shoot; the decrease of the leaf area, LAR, stomatal aperture, stomatal size, intercellular spaces and the presence of osmotic adjustment through the increase in the proline content.

Key words: water stress, anatomy, growth, woody species, Atlantic Forest.

RESUMO

Os estudos sobre o estado hídrico de plantas lenhosas da Mata Atlântica são escassos, apesar de seu gradiente altitudinal que refletem em cinco formações topográficas com diferentes disponibilidades hídricas e do mal estado de conservação deste bioma e alto

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nível de fragmentação deste habitat exigir um profundo conhecimento científico sobre a resposta de espécies deste bioma à heterogeneidade ambiental. Plântulas de *Hedyosmum brasiliense* Mart. Ex Miq., presentes em todo o gradiente altitudinal da Floresta Atlântica foram submetidas ao estresse hídrico e os resultados indicaram que a espécie apresenta estratégias que prevêem atraso de dessecação dos tecidos através da adoção de mecanismos que possam promover maior absorção e retenção de água, o que possivelmente permite esta distribuição altitudinal na Mata Atlântica. Esses mecanismos foram o aumento do comprimento da raiz, densidade estomática, espessura da epiderme e do parênquima paliçádico, arcos do xilema da raiz, a maior distribuição do carbono em sistema radicular em relação a gema, a diminuição da área foliar, RAF, abertura estomática, tamanho dos estômatos, espaços intercelulares e a presença de ajustamento osmótico através do aumento do teor de prolina.
Palavras-chave: estresse hídrico, anatomia, crescimento, espécies lenhosas, Mata Atlântica.

INTRODUCTION

The Atlantic forests are of particular interest to biologists because of their high degrees of endemism in all groups of organisms, and have been recognized as a top priority for conservation in South America (Dinerstein *et al.* 1995). The Atlantic Rain Forest is one of the two major vegetation types of the Brazilian Atlantic Forest, covering mostly the low to medium elevations (≤1000 m elev.) of the eastern slopes of the mountain chain that runs along the coastline from southern to northeastern Brazil; experiences warm and wet climate without a dry season; light and temperature are the key factors regulating seasonal patterns of trees (Morellato & Haddad 2000). Despite of absence of dry season, the marked heterogeneity of its relief generate altitudinal gradient which reflect in five topographic formations, as Alluvial, Low Lands, Submontana and High Montana Atlantic Rain Forest (Veloso *et al.* 1991), whose vegetation physiognomy can be influenced by environmental water regime (Roderjan *et al*. 2002). The studies about water status for woody plants of tropical South America are very little (Lemos Filho & Mendonça Filho 2000), mainly about the Atlantic rain forest and the poor conservation status of this biome and high level of habitat fragmentation demands a deeper scientific knowledge regarding species response to environmental heterogeneity (Rôças *et al*. 2001).

The seedling stage is generally considered to be the most important bottleneck for successful regeneration in low water availability areas, as seedlings, with their limited root system are most vulnerable to drought (Poorter & Markesteijn 2008). Species that possess mechanisms to restrict the loss of water and maintain the growth with the decrease of water, is probable that it have more success in colonizing different environment in terms of humidity that species without these mechanisms (Dickison 2000). The capacity of a species to cope with low water availability involves a combination of strategies that make it possible the increase in the capacity of absorption, transport and retention of water (Chaves *et al*. 2002).

The aim of this work is to verify in a species that is distributed in all altitudinal gradient of Atlantic rain forest if it present adjustments to alterations in water regime and if so, what kind of adjustments occurred. The species chose was *Hedyosmum brasiliense* Mart. ex Miq., present at all altitudinal gradient of Atlantic rain forest (Iserhagen *et al* 2002, Carvalho *et al*. 2005, Pereira *et al.* 2006), and of economical interest because its medicinal utilization (Zaniolo *et al*. 2001).

