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Different levels of water deficit induces changes in growth pattern but not in chlorophyll fluorescence and water relations of *Hancornia speciosa* Gomes seedlings

Diferentes níveis de déficit hídrico induzem mudanças no padrão de crescimento, mas não nas relações hídricas e fluorescência de clorofila em mudas de *Hancornia speciosa* Gomes

E. C. da Silva[1*], T. B. Prado[1], R. N. de Alcântara[1], C. D. da Silva Junior[2], L. C. Nogueira[3]

[1]*Departamento de Biologia/Laboratório de Fisiologia Vegetal/Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, CEP 49100-000, São Cristóvão-Sergipe, Brasil*
[2]*Departamento de Biologia/Laboratório de Botânica Aplicada/ Centro de Ciências Biológicas e da Saúde, Universidade Federal de Sergipe, CEP 49100-000, São Cristóvão-Sergipe, Brasil*
[3]*Embrapa Tabuleiros Costeiros/ Av. Beira Mar, 3250, Aracaju, SE, Brasil.*

**elizaciriaco@gmail.com*

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The tree *Hancornia speciosa* Gomes is commonly known in Brazil as "mangabeira", the fruit of which has high nutritional value. The knowledge about its physiology is still scarce, mainly during the initial phase of development. Thus, the aim of the present study was to evaluate the effects of different water deficit levels on growth pattern, chlorophyll fluorescence and water relations in *H.speciosa* seedlings. A factorial experimental design (water treatment x evaluation time) was used with four water treatments based on the field capacity (FC) (80%, 60%, 40% and 20%) with five replications. Plant height, number of leaves, stem diameter, dry biomass production and partitioning, quantum efficiency of photosystem II (PSII), water potential (Ψw), relative water content (RWC), carbohydrates, proteins and proline content were analyzed. Severe water stress (20% FC) led to a reduction in growth and altered the biomass partitioning pattern in the seedlings. However, water relations were not significantly affected, as the seedlings maintain high Ψw and RWC without significant increase in organic solutes content when cultivated in 20% FC. Moreover, the quantum efficiency of PSII was unaffected by the different water levels, suggesting a lack of photoinhibition due to water stress. The change in growth pattern, with an increase in root depth and reduction in shoot emission, seems to be the main strategy of the *H. speciosa seedlings* for the maintenance of tissue hydration throughout periods of water deficit.
Keywords: biomass partitioning, proline, root to shoot ratio

Hancornia speciosa Gomes é uma espécie conhecida popularmente no Brasil como mangabeira, cujo fruto apresenta alto valor nutricional. O conhecimento sobre a sua fisiologia é ainda escasso, principalmente no que se refere ao desenvolvimento inicial. Dessa forma, o objetivo do presente trabalho foi avaliar os efeitos de diferentes níveis de déficit hídrico sobre o padrão de crescimento, fluorescência de clorofila e relações hídricas em mudas de mangabeira. Foi utilizado um esquema fatorial (tratamentos x época de avaliação) com quatro tratamentos hídricos com base na capacidade de campo (CC) (80%, 60%, 40% e 20%), com cinco repetições. Foram avaliados a altura das plantas, número de folhas, diâmetro do caule, produção e partição de biomassa, eficiência quântica do fotossistema II (PSII), potencial hídrico (Ψw), teor relativo de água (TRA) e teor de carboidratos, proteínas e prolina. O déficit hídrico severo (20% CC) levou a uma redução no crescimento e alterou o padrão de partição de biomassa nas mudas. No entanto, as relações hídricas não foram significativamente afetadas, pois as mudas mantiveram altos valores de Ψw e TRA, sem acúmulos significativos nos teores de solutos orgânicos quando cultivadas com 20%CC. Além do mais, a eficiência quântica do PSII não foi afetada pelos diferentes regimes hídricos, sugerindo que não houve fotoinibição devido ao estresse hídrico. A mudança no padrão de crescimento, com um incremento no aprofundamento das raízes e redução no crescimento da parte aérea parece ser a principal estratégia das mudas de *H. speciosa* para a manutenção da hidratação dos tecidos durante períodos de déficit hídrico.
Palavras-chave: partição de biomassa, prolina, razão parte aérea/raiz

1. INTRODUCTION

The "mangabeira" (*Hancornia speciosa* Gomes) is a small fruit tree found predominantly in tropical regions that produces a fruit known locally in Brazil as "mangaba". This tree belongs to the family Apocynaceae, which has about 400 genera and 3700 species, with 95 genera and 850 species occurring in Brazil [1]. The mangabeira is abundant on the coastal tablelands and coastal plains of the northeastern region of the country as well as the savanna-like *cerrado* region [2].

Mangabeira is of considerable economic importance in northeastern Brazil, especially in the state of Sergipe, which is the largest producer in Brazil [2, 3]. The cultivation of the mangabeira occurs mainly with a low degree of technology, as manual harvesting is carried out in locations of spontaneous occurrence [3]. However, these sites have been threatened by real estate and livestock interests, which have led to the replacement of native vegetation [2].

In summer, locations of the natural occurrence of the mangabeira experience drought due to scarce rainfall and high temperatures. As plant yield depends on the availability of water in the soil, drought affects all physiological processes vital to plant development when not counterbalanced by an efficient mechanism of tolerance [4]. Water participates in cell division and elongation, is responsible for the sustenance of herbaceous plants, assists in nutrient input and transport through the xylem and phloem and is associated with metabolic reactions [5]. Water also affects photosynthesis, thus, insufficient water availability leads to a reduction in stomatal conductance, with consequent reductions in transpiration and gas exchange [6, 7].

