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Physiological performance of Hancornia speciosa Gomes seedlings under different watering conditions: growth and chlorophyll a fluorescence

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Abstract – To gain a better understanding of the physiological responses of the *Hancornia speciosa* (mangaba tree) to water stress, the aim of the present study was to evaluate initial growth, chlorophyll content and chlorophyll a fluorescence in mangaba seedlings under different watering conditions (daily irrigation and intervals of two, four and six days between watering). Water cycles did not negatively affect stem diameter, leaf area or chlorophyll content. Plants grown with watering at six-day intervals had a better performance in terms of plant height and number of leaves. The differences observed in Fm, Fv, Fv/Fm and PI do not represent a negative effect, indicating no damage to PSII. The findings suggest that mangaba seedlings are tolerant to intermittent drought and six-day intervals between watering may favor growth in the early development phase.
Key Words: *mangaba tree, photosynthetic pigments, root to shoot ratio, water stress.*

Desempenho fisiológico de mudas de *Hancornia speciosa* Gomes submetidas a diferentes condições hídricas: crescimento e fluorescência de clorofila

Resumo– Para uma melhor compreensão das respostas fisiológicas da *Hancornia speciosa* (mangabeira) ao estresse hídrico, o presente estudo objetivou avaliar o crescimento inicial, teor de clorofila e fluorescência da clorofila a em mudas submetidas a diferentes condições hídricas (irrigação diária e intervalos de dois, quatro e seis dias entre regas). Os ciclos de rega não afetaram negativamente o diâmetro do caule, área foliar e teor de clorofila. Plantas cultivadas com intervalos de seis dias entre regas apresentaram melhor crescimento em altura e número de folhas. As diferenças observadas para Fm, Fv, Fv/Fm e IP não representaram efeitos negativos indicando que não houve dano para PSII. Os resultados sugerem que mudas de mangabeira são tolerantes à secas intermitentes e intervalos de seis dias entre regas podem favorecer o crescimento das mudas na fase inicial do desenvolvimento.
Palavras-Chave: *estresse hídrico, mangabeira, pigmentos fotossintéticos, razão raiz/parte aérea.*

Rendimiento fisiológico de las plántulas de *Hancornia speciosa* Gomes bajo diferentes condiciones de agua: el crecimiento y la fluorescencia de la clorofila

Resumen – Para una mejor comprensión de las respuestas fisiológicas de *Hancornia speciosa* (mangabeira) a estrés hídrico, este estudio tuvo como objetivo evaluar el crecimiento inicial, el contenido de clorofila y la fluorescencia de la clorofila en las plántulas bajo diferentes condiciones de agua (riego diario y los intervalos de dos, cuatro y seis días entre riegos). Los ciclos de riego no afectaron el diámetro del tallo, área foliar y el contenido de clorofila. Las plantas que crecen a intervalos de seis días entre riegos mostraron un mejor crecimiento en altura y número de hojas. Las diferencias observadas por Fm, Fv, Fv / Fm e IP no representaban a los efectos negativos que indican ningún daño a PSII. Los resultados sugieren que las plántulas Mangabeira son tolerantes a la sequía y los intervalos intermitentes de seis días entre riegos pueden favorecer el crecimiento de las plántulas en la etapa temprana de desarrollo.
Palabras Clave: *estrés hídrico, Mangabeira, pigmentos fotosintéticos, relacion raíz/tallo.*

Introduction

Hancornia speciosa Gomes, commonly called the "mangaba" tree, belongs to the family Apocynaceae and is found throughout Brazil (Silva Junior 2004), mainly in areas with acidic, nutrient-poor soils (Vieira Neto *et al*. 2002). Its vegetative development is greatest during periods of higher temperature and ideal rainfall is between 750 and 1600 mm annually. Fructification occurs mainly from July to October or January to April, but can be observed at any time of year in some regions (Soares et al. 2006). In the field, *H. speciosa* responds to water deficit in the dry season by being semi-deciduous (Lorenzi 2002).

The "mangaba" fruit is tasty and greatly appreciated for consumption *in natura* or in the form of sweets, juices, jams, liqueurs and ice cream (Ganga et al. 2009). Due to its considerable productive potential, "mangaba" fruit has attracted the interest of agribusiness, but the lack of modern plantation technologies does not meet the demand and simple extraction is currently main form of exploitation (Lederman et al. 2000). However, deforestation due to agricultural activities and urbanization threatens natural populations of this tree (Vieira Neto et al. 2002).

