



ORIGINAL ARTICLE

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Emergence and initial growth of *Hancornia speciosa* (Gomes) seedlings with different substrates and water availability

Emergência e crescimento inicial de plântulas de Hancornia speciosa (Gomes) em diferentes substratos e disponibilidades hídricas

ABSTRACT: *Hancornia speciosa* is a promising fruit species due to the high protein content of its fruit. However, there is little information about its production, with a lack of information concerning the germination of its seeds and initial seedling growth. The aim of this study was to evaluate water availability and suitable substrates for its seedling emergence and production. The seeds were deposited in tubes, containing the following substrates: Distoferric Red Latosol (L); Latosol+Sand (L+A); Latosol+Bioplant® (L+B); Sand+Latosol+Poultry Litter in the following proportions 2:2:1 (L+A+C₁) and 2:4:1 (L+A+C₂) associated with water retention capacities of 25%, 50%, 75% and 100%. The substrates L+A, L+B and L and a water availability of 75% to 100% are recommendable for seedling emergence and early seedling growth. Substrates that consisted of poultry litter and water availability of 25% and 50% were prejudicial to growth and quality of *Hancornia speciosa* seedlings.

RESUMO: A mangabeira é uma frutífera promissora em razão do alto conteúdo de proteínas de seus frutos. Entretanto, há poucas informações a respeito da produção dessa espécie, necessitando-se de informações referentes à germinação das sementes e ao crescimento inicial de plântulas. Assim, objetivou-se, com este trabalho, avaliar as disponibilidades hídricas e substratos adequados para emergência de plântulas e produção. As sementes foram depositadas em tubetes, contendo os seguintes substratos: Latossolo Vermelho Distroférrico (L), Latossolo + Areia (L + A); Latossolo + Bioplant® (L + B); Areia + Latossolo + Cama-de-frango nas proporções de 2:2:1 (L + A + C₁) e 2:4:1 (L + A + C₂) associados às seguintes capacidades de retenção de água do solo de 25%, 50%, 75% e 100%. Os substratos L + A, L + B e L podem ser recomendados para a emergência e crescimento inicial de plântulas de mangabeira nas disponibilidades hídricas de 75% a 100%. Substratos compostos por cama-de-frango e disponibilidades de água de 25% e 50% prejudicam o crescimento e a qualidade das mudas de mangabeira.

1 Introduction

Hancornia speciosa is a tropical fruitful, native from Brazil, spontaneously found in various regions of the country (Santos et al., 2011). It is possible to eat its edible fruit raw or use it in the industrialization of juices, sweets and ice creams, arousing interest of the agro industrial and pharmaceutical sectors (Dias et al., 2010; Soares et al., 2011). It is considered a promising species for the national fruit due to the high protein content of its fruits; however, its exploitation is carried out in an extractive form (Lobo et al., 2008).

The cultivation of a native species depends on the technical knowledge, spread and environmental variations of this specie. These are in its initial knowledge specially the ones concerning the physiological parameters against adverse situations (Nogueira et al., 2003). Thus, among other important factors of its production there are the substrate and water availability.

The substrate had the function of supporting plants during its rooting and supply of nutrients, influencing both germination and development of plants seeds, due its physical, chemical and biological variation properties (Zietemann & Roberto, 2007; Silva et al., 2011), which demonstrates the importance of choosing the ideal formulation (Silva et al., 2011).

Water is extremely important in activating different metabolic processes that culminate with the germination of seeds (Ávila et al., 2007). As the water content of the soil or substrate decreases, there is initially a reduction of speed of germination and tougher restrictions harm the percentage of germination (Marcos Filho, 2005; Ávila et al., 2007; Maraghni et al., 2010). According to Marcos Filho (2005), excess water in the soil also affects seed germination due to airtight restrictions.

During water stress condition, there is a series of adverse events in the plant, which number and gravity increases according to intensity and duration (Porporato et al., 2001). The water deficit reduces cell expansion and can affect the process of cell division and this interfere in the development of the plant. Furthermore most of the times there is a decreasing in the water potential in the leaf, which induces a stomata closure, resulting in the reduction of gas exchange and consequently in biomass production (Gindaba et al., 2004). The growth and development of plants are also reduced in environments with excess water in the soil, causing anaerobic respiration of roots, producing lactic acid and ethanol, causing the death of

root cells by acidosis. As a result, there is less growth and less absorption of water and nutrients (Floss, 2008).

It is important to take in consideration that the water availability is usually one of the main factors that determine the productivity of the species. That the substrate has direct influence on germination and initial growth of seedlings and that there is a lack of information regarding production of seedlings of native species from the Cerrado, thus the water availability and substrates suitable for the emergence and seedling production of this specie should be evaluated.