Drought tolerance in plants can be raised by physiological mechanisms that enable them to conserve water, such as stomatal closure at the hottest time of the day, or morphological changes, such as water reservoirs in the roots or trunk and the shedding or absence of leaves, which helps reduce water loss through transpiration [7, 8, 9].

Plants classified as "drought tolerant" generally have mechanisms of adjustment, especially when stress occurs in a gradual fashion. One of them is the osmotic adjustment which can occur by the accumulation of compatible organic solutes and inorganic ions within their cells to make the cellular water potential lower than that of the surrounding soil, thereby maintaining water inflow and turgor pressure [6, 10, 11, 12]. These plants can also transfer nutrients and metabolic compounds from older to younger leaves to increase photosynthetic efficiency and reduce surface transpiration through the abscission of senescent leaves. Moreover, the anticipation of blossoming ensures the perpetuation of the species. These processes allow plants longer survival in the dry season, but are not sufficient in situations of prolonged drought beyond the capacity of tolerance of the species [6].

Plant responses to drought and its effects can be measured through plant growth, especially the production and distribution pattern of dry biomass, water status and the accumulation of osmoprotectants, which indicate osmotic regulation. Moreover, photosynthesis can be evaluated by quantifying levels of photosynthetic pigments, chlorophyll *a* fluorescence and carbon assimilation [7, 13].

Chlorophyll fluorescence is a tool applied in water stress studies and has been widely applied. Fluorescence occurs when part of the energy incident in chlorophyll is dissipated by the emission of light. The fluorescence spectrum differs from the spectrum of absorbed light and the wavelength emitted is greater than that absorbed, which facilitates the measurement of fluorescence [13]. Generally, exposure to stress induces alterations in photobiological processes, resulting in stomatal limitations, such as restrictions in the supply of carbon dioxide and the loss of water vapor, and limitations to non-stomatal components, with harm caused to reaction centers of photosystems I and II (PSI and PSII), thereby compromising the photosynthesis efficiency [7, 14]. According to Bolhàr-Nordenkampf et al. (1989)[15], changes in the photochemical efficiency of plants under drought conditions may be assessed by the analysis of chlorophyll *a* fluorescence associated with PSII, which provides important information for profiling the physiological behavior of species in response to water stress in the natural environment [7].

The initial phase of development (seedling) is the most critical to survival in the field. Considering the economic importance of the *H. speciosa*, the aim of the present study was to evaluate the effects of different levels of water deficit on the physiological behavior of seedlings

for this species through the analysis of some growth parameters, water relations and chlorophyll *a* fluorescence.

2. MATERIAL AND METHODS

Plant material and experimental design

The present study was conducted in a greenhouse of the Department of Biology of the Universidade Federal de Sergipe, Brazil. *Hancornia speciosa* Gomes seedlings were propagated using seeds from fruits collected from coastal plains with sandy soils in the municipality of Pirambu, state of Sergipe, northeastern Brazil. After emergence, the seedlings were transferred to polyethylene pots containing 6 kg of topsoil (organic substrate). The seedlings were acclimated for 15 days and irrigated daily at 80% substrate field capacity (FC).

A factorial experimental design (water treatment x evaluation time) was used with four water treatments, eight evaluation time and five replications. Based on *a priori* knowledge that the mangabeira does not grow well under 100% FC, the control group was defined as the plants watered with 80% FC. The water treatments were 80%, 60%, 40% and 20% FC, determined gravimetrically by the difference in the weight of the moist soil after being saturated through capillarity and the excess water drained by gravity [16]. Over a 60-day period, the pots were weighed daily and water was replaced based on the difference in weight and the respective percentages of FC in the different treatments. Control plants received water daily. The other treatments were irrigated only enough to establish the respective FC levels (Figure 1).

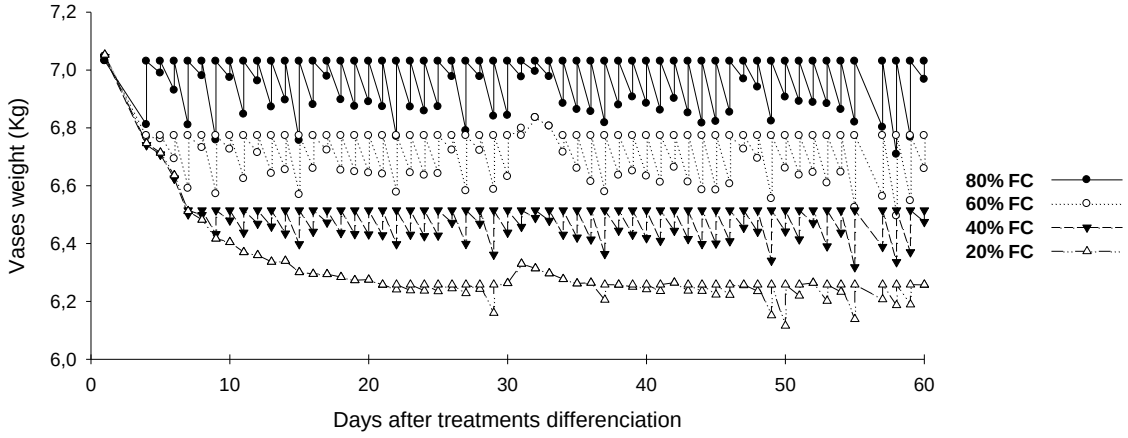

Figure 1: Water replacement in H. speciosa seedlings throughout daily weighing. Water stress treatments throughout experiment based on field capacity (80, 60, 40 and 20% FC).