The *H. speciosa* exhibits difficult sexual propagation due to its recalcitrant seeds and inhibitory effect the fruit pulp has on itself (Espíndola et al. 1993, Gricoletto 1997). Given the need for the commercial cultivation of this crop, studies on its physiological characterization are needed, especially with regard to water needs for growth and plant development under adverse conditions. The decrease in water availability in the soil constitutes an important environmental factor limiting growth. Northeastern Brazil has irregular rainfall distribution, with water shortages even in coastal regions. In summer, the high temperatures, considerable luminosity and low rainfall result in periods of stress for plants.

Water is fundamental to plant growth. For every gram of organic matter produced by plants, approximately 500 g of water absorbed by the roots is transported through the plant body and lost to the atmosphere (Taiz and Zeiger 2006). This flow is essential to nutrient absorption and transport through xylem and the maintenance of the appropriate temperature for vital processes, with the elimination of excess heat. Water is the main element that makes up the body of the plant and is responsible for the shape and structure of the organs (Larcher 2006). A lack of moisture in the soil due to evaporation and greater plant transpiration relative to absorption by the roots constitute stress factors. Other environmental aspects also affect this process, such as light, temperature, low relative humidity and vapor pressure deficit (Silva et al. 2004; Larcher 2006; Silva et al. 2009). As water deficit reduces cell expansion due to the low turgor pressure, all growth processes are affected by low water availability in the soil (Sadras and Milroy 1996; Quezada et al. 1999; Jaleel et al. 2009; Silva et al., 2013), with subsequent reductions in plant height, leaf area, the emergence of new leaves and biomass production (Cairo 1995; Larcher 2006).

In higher plants, the leaf mesophyll is the most active photosynthetic tissue. Mesophyll cells are rich in chloroplasts, which contain chlorophyll, the main pigment responsible for capturing solar energy and constituting an antenna complex with other pigments, such as carotenoids. In chloroplasts, solar radiation is converted into chemical energy in the form of ATP and NADPH during the photochemical phase (Marenco and Lopes 2005; Larcher 2006; Taiz and Zeiger 2006). The photosynthetic process in plants depends partly on the ability of light absorption by the leaf

(Salle *et al*. 2007). However, environmental factors, such as high radiation, temperature and water deficit, can cause photoinhibition and photo-oxidation in leaves (Araújo et al. 2010). The level of excitation energy in the antenna complex that drives photosynthesis is revealed by chlorophyll fluorescence yield. When a plant faces prolonged periods of water deficit, regulatory inhibition or damage of the photosynthetic apparatus can occur. Photosynthesis can be restricted by limiting the supply of CO_2 or by direct inhibition of the electron transport chain, which causes damage to photosystem II (PSII) and generates reactive oxygen species (Lemos-Filho 2000; Araújo et al. 2010). Chlorophyll *a* fluorescence is an important tool for the investigation of photosynthetic performance and responses to environmental stress using a non-destructive method (Cassana et al. 2010).

Information on the physiological responses of the *H. speciosa* to water deficit is scarce. Moreover, studies on the effect of water deficit in the initial development phase are needed, as this is the most critical phase for establishment in the field. Once *H. speciosa* is found in sandy soils, the hypothesis tested herein is that *H. speciosa* seedlings tolerate moderate drought by changing the growth pattern to facilitate water absorption and contribute to maintaining photosynthetic processes without causing damage to PSII. Thus, the purpose of the present study was to evaluate the initial growth, chlorophyll content and chlorophyll *a* fluorescence in *H. speciosa* seedlings under different watering cycles.Dicionário

Materials and methods

Plant material and soil: The experiment was carried out under greenhouse conditions at the Federal University of Sergipe (Brazil) from January to July 2010. *H. speciosa* seedlings were obtained by seeds from fruits harvested at the Itaporanga Experimental Center, city of Itaporanga D`Ajuda, state of Sergipe, Brazil (11°06'40"S and 37°11'15"W), which belongs to the Brazilian Agricultural Research Company (Embrapa-CPATC).