2 Materials and Methods

The experiment was conducted in a greenhouse with 70% brightness reduction with low-density plastic sheeting installed to avoid contact of cells with the rainwater, from the fourth quarter of 2010 to the second quarter of 2011. During the trial period, the average temperature and relative humidity were 24.1 °C and 75.2% respectively. Fifteen samples of *H. speciosa* fruits at the beginning of their ripening were previously selected and geo referenced from the remnant Cerrado vegetation, in Mato Grosso do Sul, located at 21°27'S latitude, 54°23'W longitude and 407 meters altitude. Later, those fruits were selected as their integrity, manually removed and the seeds rinsed in running water for waste pulp disposal and dried on sheets of paper towel. It was determined the degree of moisture by the oven at 105 ± 3 °C for 17 hours, with four replications of 20 seeds. The results were calculated based on the weight of the wet seeds and were expressed in percentage, observing initial humidity level of 52.1%.

The seedling was held in cells of polyethylene with capacity for 290 cm³, using as substrates: Distroferric Red Latosol (L), Latosol+Sand (L+A), Latosol+Bioplant® (L+B) at 1:1 ratio; and Latosol+Sand+Poultry litter semi decomposed in 2:2:1 (L+A+C₁) and 2:4:1 (L+A+C₂) ratio and which its chemical analyses can be seen in Table 1. All substrates were dried outdoors and sieved in a 2 mm mesh sieve before the fractions mixtures.

To evaluate the effect of different water availabilities. Water retention of 100% was determined to each substrate through water content retained after disposal and water retention of 25, 50 and 75%, were obtained through the rule of three simple (Souza et al., 2000). Then, all cells were weighed, individualized

Table 1. Chemical analysis of samples of the substrates tested for the emergence and early growth of *Hancornia speciosa* seedlings.

Tabela 1. Análise química de amostras dos substratos testados para a emergência e crescimento inicial de mudas de *Hancornia speciosa*.

Substratos	pH	P	K	Ca	Mg	H+Al	SB	CTC	V
	CaCl ₂	mg dm ⁻³	mmol _e dm ⁻³		cmol _e dm ⁻³			mmol _e dm ⁻³	%
L	4.3	1.58	0.7	1.9	0.7	2.5	27.1	5.2	51.8
L+A	4.4	0.58	0.3	1.4	0.6	1.8	19.9	3.8	52.0
L+B	5.2	51.0	39.3	10.5	5.6	7.8	199.3	27.8	71.9
L+A+C ₁	6.5	230.9	30.1	2.9	2.6	1.5	85.4	10.0	84.9
L+A+C ₂	6.6	214.5	28.6	2.6	2.1	1.5	75.7	9.1	83.7

pH in CaCl₂ - pH centimolar solution of calcium chloride, P - Phosphorus soil extracted by Mehlich, K - Potassium, exchangeable forms, Ca - calcium, exchangeable forms; Mg - Magnesium exchangeable forms, H + Al - Acidity potential; SB - Sum of bases, CTC - cation exchange capacity, V% - base saturation index. pH em CaCl₂ - pH em solução centimolar de cloreto de cálcio; P - Fósforo extraído do solo por meio de Mehlich; K - Potássio, formas trocáveis; Ca - Cálcio, formas trocáveis; Mg - Magnésio, formas trocáveis; H + Al - Acidez potencial; SB - Soma de bases; CTC - Capacidade de troca de cátions; V% - Índice de saturação por bases.

irrigation was held every two days, with tap water in quantity enough to meet the predetermined weight for each treatment.

The experiment lasted 180 days and for the evaluation of seedling emergence, counts were performed daily for 40 days, determining the percentage, average time and speed index, described by Ranal & Santana (2006). At 35, 70, 105 and 140 days after emergence, using two seedlings representing each replication, it was obtained the seedling survival record. It was taken into account the number of surviving plants on each evaluation; plant height, obtained through graduated ruler; stem diameter, with the aid of precision Digital Caliper; and number of leaves, counting the total number of leaves per plant.

At 140 days after emergence, the seedlings were removed from the substrate, washed in running water and dried on paper towels for evaluation of medium length roots, obtained through graduated ruler; leaf area was measured using the 3100 Area Meter Appliance; leaf and root dry mass, after drying kiln plant material with air renewal set at 65 °C for 72 hours and weighing precision in Analytical Balance. Dickson quality index was obtained by the formula: Dickson quality index = total dry mass (height aerial ratio and stem diameter + leaf and root dry mass) (Dickson et al., 1960).

The statistical approach adopted was entirely randomized, with four replications of 25 seeds. The percentage of emergence, speed emergence index, mean time of emergence, leaf area, average length of roots, leaf and root dry mass and Dickson quality index data were analyzed in a factorial scheme 5 x 4 (substrates x water availabilities), with two repetitions of each plant. The survival, number of leaves, plant height and stem diameter data were analyzed in sub divided plots scheme, being five substrates, four water availabilities, four times and evaluation with two repetitions of each plant. All data were submitted to analysis of variance, the 5% probability and in the case of significance, the regression analysis to 5% probability.