Plant growth

Plant growth was evaluated weekly for 60 days with the measurement of plant height and stem diameter using a digital caliper (Digimess Model, DIN 862) and counts of the number of leaves. After 60 days, the plants were harvested, separated into different organs, packed in paper bags and left to dry in an oven until reaching a constant weight. The quantification of dry mass was used to calculate the allocation of biomass and the shoot to root ratio.

Determination of water potential and relative water content

Due the small size of the leaves, after 60 days of treatment, the water potential (Ψw) of the branches was determined at midday using a Scholander pressure chamber (Model 3035, Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Relative water content (RWC) was determined in six discs taken from the leaves. Fresh mass weight (FMW) was determined and the discs were placed in Petri dishes containing distilled water. After 24 h stored in a refrigerator in the dark, the discs were weighed again to obtain the turgid mass weight (TMW). The samples were then packed in paper bags and placed in an oven with forced air circulation at 70 °C for 48

hours to obtain the dry mass weight (DMW). RWC was calculated using the equation described by Weatherley (1950) [17] and expressed as a percentage value as follows:

$$RWC\ (\%) = [(FMW-DMW)/(TMW-DMW)]\ x100.$$

Soluble sugars, proline, soluble protein and amino acids analysis

Biochemical analyses were performed colorimetrically. Samples consisted of approximately 0.2 g of leaf blade without the central vein collected at midday (the same leaves from water potential measurements). The specimens were wrapped in aluminum foil and frozen until the preparation of the extracts. The extracts were prepared by grinding the plant material in a mortar with 5 ml of 0.1 M monobasic phosphate buffer, pH 7, containing 0.01 M EDTA and filtered on nylon mesh [18]. The solutions were then centrifuged at 8000 x g for 10 minutes. The supernatants were transferred to 2 ml Eppendorff tubes and frozen until analysis.

Soluble carbohydrate content was determined colorimetrically at 490 nm in a 0.5 mL aliquot of the extract using the phenol-sulfuric acid method, with D-(+)-glucose as the standard [19]. Proline was determined at 520 nm in a 1 mL aliquot of the extract, with ninhydrin as the specific reagent and pure proline as the standard [20]. Soluble proteins were determined at 595 nm using the dye binding method in a 0.1 mL aliquot of the extract, with pure bovine serum albumin as the standard [21].

Chlorophyll fluorescence measurement

Chlorophyll *a* fluorescence measurements were taken weekly between 9 and 10 am using a saturating light pulse through a portable fluorometer (Handy PEA, Hansatech Instruments, Norfolk, UK) in mature fully expanded leaves exposed to direct sunlight. Selected leaves were submitted to a 30-min dark adaptation period, which was sufficient for all PSII reaction centers to become open [22]. Immediately following adaptation to the dark, the leaves were exposed to a pulse of saturating light at an intensity of 3000 $\mu mol.m^{-2}.s^{-1}$ with a wavelength of 656 nm for 1s. The evaluation of the kinetics of chlorophyll *a* fluorescence was based on values of initial fluorescence (F0), maximum fluorescence (Fm), variable fluorescence (Fv) and quantum efficiency (Fv/Fm).

Statistical analysis

The data were submitted to analysis of variance and the means were compared using Tukey's multiple range test ($P < 0.05$).

3. RESULTS

Water deficit induced reduction in all growth parameters of *H. speciosa* seedlings. Significant differences *(P<0.05)* among treatments were found in plant height, stem diameter and number of leaves over time (Figure 2 A, B and C). Plant height was lower after 30 days of water deficit in plants cultivated with 20% FC (severe stress) in comparison with those with 80% and 60% FC. Plants grown with 40% FC only exhibited a significant reduction in plant height in comparison to 60%FC plants after 52 days (Figure 2A).

Severe water deficit (20% FC) induced a growth stoppage related to stem elongation, with no significant increase in plant height (Figure 2A). Contrary, plants grown with 60% FC demonstrated a better performance in comparison to those grown with 40% FC at 52 days and henceforth.