The pulp of the fruit was removed by hand and the seeds were passed through sieve to remove the mucilage. After drying for 24 hours on absorbent paper in the shade, the seeds were sown in trays containing sandy soil at a depth of approximately 2 cm. Sixty days after the end of germination, uniform seedlings in height and number of leaves were selected and transferred to vases containing approximately 5.5 kg of sandy soil collected in the forest surrounding the campus of the Federal University of Sergipe. Granulometric analysis was performed at the Soil Physics Laboratory of the same university, which revealed that the soil was composed of 0.93% coarse sand, 4.52% medium-grain sand and 89.08% fine sand. The chemical analysis was performed at the Sergipe Technological Institute and revealed that the soil contained 3.93 $cmol_c\ dm^{-3}\ Ca^{2+}$, 0.80 $cmol_c\ dm^{-3}\ Mg^{2+}$, 17.2 mg $dm^{-3}\ K^+$, 11.0 mg $dm^{-3}\ P^+$, < 0.08 Al^{3+} and 0.107 $cmol_c\ dm^{-3}\ Na^+$.

Experimental design and growth analysis: The plants were irrigated daily for 60 days prior to the onset of the water deficit cycles. A randomized experimental design was employed with four water treatments and four replications per treatment: daily watering (control) and intervals of two (S2), four (S4) and six (S6) days of watering. Growth was evaluated weekly by measuring plant height, the number of leaves and stem diameter. After 57 days, the plants were collected and leaf area was estimated by the square method using a known area (Benincasa 2003). The plants were separated into leaves, stem and roots, which were dried in an oven (70 ° C) until

reaching a constant weight and the dry weight of different organs was determined. Root to shoot ratio was calculated using dry matter weight data.

Chlorophyll analysis: Chlorophyll index was measured using chlorophyll meter (CCM-200 model, OPIT-SCIENCES, USA), with five measures per seedling.

Chlorophyll fluorescence: Chlorophyll fluorescence was measured weekly in the second pair of fully healthy and completely expanded leaves located in the upper third of the plants starting 29 days after the differentiation of the treatments, always between 9:30 and 10:30 a.m. Initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv), photochemical efficiency of PSII (Fv/Fm), performance index (PI), and area were measured using a hand fluorometer (Plant Efficiency Analiser, Hanstech, King's Lynn, Norkfolk, UK). The selected leaves were subjected to a 30 min dark-adaptation period, which was sufficient for all PSII reaction centers (RCs) to become open (Rhee *et al*. 1998; Zouni *et al*. 2001). Immediately after the dark adaptation, the leaves were exposed to a pulse of saturating light at an intensity of 3000 μmol $m^{-2}s^{-1}$ at a wavelength of 650 nm for 1 s.

Statistical analysis: The data were submitted to analysis of variance (ANOVA). Means were compared using Tukey's Multiple Range Test ($P < 0.05$).