3 Results and Discussion

There was significant interaction between the substrate and water availabilities studied for percentage, mean time and speed index of seedling emergence of *H. speciosa*. There was no seedlings emergence in the substrate L+A+C₁, observing growing emergence from water retention of 50%. For the L, L+A, L+B and L+A+C₂ there was an increase in the emergence percentage to water retention of 68.4, 68.2, 74.9 and 84.6%, respectively, observing, after these maximum points, reduction of the rate according to the increasing on water availability in the substrate. The substrates L, L+A, and L+A+C₂ provided the largest seedling emergence results when compared to other substrates, showing average emergence rate of 41.3, 43.9 and 37.5%, respectively (Figure 1A). A possible cause of low percentage of *H. Speciosa* seedlings emergence was the intense predation on ants of the genre *Atta* on the seeds, causing damage to embryonic axis.

In general, it was observed a higher mean emergence time in the substrates L+A+C₁ and L+A+C₂, according to the increased water availability of the substrate, reaching greater results in 74.8 and 81.9% of water retention capabilities, respectively. Moreover, lower average emergence time in the substrate L+A (27.2 days), although there was no significant difference

between the used water availability. For the substrate L+B, there was no adjustment on the equation (Figure 1B). Water retention of 25% resulted in damage to speed emergence index in all the substrates studied, where the highest values observed were in the substrates L+A, and L+A+C₂ in water retention of 73 and 75.8%, respectively. In the substrates L and L+B an average speed emergence index of 0.162 and 0.068 were obtained, respectively, in which the first higher values, were observed in 50% of water retention and for the last, no significant difference between the treatments tested were observed (Figure 1C). For the percentage of germination of *Clitoria fairchildiana*, also a native species, Silva & Carvalho (2008) verified maximum point average around 49.1% of water retention of the substrate. From that point, the germination speed index decreased with the increasing of substrate wetting.

It was noticed a greater survival (81.4%) of *H. speciosa* seedlings in water retention calculated in 71.9% in the substrate L+A, while the substrate L+A+C₁ was not significantly influenced by water availability. The other, showed higher seedling survival substrates in 100% of water retention (Figure 1D). Over the course of evaluations, no significant variation of seedling survival to the substrate L+B was observed, while for the substrate L+A there was reduction in the survival emergence according to the assessments. In the substrate L, was observed maximum survival of 69.7 days after emergences and the substrates L+A+C₁ and L+A+C₂ showed lower survival rate along the evaluations. In substrates using poultry litter, less survival of seedlings was observed with the evaluations and the increased on water availability, when compared to other substrates (Figure 1E). The water retention of 25% provided less seedling survival when compared to other water supplies, and no adjustment in the equation was observed, while water retention of 100% was responsible for greater results, showing less survival rate with the increasing of age of the seedlings, as well as the water retention of 50 and 75% (Figure 1F).

The absorption of water is necessary for the activation of metabolic processes of seeds, unleashing a succession of events that culminate with the issuance of the primary root (Marcos Filho, 2005; Kos & Poschold, 2008; Yang et al., 2010). However, under lower humidity condition than the required by the species there is reduction of enzyme activity, culminated by the low seed germination and speed in which that occurs (Bewley & Black, 1994). On the other hand, soaked or excessively wet soils, limitations to the diffusion of oxygen can also cause the stoppage of germination, probably because the absence or scarcity of oxygen, promote ethanol production in the cells, which is toxic to normal metabolism and thus causing acidification and death (Marcos Filho, 2005; Kolb & Joly, 2010).

All aspects of growth and development of the plants are affected by the deficiency of water in the tissues, caused by excessive evaporating demand or limited water supply. Moreover, the effects of deficit of water on the development of the plants depend on the intensity, duration, stress, the stage of the growth and its orientation is genetic and can cause several morph physiological modifications and even take the plant to death. On the other hand, in flood conditions or drenching the dissolved oxygen diffuses so slowly that only few inches of soil or substrate near the surface remain bleached (Martins et al.,

2010). The seedlings produced in substrates compounded by poultry litter in its two proportions presented, quite often, collar with brown aqueous injuries, occurring a flat in the aerial part following by the fall of its leaves, which may have contributed to the high mortality of seedlings in these substrates. The same was observed by Silva et al. (2011), studying the development

of *H. speciosa* seedlings on substrate with bovine manure. Thus, the addition of poultry litter in substrate for seedling production has made possible an environment favorable to the development of plant pathogens.

The seedlings showed increasing in height according to the availability of water in the substrate L. For the substrates