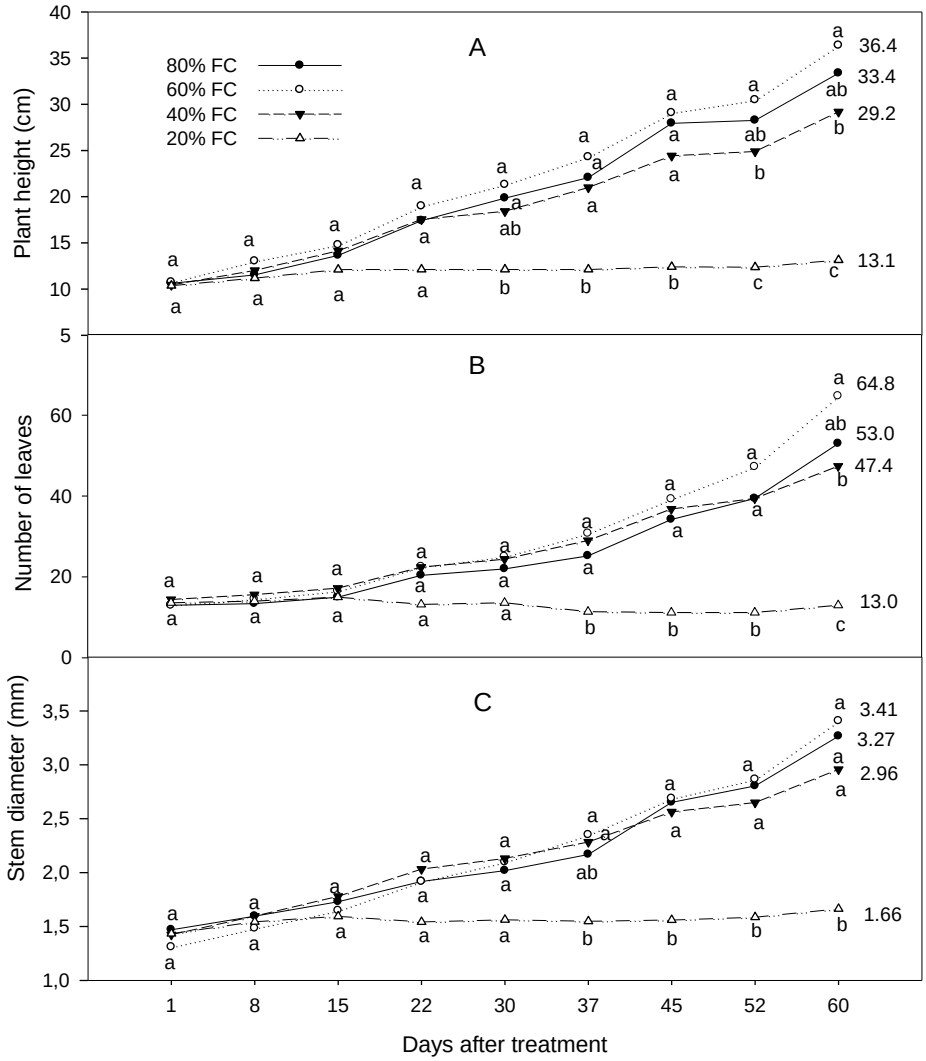

Figure 2: Plant height (A), number of leaves (B) and stem diameter (C) of H. speciosa seedlings cultivated under water deficit based on different degrees of field capacity (80, 60, 40 and 20% FC). Means followed by same letters do not differ significantly (P < 0.05, Tukey's multiple range test).

The number of leaves was reduced in response to water deficit. Fewer leaves in comparison to the control were found on plants grown with 20% FC beginning on Day 37. This was followed by shedding and the subsequent emission of new leaves at the end of the experimental period (Figure 2B). Mean values in this treatment remained lower than all other treatments and did not differ significantly from the beginning of the experimental period. Plants grown with 40% FC had an average of 47.4 leaves, which was statistically similar to the control plants. In contrast, plants grown with 60% FC had the best performance, with an average of 64.8 leaves at the end of the experiment (Figures 2A and 2B).

Stem diameter was the least affected growth parameter, with a significant reduction only in plants cultivated with 20% FC from Day 37 onward (Figure 2C).

Despite the differences in soil water availability (Figure 1), the dry matter of the stem and roots seems not to have been strongly affected by this stress factor, as no significant differences were found among treatments (Figure 3 A and B). In contrast, leaf dry matter (LDM) was affected by severe stress, demonstrating a statistically significant reduction ($P < 0.05$) in plants grown with 20% FC (Figure 3C). In the analysis of total dry matter, plants under 60% FC demonstrated the best performance, with a tendency toward an increase in dry matter production in comparison to control plants, while plants under 20% FC exhibited a reduction in dry matter, differing significantly ($P>0.05$) from plants under 60% FC, but not from the control (Figure 3D).