MATERIAL AND METHODS

Growth conditions - seedlings of *Hedyosmum brasiliense* Mart. ex Miq, obtained from the germination of seeds, were planted in plastic bags of 1L of volume, perforated in the base, containing forest soil, it polishes and composed thermophilic in the proportion of 3:1:2. The bags with one plant each, were disposed in boxes of 1 m^3, placed outdoors, and made with black polyethylene screen mesh allowing the passage of 50% of the full sun. The light intensity inside boxes was determined by a quanta meter LICOR 250. The irrigation was made with water every other day. After 30 days of growth, it was initiate the treatment of water stress, where half of the plants received weekly irrigation with distilled water or with polyethylene glycol solution (PEG - Carbowax 6000 - Synth®) of water potential of 0,9 MPa (315,67 g of PEG in 1 L of distilled water), prepared according to Willians & Shaykewich (1969). The other half of the plants (controls) continued to receive irrigation with water distilled every other day. Each box constituted an experimental unit with six plants for treatment for box. Two harvests were made, at 40 and 118 days after the beginning of the treatment. Twelve plants for treatment were collected, nine for growth evaluation and three for the anatomical analyses and proline content.

Biomass and leaf area determination - the biomass was determined by weighting after drying the plant material to 80 ^{o}C until constant mass. The leaf area was certain through digital planimeter.

Growth analysis - The mean relative growth rate (RGR) and the mean leaf area ratio (LAR) were calculated according to Hunt (1982), being $RGR=(lnM_2-lnM_1) / (T_2-T_1)$ and $LAR= (A_1/M_1 + A_2/M_2)/2$, where M_1 and M_2 correspond the total dry masses in the collection one and collection two, respectively, T1 and T2 correspond to the time slice among the collection one and collection two (40 and 118 days of growth, respectively) and A1 and A2 correspond to the leaf area in the harvest one and in harvest two.

Determination of the proline content - The determination was according Bokhari & Trent (1985). Three portions of 500 mg of leaves per plant were softened separately in 10 mL of aqueous solution of sulfosalicilyc acid 3%. From the supernatant obtained by extract centrifugation were removed three brackets of 2 mL that were put in assay tubes and to each tube were added 2 mL of glacial acetic acid and 2 mL of acid nihidrine. The tubes were put in bain-marie to 100ºC and after one hour reactions were finished the tubes in ice. After that, 4mL of toluene were added to each tube, which were agitated and put in rest for the separation of the phases. The absorbance of the toluene fraction was measured and compared with the absorbance of proline standard solution.

Histochemical tests - the reagents utilized were reagent of Steimetz, for suberine, lignin, cutin, cellulose, mucilage, starch and phenolic compounds identification (Costa 1982); sudan IV, for detection of oils and cutin (Costa 1982); fluoroglucine / HCl, for lignin detection (Costa, 1982) and thionine for mucilage detection (Purvis *et al*. 1964 apud Kraus & Arduim 1997).

Quantitative analyses – For quantitative data, the number minimum amostral was calculated by the equation $n=(t_2.s_2)$. d-2, where "t" is given by Table of Student (considering n-1, for significance of 0,05)," s" is the standard deviation and "d" is same E/100.average, where E=10, for 10% of probability, value considered satisfactory (Sokal & Rohlf 1969). Of three plants per treatment, samples of two roots and two leaves totally expanded were removed and immersed in solution of Jeffrey (Johansen 1940), for analyses of epidermal structures and dimensions of vessel elements. The stomata number per unit area was determined projecting images, with aid of clear camera coupled to the optical microscope, on a delimited area and checked with micrometrical scale. The stomatal index was calculated by the equation n=(ne/nc+ne) x100, where "ne" is the stomatal number per unit area and "nc" is the number of epidermal cells in the same area (Wilkinson 1979). The determination of the guard cells and stomatal pore dimensions was made in optical microscopy trough paradermic sections, considering the longitudinal and the traverse axis.

Structural analyses - Samples of leaves, stems and roots were infiltrated in paraffin and stained with safranin and fast-green, according Johansen (1940), or infiltrated in hidroxietilmetacrilato and stained with toluidine blue (Gahan & Onyia 1984). The photomicrographs were made in optical microscope Leica MPS30.

Statistical analysis - The comparison among the treatments was made with standard *t* test, at the level of significance of 5%.

RESULTS

Several traits of *H. brasiliense* seedlings under high and low water availability are shown on table 1. The seedlings in response to the drought presented reduction of the leaf area, the number of leaves and the expansion of each leaf. The seedlings submitted to water stress presented larger value of root to shoot ratio (R:S), decrease of the leaf area ratio (LAR) than to those without stress and a significant increase in the proline content in relation to the not stressed plants.