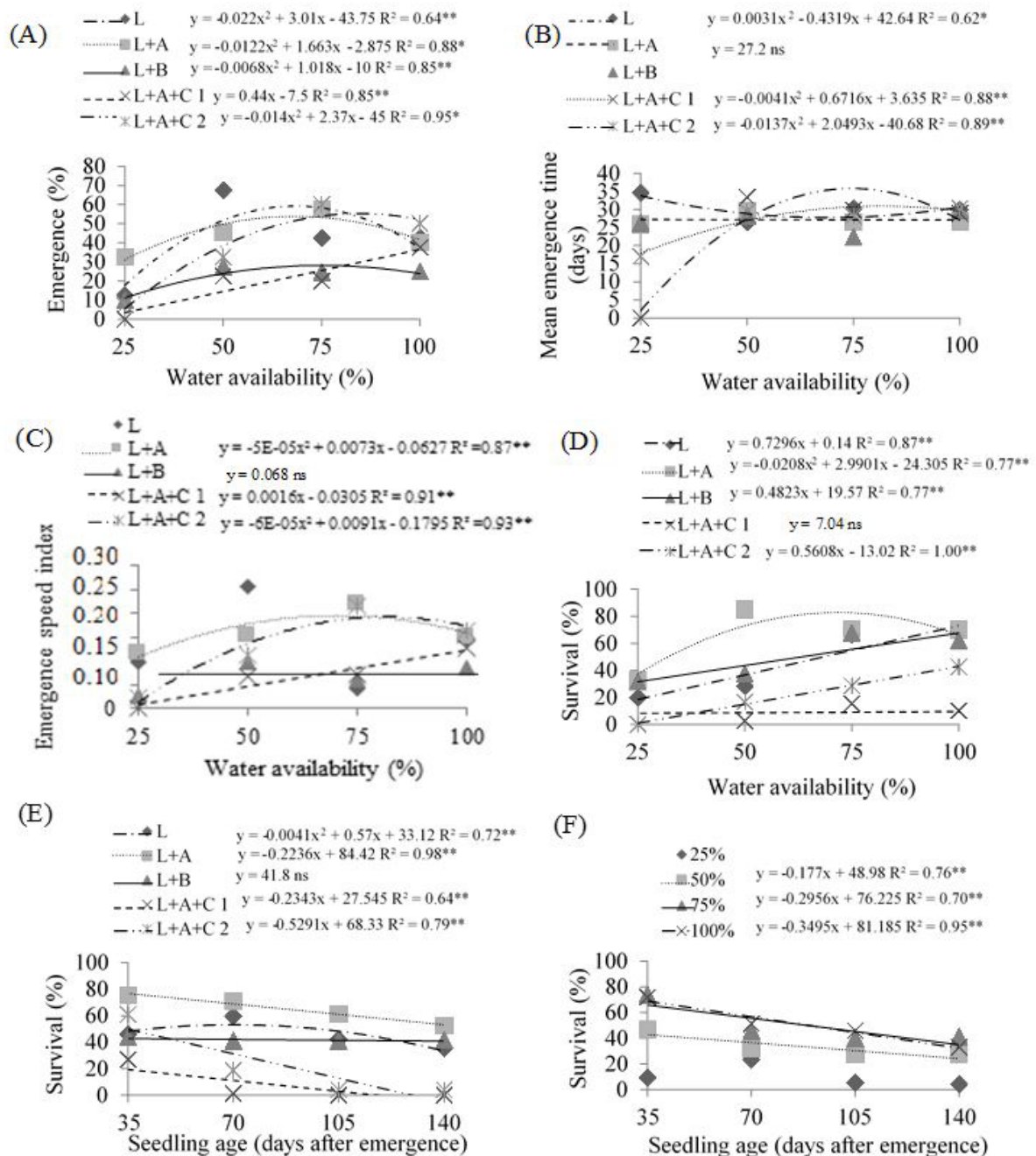


Figure 1. Emergence rate (A), mean emergence time (B), emergence speed index (C) and survival (D, E and F) of *Hancornia speciosa* seedlings exposed to different substrates and water availabilities. ns = not significant at 5% of probability, * significant at 5% of probability and **significant at 1% of probability.

Figura 1. Emergência (A), tempo médio de emergência (B), índice de velocidades de emergência (C) e sobrevivência (D, E e F) de mudas de *Hancornia speciosa* expostas a diferentes substratos e disponibilidades de água. ns = não significativo a de 5% de probabilidade, *significativo a 5% de probabilidade e **significativo a 1% de probabilidade.

L+A, L+B and L+A+C₁, the maximum height was obtained was 83.2, 128.1 and 94.7% of water retention, respectively. The substrate compound of poultry litter provided less seedling height when compared to the others along the evaluations. The greater heights were observed in the substrates L+A and L+B

(Figure 2A). There was no adjustment of the equation along the evaluations for L substrate, while the seedlings produced in the substrates L+A and L+B presented greater height as the increase of their age (Figure 2B). It was not observed significant interaction between water resources and evaluation