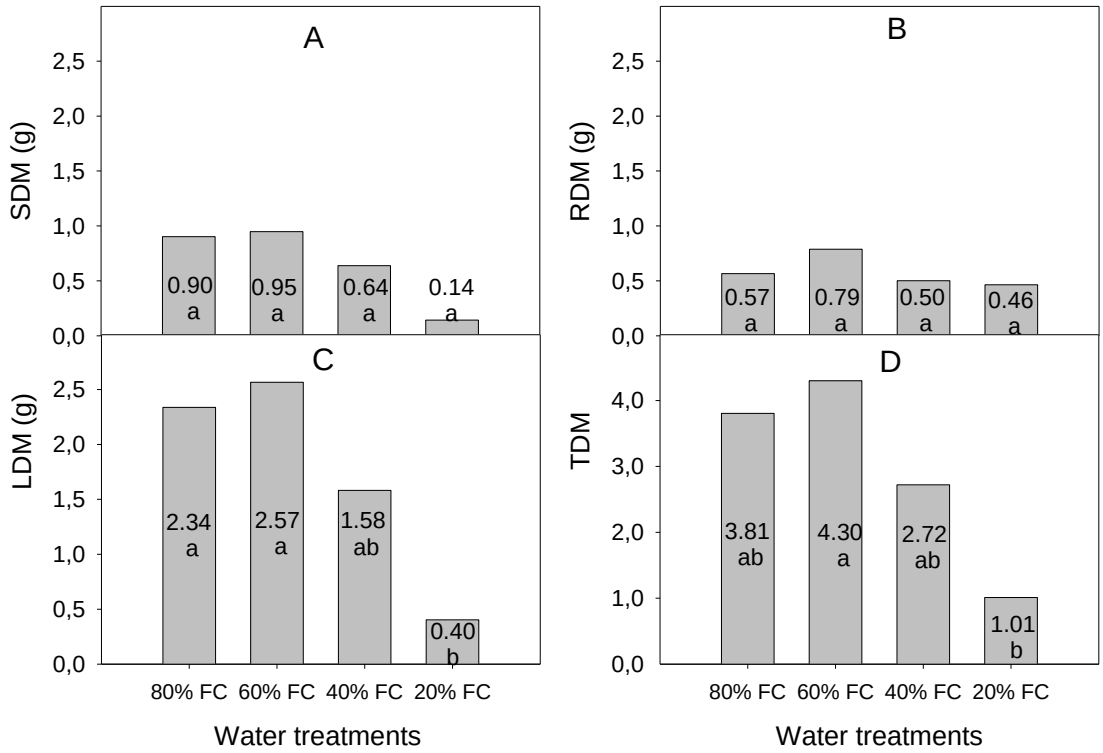

Figure 3: Stem (SDM), root (RDM), leaf (LDM) and total (TDM) dry matter (respectively A, B, C and D) of H. speciosa seedlings cultivated under water deficit with different degrees of field capacity (80, 60, 40 and 20% FC). Means followed by same letters do not differ significantly (P < 0.05, Tukey's multiple range test).

A change in the pattern of biomass partitioning was observed in plants under 20% FC in comparison with the other treatments, with an increase in root dry matter in detriment to the shoot nearly threefold greater than that found in control plants (Figure 4 A). The shoot to root ratio was significantly changed to 1:1 in plants under 20% FC, in comparison with about 5:1 in the control plants and 4:1 in the other treatments (Figure 4 B), although these last treatments did not differ from control plants.

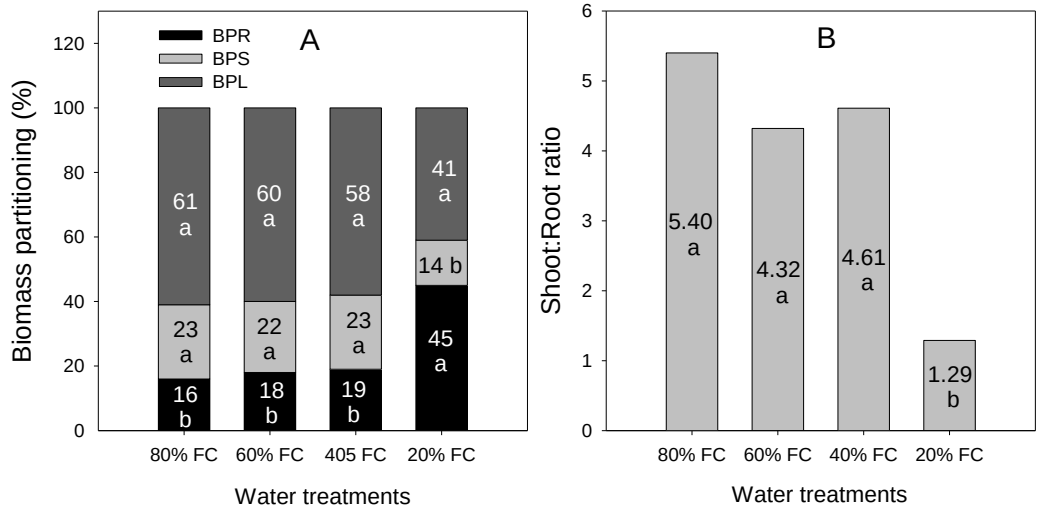

Figure 4: Biomass partitioning to roots (BPR), stem (BPS) and leaves (BPL) (A) and shoot to root ratio(B) of H. speciosa seedlings cultivated under water deficit with different degrees of field capacity (80, 60, 40 and 20% FC). Means followed by same letters do not differ significantly (P < 0.05, Tukey's multiple range test).

In contrast to the evident effects of drought on growth, little variation was found regarding water relations. No significant difference among treatments was found regarding RWC, which ranged from 73.4% in plants cultivated with 80% FC to 60.4% in plants grown with 20% FC (Figure 5A). Moreover, Ψw only differed between plants submitted to 40% and 20% FC, but not when compared to control plants. The lowest value was found in plants cultivated with 20% FC (-0.12 MPa) and the highest was found in plants grown with 40% FC (-0.06 MPa) (Figure 5B).