Anatomical response to water stress is shown in table 2. The data regarding the stomatal index that is the percentage of the stomata number related to the number of cells per area, did not present significant differences among the treatments. It was verified that in water stressed plants the thickness of the epidermal cells, in both faces, was larger than in control plants. The number of epidermal cells per unit area in

Table 1. Data of *Hedyosmum brasiliense* seedlings submitted to different water regimes.

Days of treatment	40		118	
	Water stress	Control	Water stress	Control
Root DM (mg)	16.78 ± 5.91b	28.33 ± 7.86a	271.00 ± 93.49b	620.00 ± 140.96a
Steem DM (mg)	11.44 ± 8.44b	16.00 ± 3.54a	76.00 ± 36.59b	284.00 ± 77.92a
Leaf DM (mg)	22.00 ± 10.45b	38.11 ± 11.50a	228.00 ± 93.43b	918.00 ± 99.33a
Shoot DM (mg)	33.40 ± 12.32b	54.11 ± 14.10a	304.00 ± 121.72b	202.00 ± 87.42a
Total DMs (mg)	50.22 ± 17.87b	82.44 ± 13.71a	575.00 ± 187.25b	822.00 ± 124.02a
Root/Shoot ratio	0.50 ± 0.09a	0.52 ± 0.17a	0.94 ± 0.32a	0.53 ± 0.06b
Leaf number	6.33 ± 1.30a	9.33 ± 2.08a	9.67 ± 0.58b	2.00 ± 0.52a
RGR (mg mg^{-1} dia^{-1})	0.020 ± 0.003b	0.032 ± 0.009a	0.026 ± 0.003b	0.036 ± 0.004a
LAR (m g^{-1} cm^{-1})	-	-	0.023 ± 0.004a	0.038 ± 0.007b
Proline content (µg g^{-1} DM)	-	-	3.31 ± 0.57b	38.51 ± 8.06a

Letters compare lines within the same time. Different letters indicate statistical difference (*t* test p< 0.05). Dry mass (DM), Relative growth rate (TCR), Leaf area ratio (LAR).

both faces, increased in water stressed plants and the ordinary epidermal cells appeared visibly smaller in water stressed plants (Fig. 1-2). Some epidermal cells rounding the stomata showed radiated grooves in the cuticle.

However, in the present study the presence of cuticular grooves was observed both in plants under water stress and with good water supply, indicating that this characteristic was not sensible to water variation.

The histological structure of the mesophyll (Tab. 2) was not altered in water stressed plants, however its thickness was altered due an increase of the palisade parenchyma. Under water stress the intercellular spaces of the spongy parenchyma were visibly

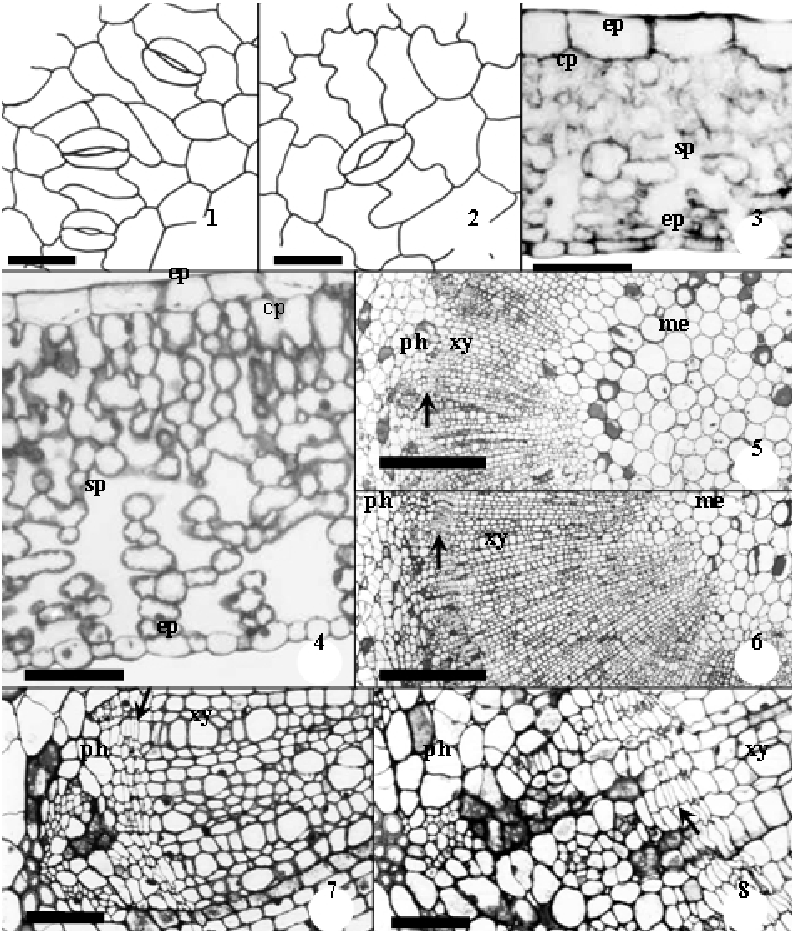
The stem diameter in water stressed plants was reduced (Tab. 2) and the vascular system presented visible smaller width (Fig. 5-7) compared to control plants (Fig. 6-8). The stem longitudinal growth was not affected by water stress (Tab. 2).