Results

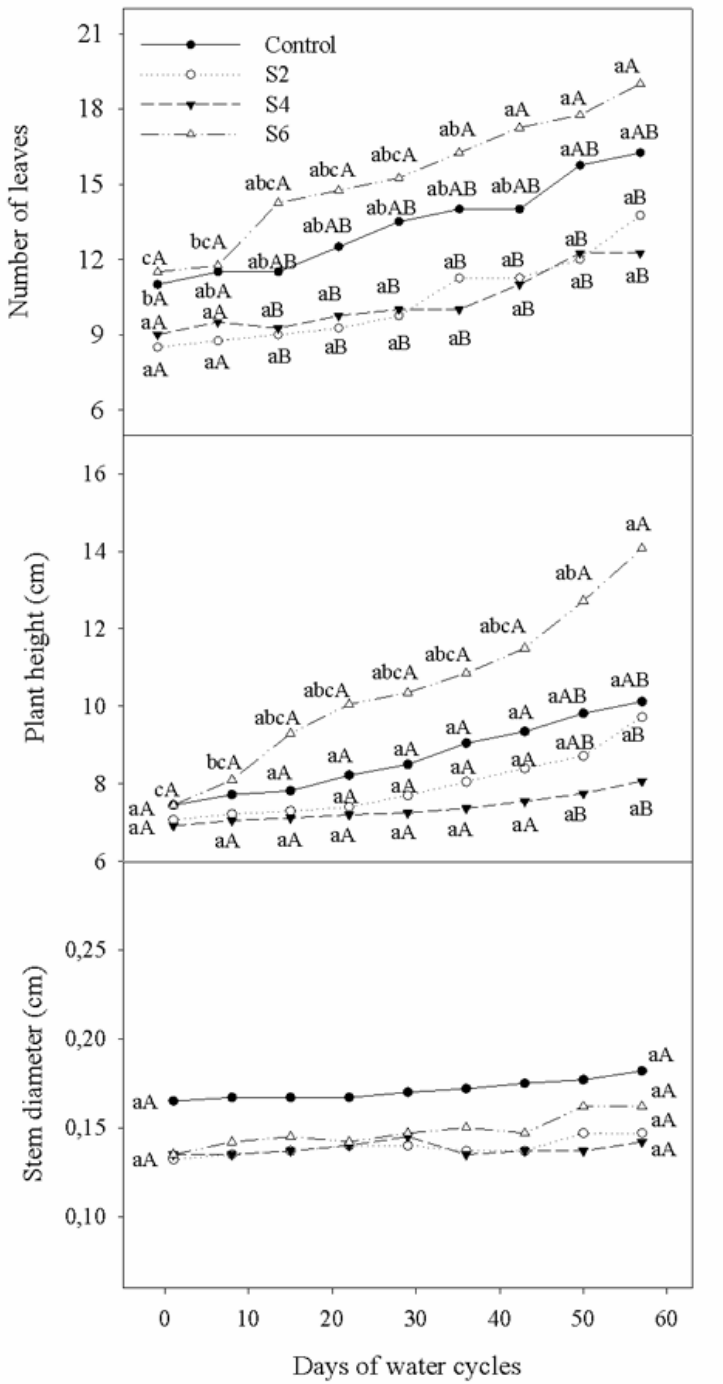
Growth parameters: Water deficit significantly affected some growth parameters in *H. speciosa* seedlings. Number of leaves and plant height were significantly ($P < 0.05$) affected by the watering cycles intervals (Fig. 1). Plants submitted to six-day watering interval (S6) treatment had a greater number of leaves in comparison to two-day and four-day watering intervals (S2 and S4) beginning on Day 22 through to the end of the experimental period, but did not differ significantly from the control (Fig. 1). The emergence of new leaves was slow throughout the experimental period. In the comparison of evaluation times, control plants and those submitted to S6 cycle exhibited a significant increase in new leaves at the end of the experimental period (57 days) and after 47 days, respectively, whereas no significant difference was found in plants submitted to S2 and S4 between the beginning and end of the period of stress (Fig.1).

Significant differences were found in plant height after 50 days of water deficit induced by different watering cycles (Fig. 1). Plants grown under the six-day watering interval (S6) exhibited a better performance, with greater plant height in comparison to the other treatments. Control plants and those submitted to the two-day and four-day watering intervals (S2 and S4) exhibited no significant growth in height over time (Fig. 1).

However, stem diameter was not affected by the different watering cycles (Fig.1). Throughout the experimental period, stem diameter exhibited slow growth, with no significant differences between the beginning and end (57 days) of the experiment.

Leaf area was not significantly affected by the different watering cycles in comparison to the control plants (Fig. 2). However, significant differences were found between S4 and S6, with a larger mean area in the latter treatment (47.29 cm^2 vs. 23.87 cm^2).

Figure 1 - Number of leaves, plant height and stem diameter of *H. speciosa* seedlings cultivated under greenhouse conditions and submitted to different watering cycles. Equal letters (lowercase among evaluation days and uppercase among treatments) do not differ significantly (Tukey's Multiple Range Test; $P < 0.05$).



On the other hand, dry matter production was not significantly affected ($P < 0.05$) by the different watering cycles. No statistical differences were found to LDM, SDM, RDM and TDM (Tab. 1). The mean root-to-shoot ratio was approximately 0.72 despite de water treatment. Although no statistically significant differences were found among the treatments, plants submitted to S6 exhibited a tendency toward greater dry matter production in comparison to the other treatments (Tab. 1).

Figure 2 - Leaf area (cm2) on *H. speciosa* seedlings cultivated under greenhouse conditions and submitted to different watering cycles. Equal letters do not differ significantly (Tukey's Multiple Range Test; $P < 0.05$).