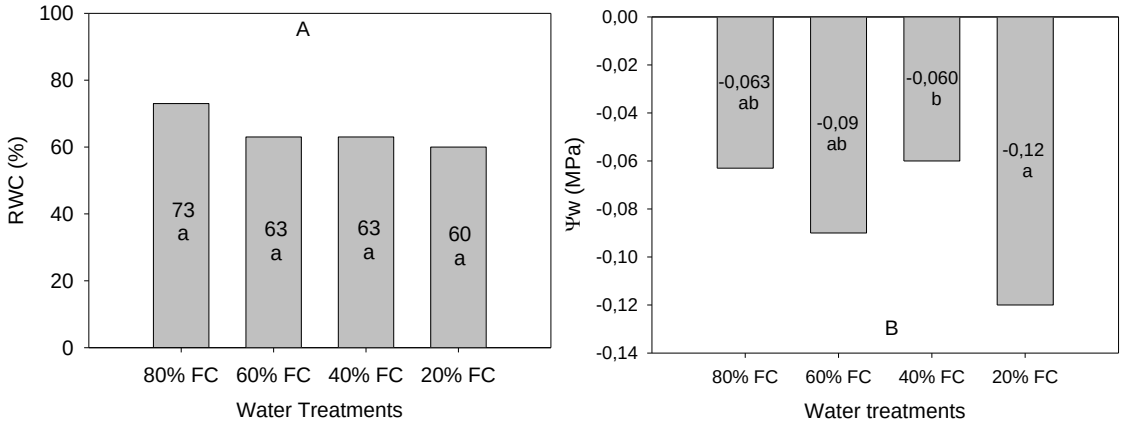

Figure 5: Relative water content (RWC) (A) and branch water potential (Ψw) (B) of H.speciosa seedlings cultivated under water deficit with different degrees of field capacity (80, 60, 40 and 20% FC). Means followed by same letters do not differ significantly (P < 0.05, Tukey's multiple range test).

The biochemical analysis revealed little change in the accumulation of organic solutes in the leaves of *H. speciosa* seedlings. Moreover, none of the treatments demonstrated a significant accumulation of soluble proteins or carbohydrates (Figure 6). The variation in the amount of proteins (Figure 6A) and carbohydrates (Figure 6B) ranged from 16.24 to 21.43 $mg.g^{-1}$ FM and 225.09 to 343.44 $\mu mol.g^{-1}$FM, respectively. However, proline accumulation was found in plants grown with 60% FC in comparison to control plants (Figure 6C). Moreover, plants grown with 40 and 20% FC also demonstrated a tendency toward the accumulation of proline, although the values did not differ significantly from the control plants.

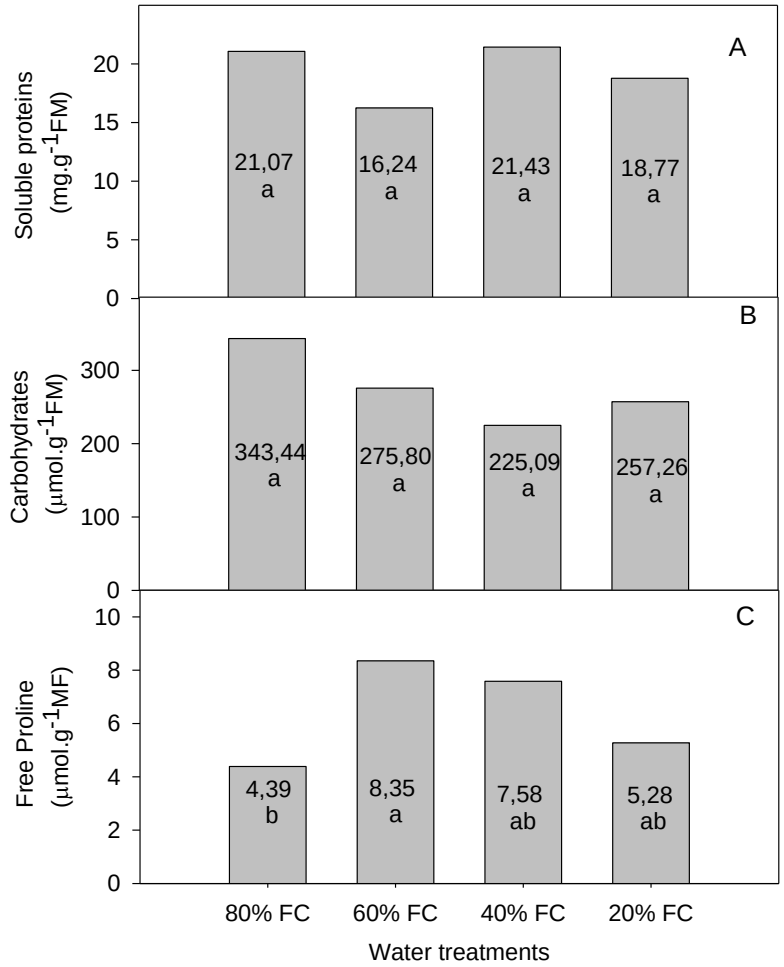

Figure 6: Soluble proteins (A), carbohydrates (B) and free proline (C) content in H. speciosa seedlings cultivated under water deficit with different degrees of field capacity (FC). Means followed by same letters do not differ significantly (P < 0.05, Tukey's multiple range test).

Regarding chlorophyll *a* fluorescence, no significant difference in initial fluorescence (F0) was found among treatments or over time (Table 1), except for a significant drop on Day 30, followed by a recovery. A 26% reduction in maximum fluorescence (Fm) occurred in plants subjected to 20% FC in comparison to the control group on Day 37 (Table 1). However, this difference did not persist through to the end of the experiment, as Fm recovered in the subsequent evaluations, reaching statistically similar values to those found in the other groups.

On Days 30 and 37, significant differences were found in variable fluorescence (Fv) and quantum efficiency (Fv/Fm) between plants submitted to 20% FC in comparison to the other treatments (Table 1). Throughout the experiment, plants treated with 20% FC had quantum efficiency of 0.69 on Day 30, which is indicative of photoinhibition, rising thereafter to 0.74 and 0.76 on the two subsequent evaluations (Days 45 and 60).

Table 1: Initial (F0), maximum (Fm) and variable chlorophyll a fluorescence (Fv) and quantum efficiency (Fv/Fm) in H. speciosa seedlings grown under greenhouse conditions with different percentages of soil water availability. Equal lower case letters denote absence of significant difference comparing treatments and equal upper case letters denote absence of significant difference comparing evaluations (P < 0.05, Tukey's multiple range test).