The root cortical parenchyma in water stressed plants (Fig.9) presented narrower intercellular spaces than in control plants (Fig. 10). Stressed plants (Fig. 9) presented larger number of xylem arches than control plants (Fig. 10). The vessel diameter was not affected by the water stress (Tab.2).

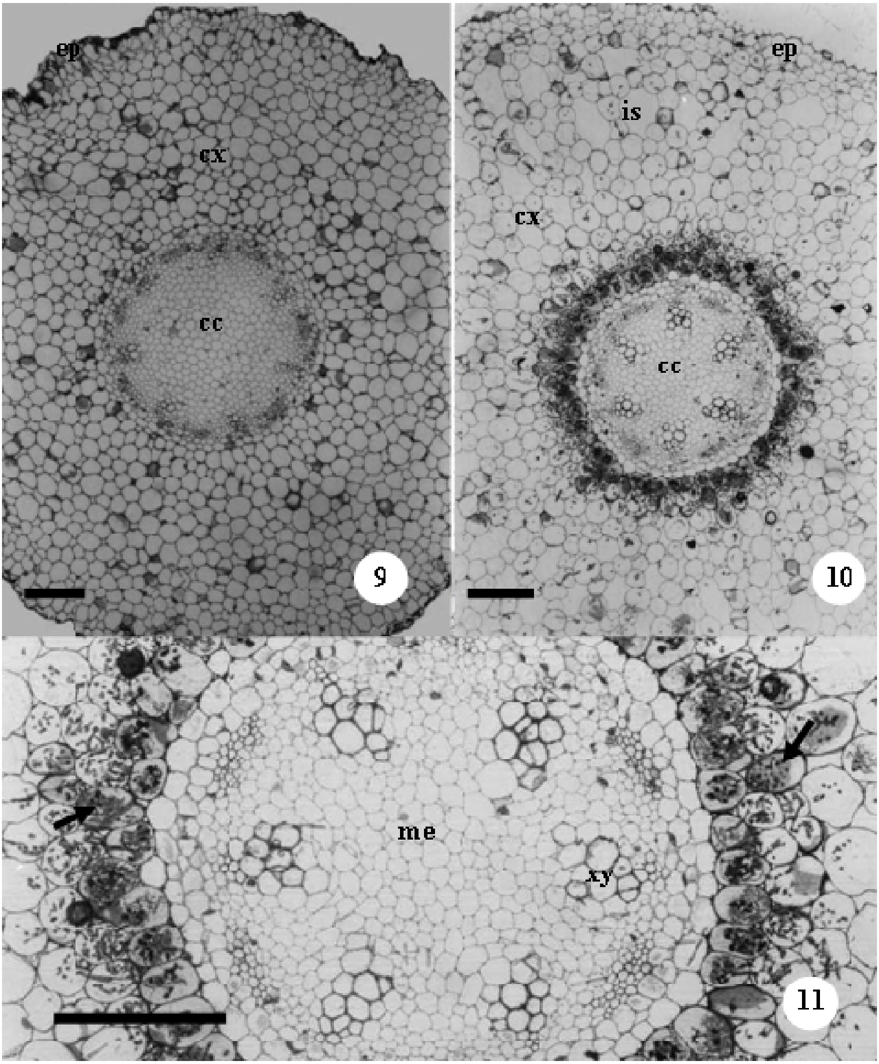
Table 2. Anatomical and morphological measurements of structures from *Hedyosmum brasiliense* seedlings submitted to different water regimes.