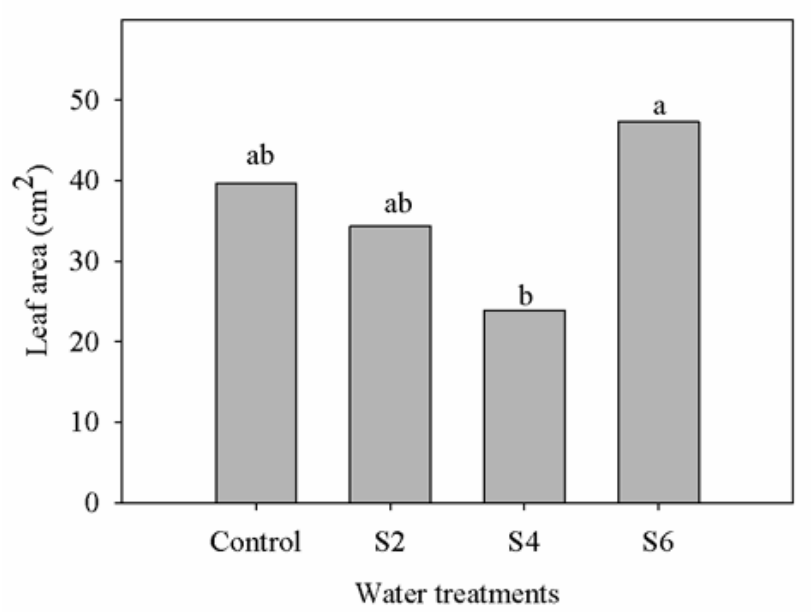


Table 1 - Leaf (LDM), stem (SDM), root (RDM) and total (TDM) dry matter and root to shoot ratio of *H. speciosa* seedlings cultivated with different watering cycles under greenhouse conditions

Water treatments	LDM(g)	SDM(g)	RDM (g)	TDM (g)	Root:shoot ratio
Control	0.30 a	0.07 a	0.33 a	0.70 a	0.89 a
E2	0.23 a	0.06 a	0.18 a	0.47 a	0.65 a
E4	0.18 a	0.05 a	0.17 a	0.40 a	0.73 a
E6	0.41 a	0.10 a	0.33 a	0.85 a	0.63 a

Equal letters among treatments do not differ significantly (Tukey Multiple's Range Test; $P < 0.05$).

In general, the different watering cycles did not significantly affect the chlorophyll index in the *H. speciosa* seedlings. According to measurements of the chlorophyll meter (SPAD), total chlorophyll index ranged from 41 to 67.5 (Tab. 2).

Table 2 - Total chlorophyll SPAD index in *H. speciosa* seedlings cultivated with different watering cycles under greenhouse conditions.

Water Treatments	Days after watering cycles				
	29	36	43	50	57
Control	67.1 aA	50.8 aA	49.9 aA	59.3 aAB	57.6 aA
S2	60.2 aA	56.3 aA	62.6 aA	74.4 aA	54.8 aA
S4	47.0 aA	57.4 aA	61.6 aA	41.0 aB	52.5 aA
S6	62.6 aA	63.8 aA	67.5 aA	58.5 aAB	57.7 aA

Equal letters (lowercase among evaluation days and uppercase among treatments) do not differ significantly (Tukey's Multiple Range Test; $P < 0.05$).

Table 3 - Initial (F0), maximum (Fm) and variable (Fv) chlorophyll fluorescence, quantum efficiency of PSII (Fv/Fm) and performance index (PI) of *H. speciosa* seedlings cultivated under greenhouse conditions and submitted to different watering cycles.

Treatments	Days of watering cycle				
	29	36	43	50	57
	F0				
Control	618.0 aA	628.0 aA	621.0 aA	611.7 aA	613.0 aA
S2	633.7 aA	627.2 aA	640.2 aA	642.2 aA	612.5 aA
S4	629.7 aA	656.0 aA	636.7 aA	632.0 aA	620.5 aA
S6	625.2 abA	613.7 abA	650.7 aA	596.2 bA	611.7 abA
	Fm				
Control	3159.5 bAB	3144.7 bAB	3531.7 aB	3213.0 bB	3289.0abAB
S2	2961.5 cB	3063.2 cB	3783.5 aA	3462.5 bA	3188.2 cB
S4	3232.2 bA	3322.5 abA	3228.5 bC	3137.7 bB	3494.7 aA
S6	3150.5 aAB	3112.5 aAB	3353.5 aBC	3162.7 aB	3246.0 aB
	Fv				