Water degree (FC)	F0							
	Days of evaluation							
	1	8	15	22	30	37	45	60
80%	598.7 aA	573.5 aA	544.5 aA	544.2 aA	311.2 aB	537.2 aA	537.2 aA	530.2 aA
60%	639.7 aA	577.7 aA	591.5 aA	540.0 aA	369.2 aB	530.0 aA	534.5 aA	532.3 aA
40%	599.2 aA	560.2 aA	569.7 aA	541.7 aA	392.0 aB	546.2 aA	543.7 aA	529.2 aA
20%	575.5 aA	543.5 aA	577.7 aA	537.7 aA	404.5 aB	546.7 aA	548.5 aA	534.7 aA
	Fm							
80%	2725 aA	2861aA	2788 aA	2912aA	1522aB	2903 aB	2909 aA	2669 aA
60%	2880 aA	2841aA	2942 aA	2640 aAB	1972 aB	2669abAB	2717aAB	2701aAB
40%	2693aAB	2874aA	2770aAB	2740 aAB	2032 aB	2470abAB	2777aAB	2596aAB
20%	2762aA	2808aA	2760 aA	2393 aA	1349 aB	2151bAB	2347 aA	2237 aA
	Fv							
80%	2126 aA	2288 aA	2244 aA	2368 aA	1211abB	2365 aA	2372 aA	2138 aA
60%	2240 aA	2263 aA	2351 aA	2100 aA	1602 aA	2139 abA	2183 aA	2168 aA
40%	2094 aA	2313 aA	2200 aA	2198 aA	1640 aA	1924 abA	2233 aA	2067 aA
20%	2187 aA	2265 aA	2182 aA	1855 aA	945 bB	1604 bAB	1798 aA	1702aAB
	Fv/Fm							
80%	0.779 aA	0.799 aA	0.805 aA	0.811 aA	0.795 aA	0.814 aA	0.815 aA	0.801 aA
60%	0.773 aA	0.795 aA	0.797 aA	0.790 aA	0.808 aA	0.801 abA	0.802 aA	0.803 aA
40%	0.777 aA	0.805 aA	0.792 aA	0.801 aA	0.802 aA	0.746 abA	0.804 aA	0.795 aA
20%	0.791 aA	0.806 aA	0.790 aA	0.773 aA	0.689 bB	0.735 bAB	0.765aAB	0.760aAB

4. DISCUSSION

Water deficit is a major problem worldwide, reducing plant yield in both agricultural and natural environments [7, 23, 24]. While the effect of water deficit on plants has been well studied, each species exhibits different mechanisms to overcome this stress factor. In the present study, severe stress (20% FC) affected growth, paralyzing plant height and stem diameter and causing the leaves to shed in *H. speciosa*. Shedding was followed by the emission of new leaves, but to a lesser extent. This demonstrates that a severe reduction in soil moisture affects the processes of cell division and elongation [6, 25]. Interestingly, the onset of differences in plant height occurred only after 30 days of treatment and the plants remained nearly 22 days without watering until the field capacity reached 20%. Moreover, plants grown under 60% FC demonstrated a better performance in comparison to 40% FC, confirming the better growth of this species in well drained soils. Thus, mild stress favored the growth of the *H. speciosa* seedlings.

The dry matter yield of the stem and roots was not severely affected by water stress, as no significant differences were found among the treatments. In contrast, leaf dry matter and total dry matter were markedly reduced. These findings demonstrate the shedding of leaves induced by water deficit and also suggest variability among individuals with regard to dry matter production in each treatment. A reduction in dry matter generally reflects less carbon assimilation [26]. Although gas exchange was not evaluated in the present study, it is possible that stomatal conductance had been reduced in the plants under severe stress.

The data found on dry matter production reflect a change in the pattern of biomass partitioning in plants under 20% FC, with an increase in root dry matter in detriment to the shoot, reducing the shoot to root dry matter ratio to 1:1, whereas this ratio was approximately 5:1 in the control

plants. These findings suggest that plants experiencing drought maximized biomass partitioning to root growth, while carbon assimilation in the shoot was used more to maintain previously formed structures. The deepening of the root system allows plants to find water in deeper regions of the soil. This behavior is considered a xeromorphic characteristic [4, 25], which allows plants to maintain the inflow of water and adequate water status.

Reduced growth as a survival strategy has been reported by Silva et al. (2010) [27] for seedlings of *Erythrina velutina* Willd, known locally as "mulungu". The authors found that, despite the absence of change in the dry matter distribution pattern, the reduction in growth allowed the seedlings to maintain turgor pressure. Indeed, the reduction in plant growth parameters is both a strategy and consequence. The control of the loss of water through transpiration by partially or completely closing the stomata during times of high temperature and in response to a low degree of soil water availability allows better water management, but, as a consequence, the inflow of CO_2 is insufficient to maintain the structures and physiological processes for the synthesis of the carbon skeleton to maintain growth in height and new structures. Thus, smaller plants need less water to maintain functional processes and keep the cells hydrated [27].