Tratamento	Deficiência Hídrica	Controle
Epidermal cells (number mm^{-2})		
Adaxial surface	244.17 ± 31.27a	169.17 ± 21.46b
Abaxial surface	385.83 ± 48.99a	248.33 ± 22.68b
Guard cells dimensions (□m)		
Length	47.47 ± 9.86b	54.87 ± 4.89a
Width	10.87 ± 1.34b	14.10 ± 1.54a
Stomatal pore dimensions (□m)		
Length	24.74 ± 2.34b	33.75 ± 2.75a
Width	5.13 ± 1.39b	12.60 ± 1.88a
Stomatal density (number mm^{-2})	644.37 ± 43.72a	444.79 ± 37.17b
Stomatal index	14.60 ± 2.99a	15.35 ± 2.61a
Espessuras (□m)		
Epiderm – adaxial surface	42.31 ± 5.15a	35.90 ± 4.88b
Epiderm – abaxial surface	20.26 ± 4.51a	17.82 ± 3.11b
Palisade parenchyma	121.92 ± 13.96a	92.82 ± 17.92b
Spongy parenchyma	95.26 ± 10.63b	221.28 ± 21.91a
Mesophyll	214.74 ± 19.64b	314.10 ± 25.46a
Foliar blade	272.56 ± 20.50b	370.26 ± 24.72a
Steem dimensions		
ength (cm)	9.00 ± 2.00a	10.67 ± 0.76[a]
Diameter (mm)	4.00 ± 0.04b	5.60 ± 0.04a
Root dimensions		
Length (cm)	22.33 ± 1.26a	18.57 ± 0.60b
Diameter (mm)	1.94 ± 0.08b	2.39 ± 0.04a
Vessel elements dimensions (□m)		
Length	886.52 ± 92.54a	890.19 ± 139.57a
Diameter	12.70 ± 2.97a	12.70 ± 3.32a

Letters compare horizontal lines. Different letters indicate statistical differences (*t* test $p < 0.05$).



Figures 1-8. Photomicrographs of root cross section of Hedyosmum brasiliense. Frontal view of ab axial Epidermis surface of Hedyosmum brasiliense leaves after 40 days under water stress (1) and control (2). Photomicrographs of mesophyll cross sections of Hedyosmum brasiliense leaves 118 days under Water stress (3) and control (4). Photomicrographs of cross sections show ing vascular system of Hedyosmum brasiliense tem after 118 days under water stress(5) and control (6). Detail of vascular System and cambium of stems in plants under water stress (7) and control (8). Epidermis (ep), phloem (ph), medulla (me), compact parenchyma (cp), spongy parenchyma (sp), xylem (xy), and anow indicates Vascular cambium. Bars = 50 Um (1-2), 100 Um (3-4, 7-8) and 500 Um (5-6).



Figures 9-11. Photomicrographs of root cross section of *Hedyosmum brasiliense*. General aspects of plants after 118 days under water stress (9) and control (10). Detail showing central cylinder of plant control and parenchymatic cells of cortical region, showing mychorryza. Central cylinder (cc), epiderm (ep), cortex (cx), intercellular space (is), medulla (me), xylem (xy) and arrows indicate mychorriza. Bars=200µm. Smaller and there was the mucilage presence among the epidermal cells (Fig. 3 and 4).