Control	2541.5 bAB	2516.7 bAB	2910.7 aB	2601.2 bAB	2676.0 bAB
S2	2327.7 dB	2436.0 cdB	3143.2 aA	2820.2 bA	2575.7 cB
S4	2602.5 bA	2666.5 abA	2591.7 bC	2505.7 bB	2874,2 aA
S6	2525.2 aAB	2498.7 aAB	2702.7 aBC	2566.5 aB	2634,2 aB
			Fv/Fm		
Control	0.804 bA	0.800 bA	0.824 aA	0.809 abA	0.813 abA
S2	0.785 dB	0.795 cdA	0.831 aA	0.814 abA	0.808 bcA
S4	0.805 abA	0.802 bA	0.802 bB	0.798 bA	0.822 aA
S6	0.801 aA	0.803 aA	0.805 aB	0.811 aA	0.811 aA
			PI		
Control	2.430 aA	2.317 aA	2.336 aB	2.750 aA	2.866 aA
S2	1.918 aA	2.029 aA	2.340 aB	2.801 aA	2.579 aA
S4	2.436 bA	2.335 bA	3.713 aA	2.144 bA	3.320 abA
S6	2.626 aA	2.696 aA	3.469 aAB	2.942 aA	2.608 aA

Equal letters (lowercase among evaluation days and uppercase among treatments) do not differ significantly (Tukey's Multiple Range Test; $P < 0.05$).

In general, no pattern was found in the response of the plants to the different watering cycles with regard to chlorophyll fluorescence. Initial chlorophyll fluorescence (F0) did not differ among watering treatments, but differed among evaluation time to S6 plants after 43 and 50 days of treatment. Maximum chlorophyll fluorescence (Fm) differed significantly among treatments and evaluation time. Significant differences were found on the first two evaluations between the plants submitted to S2 and S4, but these differences did not achieve statistical significance in comparison to the control. However, statistically significant differences in comparison to the control occurred at Day 50, with higher Fm found in the plants submitted to S2 and lower Fm in the plants submitted to S4. Nonetheless, no defined pattern was found regarding variation throughout the evaluation days in any treatment, except S6, which maintained constant values during the experimental period (Tab. 3).

The variable chlorophyll fluorescence (Fv), which is the difference between maximum and initial fluorescence, also differed significantly among treatments and evaluation days, but without a defined pattern, probably due to variations in Fm among the days. The lowest values were found in S4 after 43 days of stress, with a subsequent recovery. Plants submitted to S6 were the only ones with no significant variation in Fv throughout over experiment. The greatest variation was found in S2 (Tab. 4).

Quantum efficiency of PSII (Fv/Fm) differed among evaluation days and watering cycles, but without a defined pattern (Tab. 4). However, water stress did not affect PSII, as all values remained above 0.75. The greatest variation was from 0.78 to 0.83 in S2 treatment. Once again, only plants submitted to S6 exhibited no significant variations throughout the experimental period. Similarly, the performance index (PI) differed among evaluation days only in S4, but without a defined pattern and differences among watering cycles only occurred on Day 43 (Tab. 3).

The area was reduced in almost all watering cycles' treatment when compared with control plants in three evaluations days (after 29, 36 and 57 days), and no significant differences was found only after 50 days (Tab. 4).

Table 4 - Area and maximum density of reaction center per transversal section (RC/CSm) of *H. speciosa* seedlings cultivated under greenhouse conditions and submitted to different watering cycles.

Treatments	Days of watering cycle				
	29	36	43	50	57
			Area		
Control	68081.2 aA	67268.7 aA	66650.0 abA	75612.5 abA	68081.2 aA
S2	60745.0 aB	59409.0 aB	63861.0bAB	80203.0abA	60745.0 aB
S4	57887.0 aB	62950.0 aB	84358.7 aA	88912.0 aA	57887.0 aB
S6	66684.0 aB	67952.7aAB	65482.5abB	85084.5aA	66684.0 aB

Equal letters (lowercase among evaluation days and uppercase among treatments) do not differ significantly (Tukey's Multiple Range Test; $P < 0.05$).

Discussion

Drought exerts a negative effect on plant growth and development (Al-Absi 2009; Silva et al. 2011, Silva et al. 2013). For a large number of species, initial growth is the most critical phase of plant development in the field and is severely affected by water deficit (Jaleel et al. 2009; Martins et al. 2010). In the present study, *H. speciosa* seedlings demonstrated slow growth, which likely explains the only slight effects on growth with the extension of water stress. This characteristic is intrinsic to this species and may contribute to better water management in the initial growth phase, when the root is exploring the soil depth.