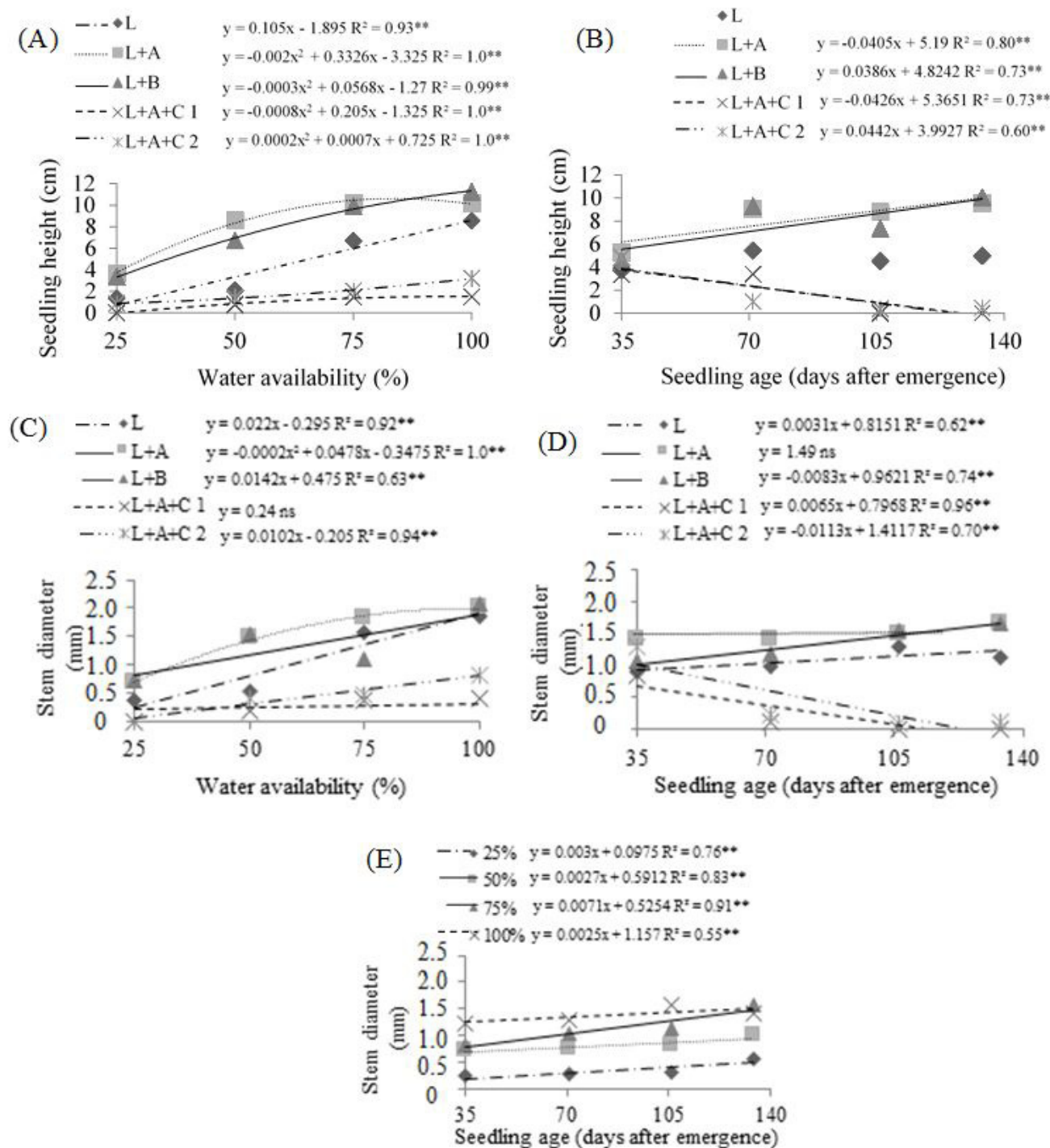


Figure 2. Seedling height (A and B) and stem diameter (C, D and E) of *Hancornia speciosa* seedlings exposed to different substrates, water availabilities and days after emergence. ns = not significant at 5% of probability and **significant at 1% of probability.

Figura 2. Altura (A e B) e diâmetro do colo (C, D e E) de mudas de *Hancornia speciosa* submetidas a diferentes substratos, disponibilidades de água e dias após a emergência. ns = não significativo a de 5% de probabilidade e **significativo a 1% de probabilidade.

times, only water effects regarding the height of seedlings, greater heights in 75 (5.61 cm) and 100% (6.94 cm) of water retention were observed.

As well as for the height of the seedlings, there was an increase in the stem diameter in greater wetting on the substrate, observing a linear fit in the equation for the substrates L, L+B and L+A+C₂ and quadratic for the substrate L+A, with greater results in water retention of 119.5%. For the substrate L+A+C₁, which showed an average diameter of 0.24 mm, there was no statistical difference among the water availabilities studied. Again it was observed poorer performance of substrates consisted of poultry litter and larger stem diameters in substrate L+A (Figure 2C). There were no changes in the stem diameter throughout the period of exposure to stress in the substrate L+A (1.49 mm), whereas the diameter increased at L+B and reduced in the substrates compounded by poultry litter. In the latter case, the drastic reduction verified occurred in low seedlings survival (Figure 2D). All water availabilities showed greater stem diameter along the evaluations, observing minors results in water retention of 25% (Figure 2E).

Although the addition of organic matter in the substrate for seedling production to improve chemistry and physics of the substrates is a trend, possibly the use of poultry litter undermined the development of *H. speciosa* seedlings. Rosa et al. (2005) also noticed that the addition of sugar cane bagasse in the substrate for seedling production of *H. speciosa* has not provided further development of seedlings. According to Vieira Neto (1998) these seedlings showed a better development in more acidic substrates, unlike the substrates containing manure, component that provided elevation of the pH, causing in some cases alkalinity.