Regardless of the differences found among treatments, the *H. speciosa* seedlings had high degrees of branch water potential even in situations of severe water stress (-0,12 MPa to treatment 20% FC), with higher values in comparison to those reported for other plant species. In *Malpighia emarginata* DC, known locally as "acerola", Ψw of -4.3 MPa was found in plants propagated by seeds after five days of withholding water and -4.5 MPa was found in grafted plants after 20 days of withholding water [28]. Silva et al. (2003) [29] found a reduction in Ψw from -0.34 MPa to -0.87 MPa in *Mimosa caesalpiniifolia* grown with 50% FC. Several other species with anisohydric behavior experience a reduction in water potential under conditions of water stress, such as soybean, sunflower and wheat [30]. Silva et al. (2009) [31] classify *Spondias tuberosa* Arruda as an isohydric species due to the little variation in leaf water potential despite the reduction in soil moisture. Although further studies are necessary to determine the type of behavior *H. speciosa* seedlings use to overcome water deficit, the present findings also suggest isohydric behavior. Studies performed under field conditions show that the mangabeira exhibits little variation in branch water potential throughout the year (unpublished data). The present investigation was performed in pots, in which low soil moisture is more evident than in the field, with 1.21% of soil moisture for plants with 20% FC and 12.69% for control plants (data not shown). This low soil moisture may explain the small difference in branch water potential in stressed plants.

Regarding RWC, no significant differences were found among treatments and the values demonstrate that the species maintained the tissue hydrated. As water potential was only slightly reduced and RWC was not significantly affected, the reduction in growth in plants cultivated with 20% FC seems to be the strategy for overcoming severe water deficit and maintaining water status in the tissues.

Photochemical inhibition is often found in plants under severe stress. Drought can induce alterations in photobiological components, such as the reaction centers of PSI and PSII, thereby compromising photosynthesis efficiency [7, 14]. *H. speciosa* seedlings exhibited little variation in photochemical parameters in the present study. Bacarin and Mosquim (2002) [32] stated that changes in fluorescence can occur as a result of plant growth.

A decrease in quantum efficiency occurred after 30 days of treatment. The value of 0.69 at this evaluation time is indicative of photoinhibition and was followed by a recovery to values reaching 0.74 and 0.76 in the two subsequent evaluations. These data suggest that the structure of PSII was unaffected by mild to moderate stress, but momentarily affected by severe stress (20% FC), indicating a possible adjustment of the plants to stress conditions, with transient or dynamic photoinibition and subsequent recovery. Transient photoihibition generally occurs under natural conditions in times of high solar radiation, such as midday. By the end of the experimental period, the photochemical stage of photosynthesis did not appear to have been affected by the water stress applied. Values of Fv/Fm from 0.75 to 0.85 [15, 33] indicate that the PSII is intact, as such values are not found in plants subjected to severe stress.

Under stress, a decrease in the Fv/Fm ratio has been attributed to the inactivity of reaction centers due to the degradation of D1 and D2 proteins, which are responsible for the transfer of

electrons to chlorophyll *a* associated with the PSII reaction center [34]. Thus, the lack of a reduction in the Fv/Fm ratio in the *H. speciosa* seedlings under different water deficit treatments indicates an absence of harm to the protein structures of the photochemical complexes of the thylakoid membranes. Therefore, the capacity of plastoquinone complexes, which are responsible for the photochemical transport of electrons between PSI and II, to carry out oxidoreduction reactions may not have been affected by the mild, moderate and severe water stress applied.

The accumulation of osmotically active solutes is another tool for studying tolerance to water stress. Organic compounds (carbohydrates, amino acids and soluble proteins) and quaternary ammonium compounds as glycine betaine play an important role as osmoregulators and osmoprotectants [11, 18, 35]. The solutes studied in the present investigation were not significantly accumulated as a response to water deficit (Figure 6). However, the amount of proline accumulated in plants subjected to 60% FC (8.35 μmol g^{-1} FM) was approximately twofold greater than that in the control plants (4.39 μmol g^{-1} FM) (Figure 7). Moreover, plants submitted to moderate to severe water deficit (40% and 20% FC) exhibited a tendency toward the accumulation of proline, although the difference in comparison to control plants was non-significant. The level of intracellular proline is determined by biosynthesis, catabolism and transport between cells and different cellular compartments and has been associated with drought tolerance in plants [36]. As there were no changes in RWC or any significant reduction in water potential when cultivated under mild stress (60% FC), the results suggest a role for proline other than osmoregulation in *H. speciosa* seedlings. Indeed, the increase in proline concentration may be related to the maintenance of the integrity of membranes and macromolecules through the removal of radical oxygen species; or perhaps proline plays a role in the regulation and control of plant development or as a signaling molecule [36, 37], since there was no indication of osmotic adjustment in response to drought in the present study.

Although carbon fixation was not studied in the present investigation, the results suggest that the reduction in growth under water deficit could be a consequence of strong stomatal control, which reduces the input of CO_2 for photosynthetic assimilation and reduces the availability of carbon skeleton for vegetative growth. This reduction in stomatal opening, however, assists in maintaining the water content in the tissue, thereby avoiding desiccation. Further studies are needed about gas exchange to gain a better understanding of the mechanism used by *H. speciosa* seedlings under water deficit. In the present study, these seedlings tolerated drought with high water potential and a change in the growth pattern, with a decrease in the shoot to root ratio. This change seems to be the strategy for maintaining water status and the survival of the species under conditions of severe water stress.

5. CONCLUSION

The change in growth pattern, with an increase in root depth and a reduction in shoot emission, seems to be the main strategy of mangabeira seedlings for maintaining tissue hydration and tolerating severe water deficit throughout the period of imposed water stress.

6. ACKNOWLEDGMENT

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