Unexpectedly, plants grown with the six-day watering interval (S6) exhibited a significant increase in plant height throughout the experimental period, while control plants and those submitted to S2 and S4 exhibit no significant increase in plant height over time (Fig. 1). Thus, the six-day watering interval seems to have induced a better performance in the cell elongation, favoring growth in the height of *H. speciosa* seedling, which was not seen with the other watering cycles (two and four days). The *H. speciosa* tree is not found in dry lands, but in coastal areas with sandy soils (as used in the present study). Six days without watering significantly reduces the amount of moisture in the soil due the evapotranspiration,

which confirms statements found in the literature on the better performance of this plant in well-drained soil (Soares et al. 2006).

Albuquerque (2004) studied *H. speciosa* seedlings submitted to four levels of water deficit ranging from 100% to 25% of field capacity (FC) and found reductions in plant height, stem diameter, the number of leaves and leaf area in plants cultivated with 25% FC after 90 days of treatment. However, the other treatments (75% and 50% FC) remained similar to control plants and the author classified the *H. speciosa* tree as a drought-tolerant species, since the reductions were only found in seedlings submitted to severe stress. The findings in the study cited as well as the present investigation demonstrate that the *H. speciosa* seedlings tolerates periods of water deficit and develops better at high temperatures, as those found in summer in northeastern Brazil (Vieira Neto et al. 2002).

In addition, stem diameter was not significantly altered by the different water treatments (Fig. 1), but a greater number of leaves were found in plants submitted to the S6 treatment (Fig. 1). These variables are generally reduced when plants are submitted to water stress, as observed for seedlings of *Azadiractha indica* (Martins et al. 2010), *Malaleuca alternifolia* (Silva et al. 2002), *Erythrina velutina* (Silva et al. 2010a) and a number of other species. The reduction in the number of leaves is mainly due to abscission (Taiz and Zeiger 2006). However, the S6 treatment favored the emergence of new leaves on the seedlings, as plants in this treatment had the greatest number of leaves at the end of the experimental period, while those submitted to S2 and S4 did not differ significantly from the control plants (Fig. 3). The fact that water stress favored shoot growth in *H. speciosa* tree seedlings is an important finding, as the leaves are the main organs responsible for acquiring CO_2 for the production of dry matter, although leaves are also the organs by which water is lost through transpiration.

Dry matter was not significantly affected by the different watering cycles (Tab. 1). This may be explained by the variation among the replicates and the slower growth of *H. speciosa* seedlings in comparison to other tropical species. However, in absolute values, the plants submitted to S6 exhibited greater amounts of dry matter in the leaves, stem and roots, with a consequently greater amount of total dry matter (Tab. 1). These findings suggest a tendency toward an increase in dry matter over time. Thus, the prolongation of the experimental period would be expected to induce changes in the production of dry matter.

The literature is scarce regarding the effects of water deficit on the growth of *H. speciosa* seedlings. However, the slow growth of the *H. speciosa* must favor the maintenance of water management, as observed for *Erythrina velutina* (Silva et al. 2010a). The authors cited state that small plants have few leaves and a small leaf area, which translates to less water loss through transpiration and enhances the chances of survival under conditions of water deficit. However, to *H. speciosa* seedlings, the two-to-six-day watering cycle seems insufficient to produce significant morphological changes. Indeed, the six-day watering cycle induced a better performance with regard to plant development.

To a better understanding about this performance, we hypothesized that the species has an efficient photosynthetic mechanism. Plant growth has a direct correlation with photosynthesis, which is the principal process of carbon fixation. Thus, any factor that affects photosynthesis also affects growth. Photosynthetic pigments, especially chlorophylls, are important to the process of light harvesting and the production of reducing power for photosynthesis. Thus, the evaluation

of pigment content is important in plants under stress conditions. Water deficit often reduces chlorophyll content and induces changes in the chlorophyll *a*/*b* ratio and carotenoids (Jaleel et al. 2009). According to Walter et al. (2011), in the short-term acclimation to drought, changes occur in pigment content and the xanthophyll cycle to prevent photodamage. But, in the present study, chlorophyll content did not decrease with the water stress cycles applied, which suggests an efficient photoprotection mechanism in *H. speciosa* seedlings (Tabs. 2 and 3).