According to Rosa et al. (2005) pH values ranging from 5.2 to 5.5 promoted better development of *H. speciosa* seedlings while values from 6.0 and 6.8 caused reduction in its growth. In this study, as can be seen in Table 1, the substrates compound by poultry litter showed in average a pH of 6.5, while the substrates L, L+A and L+B presented pH of 4.3, 4.4 and 5.2, respectively, suggesting that the pH is an important factor for the production of *H. speciosa* seedlings. In addition, the sand may have improved the physical conditions of the substrate when mixed to distroferic red latosol created porous spaces and increased the graininess in the substrates, regulating the fluid retention and drainage, favoring the growth of roots (Schmitz et al., 2002; Zietemann & Roberto, 2007).

Its low availability can therefore cause several morph physiological changes in plants and reduce the speed of physiological and biochemical processes reducing or stopping the growth of seedlings in these conditions. On the other hand, conditions of hypoxia or anoxia, caused by moisture excess, can affect the energy production to the roots sustain the physiological processes that depend on the shoot. Thus, the saturation of the soil also affects the growth of the aerial part either by inhibition of stretching or initiation of leaf expansion and internodes (Santos & Carlesso, 1998).

The growth in diameter depends on gas exchange activities, and thus it is a good indicator of liquid assimilation because it depends on the current photosynthesis (Larcher, 2004). This way, at lower water availabilities there is, less development

of aerial part and less assimilation of CO₂, where one can observe smaller stem diameter.

The *H. speciosa* seedlings also presented a higher number of leaves in accordance with the increased water availability in the substrates L, L+B and L+A+C₂. For the substrate L+A there was a higher emission of leaves in water retention of 80.9%, while in the substrate L+A+C₁ there was no influence of water availability, where the seedlings presented an average of 0.93 leaves. As well as to the height of seedlings and diameter of the collar, the substrate compounded by poultry litter provided seedlings with fewer leaves (Figure 3A), with a significant reduction in the number of leaves during the evaluations, observing null values in the substrate L+A+C₁ from 105 days after emergence because of high mortality of seedlings affected by fungal diseases.

At the end of the assessments (140 days after emergence), for the substrates L, L+A e L+B, it was found more leaves at 95, 84 and 106 days after emergence, respectively (Figure 3B). The interaction between water availability and evaluation times were not significant for the number of leaves of *H. speciosa* seedlings, noticing isolated effects of these factors, which water retention of 100% and assessment of 70 days after emergence presented higher values (Figure 3C). This behavior could be a reflection of lower rate of cell division, thus reducing the appearance of new leaves. Another explanation is related to the decrease on the number and rate of growth of branches of plants exposed to water stress and thus a limitation in the number of leaves.

Nascimento et al. (2011) also found higher number of leaves in water retention of 100% and reduction of emission of *Hymenaea stigonocarpa* leaves with water deficit, suggesting that

It was observed seedlings with smaller leaf area produced in the substrates L, L+A+C₁ and L+A+C₂ compared with the other substrates, observing leaf area up to water retention of 73.3, 118.8 and 75% respectively. The seedlings cultivated on the substrate L+A presented higher results in water availability of 91.3%, while in the substrate L+B the leaf area increased in addition of water availability (Figure 4A). The length of *H. speciosa* seedlings roots was higher in the substrate L+B, presented maximum point in 70.3% of water retention in the substrate, and the substrates L+A+C₁ and L+A+C₂ provided smaller lengths of roots. The substrates L+A and L+A+C₁ showed maximum roots length in water availability of 81 and 82.7%, respectively, with decreasing in higher water availability (Figure 4B).

In general, the substrates compounded by poultry litter provided seedlings with lower aerial biomass. The substrate L+B provided greater results of dry mass of shoot with maximum production at 116.5% of water availability. For the substrates L and L+A a higher dry mass of shoot of 100% water availability was observed (Figure 4C).

Also to the dry mass of roots, it was noted greater results in seedlings produced in substrate L+B, verifying dry mass increased with the water supply from the substrate. For the substrate L the higher production occurred in 83.8% of water availability. For the substrate L+A there was only an increasing of dry mass of roots in 38.3% of water availability. Lower results were obtained in seedlings grown on substrates L+A+C₁ and L+A+C₂, when compared to other substrates (Figure 4D).