DISCUSSION

One of the strategies of the plants for the survival in dry environments is the decrease of the total leaf area for reduction of the transpiration of the plant and the distribution of carbon between roots and shoot in way to favor the root and with this favor the absorption of water (Pereira & Pallardi 1989). Seedlings of H. *brasiliense* presented these adjustments, indicating an adaptation of the morphology of the plant to the conditions of drought of the soil. The decrease of the leaf area ratio (LAR) due to the water stress occurred in seedlings is as indication of the capacity of a species in optimizing the use of water in such situations (Lambers *et al*. 1998). This decrease in LAR can result in smaller relative growth rate (RGR), resulting in smaller dry mass of these plants (Lambers *et al*. 1998), as occurred in *H. brasiliense.*

The intracellular accumulation of solutes active osmotically in response to the water stress, as proline, showed by *H. brasilense* seedlings has been reported as is an important mechanism of drought tolerance and it has been verified in several species (Chaves Filho & Staciarini-Seraphin 2001; Pinior *et al*. 2005). The increase in the proline content indicates that the species possesses capacity of osmotic adjustment, favoring the maintenance of the turgidity cell under deficiency, allowing the functioning of the photosynthetic apparatus (Pinior *et al*. 2005).

Studies have been showing that the water deficiency gets an increase of the stomatal density (Zhang *et al*. 2006) and a decrease in the stomata size (Sam *et al*., 2000) and these alterations can elevate the adaptation of the plant to the drought (Melo *et al*. 2007). Opposed data, decrease of the stomatal density with an increase of the drought was observed in other species, but just under severe water stresses, under moderate stress, these species also showed an increase of the stomatal density (Xu & Zhou 2008). The leaves water stressed seedlings of *H.brasiliensis* presented a reduction of the guard-cells dimensions and of the stomatal pore and an increase of the stomatal density, showing capacity to adapt to the drought, but the stomatal index, that is the percentage of the stomata number related to the number of cells per area, were not altered in water stressed seedlings, what indicates that occurred a reduction of the size of the epidermal cells in function of the water stress.

The guard-cells position above the ordinary epidermal cells is frequently described as a hydro morph characteristic (Dickison 2000), while under limitation of the water supply, the plants tend to protect the stomatal opening, deepening the stomata, in way to reduce the transpiration (Fahn & Cutler 1992). In *H. brasiliense* seedlings under water stress, the guard-cells keeps its position above the ordinary epidermal cells, showing that this characteristic did not present plasticity, at least at the water stress imposed here to the plants. Some epidermal cells rounding the stomata showed radiated grooves in the cuticle. This characteristic can be used as diagnostic to separate species (Metcalfe & Chalk 1979) or can indicate a possible adaptation to environmental conditions, as those of the Brazilian savannah, where high light intensities and high transpiration taxes prevail (Salatino *et al*. 1986). However, in the present study the presence of cuticular grooves was observed both in plants under water stress and with good water supply, indicating that this characteristic was not sensible to water variation.

It was verified that in water stressed plants the thickness of the epidermal cells, in both faces, was larger than in control plants. The literature usually associates large cell thickness with the capacity of light reflection (Delucia *et al*. 1996). One of the main effects of water deficit is diminish the cellular size (Levitt 1980) and in *H.brasiliense* under water stress this effect lead an increase in the number of epidermal cells per unit area in both face due the smaller size of these cells.

The increase in the palisade layer seen in water stressed seedlings of *H. brasiliense* has been found in several plant species and it is associated to the strategy used by plants to maintain the photosynthetic capacity at low water potentials (Oguchi *et al*. 2003). Under water stress the intercellular spaces of the spongy parenchyma were visibly smaller and there was the mucilage presence among the epidermal cells. Presence of mucilage inside cells or in cell walls is believed to aid the retention of water (Fahn & Cutler 1992).

The decrease of the intercellular spaces of the root cortical parenchyma showed by seedlings of *H. brasiliense* under water stress, a response found in other woody species, has been seen as an adaptation in response to the drought (North & Nobel 1991). The vessel diameter was not affected by the water stress, although narrow vessel diameter has been associated to plants leaving in dry environments (Melo *et al*. 2007). The total length of the roots in water stressed plants was larger than in control plants fact also observed in other forest species and related to scavenge of water in dry soils (Price *et al*. 2002).

Tyree *et al.* (2003) indicate that the mechanism of resistance of plants to the drought can be divided in characteristics adopted by the plant that delays the dissection and in those that tolerate the dissection. The results found for *H. brasiliense* indicated that this species has the ability cope with drought delaying dissection, through mechanisms that can promote larger reception of water or larger water use efficiency. This capacity to resist to the drought could be advantageous for the wide distribution of H. *brasiliense* in several altitudes of the Atlantic Forest.

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