The decrease in photosynthesis during periods of water stress occur by limiting the CO_2 supply through stomatal closure (Flexas et al. 2007; Silva et al. 2010b), the inactivation of Rubisco or other enzymes involved in carbon fixation (Flexas et al. 2004) and the inhibition of electron transport in the photochemical phase, which affects PSII (Silva et al. 2013). Carboxilation was not studied in the present study, but the photochemical phase was evaluated and photodamage was not found in *H. speciosa* seedlings in response to watering cycles.

Severe photoinhibition can give rise to photodamage in PSII, with the formation of reactive oxygen species, which are toxic, destroy pigments and affect the stability of the membrane (Lemos-Filho, 2000; Araújo et al. 2010). Damage in PSII is measured by chlorophyll fluorescence. F0 represents the initial fluorescence when all reaction centers (RCs) are open and refers to the fluorescence emission by chlorophyll molecules in the complex of the PSII light collector (Krause and Weiss, 1984). During experimental period, water deficit did not significantly affect F0 in *H. speciosa* seedlings, but variations were observed over time (Tab. 4). Bacarin and Mosquim (2002) also observed this kind of variation in two bean genotypes, coinciding with the increase in dry weight in the pods and nodules. Moreover, Tatagiba and Pezzopane (2007) found reductions in F0 in a eucalyptus clone during the dry season. The other variables of chlorophyll fluorescence had the same tendency.

Maximum fluorescence (Fm) is defined as the intensity of fluorescence when all PSII RCs are open, that is, the photochemical quenching is equal to zero and all non-photochemical processes of extinction are minimum (Van Kooten and Snel, 1990), which indicates the complete reduction of quinone A (QA) from the incidence of a light pulse in the QA RC, generating maximum fluorescence. The difference between Fm and F0 results in variable fluorescence (Fv), which represents the flow of electrons from the PSII RC (P680) to plastoquinone (PQH2) (Björkman and Demming, 1987). Maximum quantum yield is calculated as Fv/Fm = (Fm-F0)/Fm. When the photosynthetic apparatus is intact, the Fv/Fm ratio should be between 0.75 and 0.85 (Bolhàr-Nordenkampf et al. 1989), while a reduction in this ratio reflects photoinhibitory damage in PSII RCs (Björkman and Demming, 1987).

The performance index (PI) has been used as a consistent parameter for the evaluation of plant performance regarding the absorption of light energy, excitation energy trapping and the conversion of excitation energy to electron transport by photosynthesis under conditions of environmental stress, such as high irradiance, drought, heat, dark chilling, ozone and salt (Clark et al. 2000; Mishra et al. 2001; De Ronde et al. 2004; Strauss et al. 2006). Furthermore, the PI ABS allows broader analysis of photosynthetic performance, such as the relationship between photon absorption efficiency and the capture of excited energy in PSII, as well as an analysis of the density of active RCs and the probability that excited energy moves an electron further than QA (Gonçalves and Santos Junior 2005). Therefore, PI ABS is a better parameter for evaluating the responses of PSII to stressful conditions than Fv/Fm alone.

Variations among evaluation days and treatments in *H. speciosa* seedlings were also found with regard to Fv, Fm, Fv/Fm and PI, but with no well-defined pattern in these differences. Thus, such variations could be attributed to other environmental factors rather than a response to the watering cycles alone, such as the maintenance of RCs per active leaf area, the high maximum quantum efficiency of PSII and the high rate of electron transport beyond QA. Furthermore, high RC and Fv/Fm values suggest that a small proportion of the absorbed energy was dissipated as heat and fluorescence.

The findings demonstrate that the different watering cycles applied to *H. speciosa* seedlings did not damage PSII, as no Fv/Fm ratios were lower than 0.75 (Tab. 4). Indeed, the stressed plants, especially those submitted to S6, had Fv/Fm ratios greater than 0.80, demonstrating that PSII remained intact throughout the experimental period. Other chlorophyll fluorescence parameters were investigated and no statistical differences were found (data not shown), further demonstrating the lack of photodamage in the *H. speciosa* seedlings.

Conclusions

Based on the present findings, *H. speciosa* seedlings tolerate intermittent drought during the initial developmental phase with no significant reduction in growth parameters. The hypothesis about changing in the growth pattern was refuted. The best performance in terms of plant growth was achieved with the six-day watering interval. Moreover, photosynthetic pigments and chlorophyll *a* fluorescence were not affected by the water deficit cycles, demonstrating a lack of damage in photochemical phase when *H. speciosa* seedlings are cultivated using until six-day watering cycle intervals.

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