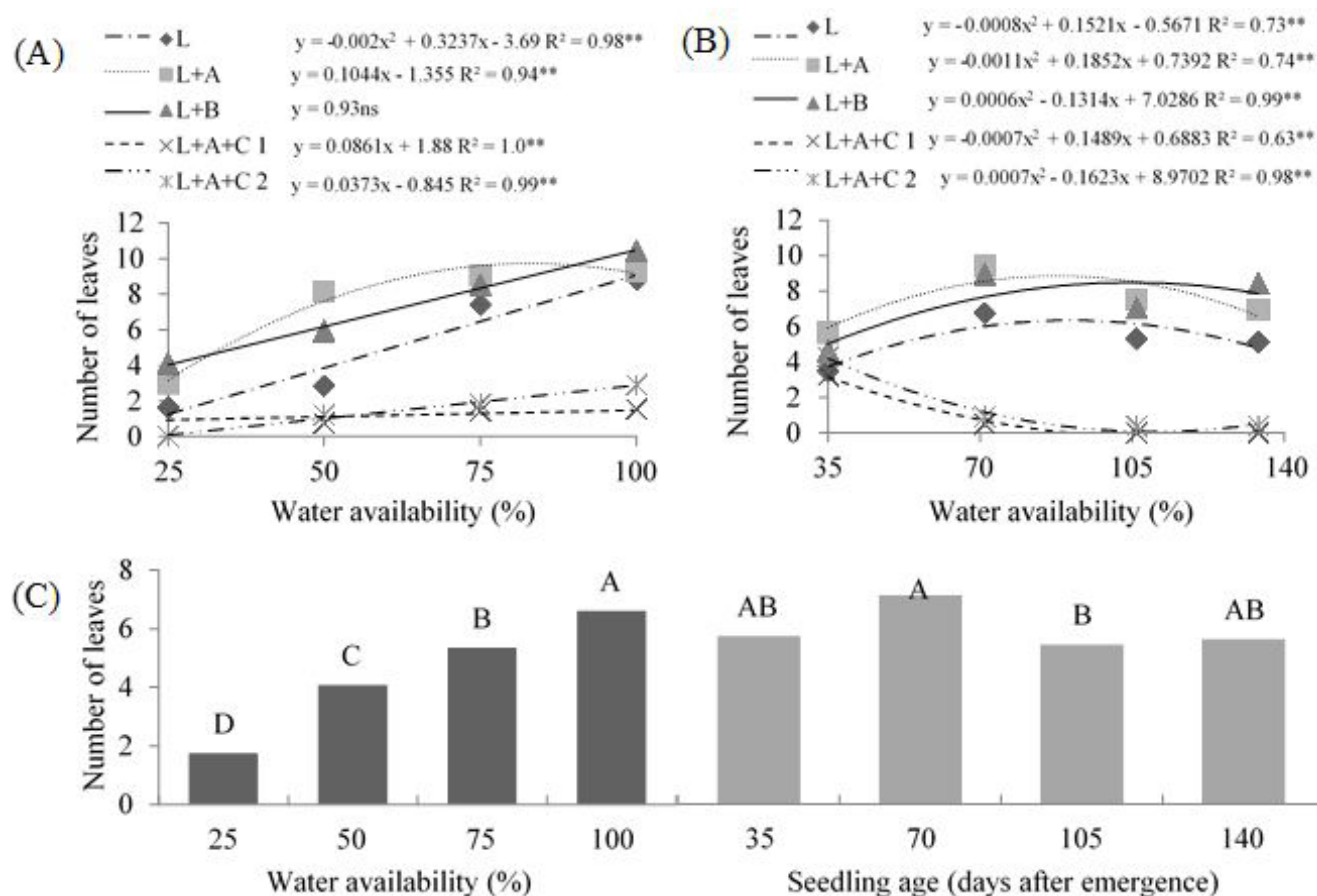


Figure 3. Number of leaves (A, B and C) of *Hancornia speciosa* seedlings exposed to different water availabilities and days after emergence. ns = not significant at 5% of probability and **significant at 1% of probability.

Figura 3. Número de folhas (A, B e C) de mudas de *Hancornia speciosa* submetidas a diferentes disponibilidades hídricas e dias após a emergência. ns = não significativo a de 5% de probabilidade e **significativo a 1% de probabilidade.

In the substrate L+B there was an increasing in the Dickson quality index from 72.5% of water availability, reaching maximum values of higher water availability (100%). In the substrate L+A+C₁ the Dickson quality index of seedlings was larger with the increased of water availability in the substrate, while the substrate L+A+C₂ there was no significant influence water availability for the quality index of seedlings. It was observed that the seedlings grown in these substrates showed lower Dickson quality index, which was greatest in seedlings grown in distroferic red latosol with or without sand (Figure 4E). The higher the value of this index, a better quality of the changes was produced, indicating the robustness and balance distribution of biomass in the seedlings (Fonseca et al., 2002). Thus, higher quality seedlings were produced in substrates L, L+A, and L+B, in water resistance of 66, 69 and 100%, respectively.

Leaf expansion is a process governed by cell turgor. Thus, under water stress, there is a reduction in expansion and size of the leaves, which would explain the results obtained in this study (Lima & Torres, 2009). Furthermore, the reduction in leaf area can lead to a decrease in photosynthesis and, consequently, the growth, becoming one of the most drastic effects of water stress (Cabral et al., 2004).

The elongation of roots is also a process dependent of turgor cell. The reduction of growth of shoots and leaf expansion inhibition reduces carbon and energy consumption and a higher proportion of assimilated plant can sustain the growth of later roots, leading to a preferential growth toward the same areas of soil that remain moist. Cabral et al. (2004) suggests that this behavior is of an adaptive character, common to plants subjected to water stress, being advantageous for allowing that the same obtained water even after the surface of the soil lost moisture after the dry season and frequently observed in the Cerrado. On the other hand, the excess water in the soil causes anaerobic respiration of roots, causing death of root cells by acidosis. With this, there was less growth and less absorption of water and nutrients (Floss, 2008).

Smaller lengths of plants and less accumulation of dry mass occur depending on the closing of stomata rich in stress conditions, reducing the production of biomass and productivity of the plants (Silva et al. 2008; Lima & Torres, 2009). Other factors such as the reduction of absorption of soil elements, changes in hormonal balance and plant respiration also contributed to the growth reduction of the plants (Larcher, 2004). And with all these there is a justification for the reductions of dried mass of shoots and roots as water stress becomes more severe (Martins et al., 2010).

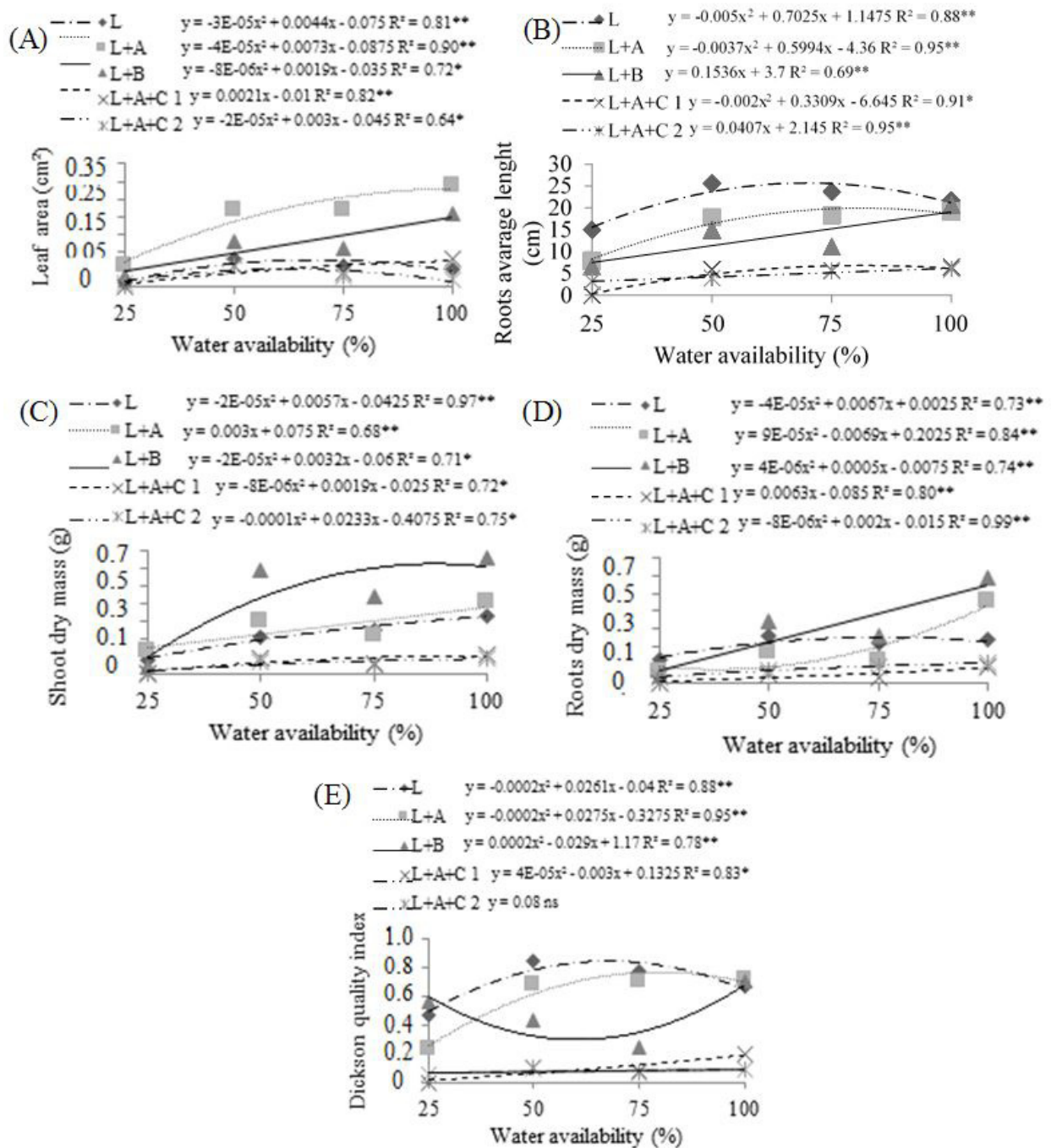


Figure 4. Leaf area (A), roots average length (B), shoot (C) and roots (D) dry mass and Dickson quality index (E) of *Hancornia speciosa* seedlings exposed to different substrates and water availability. ns = not significant at 5% of probability, * significant at 5% of probability and **significant at 1% of probability.

Figura 4. Área foliar (A), comprimento médio das raízes (B), massa seca da parte aérea (C) e das raízes (D) e índice de qualidade de Dickson (E) de mudas de *Hancornia speciosa* submetidas a diferentes substratos e disponibilidades hídricas. ns = não significativo a 5% de probabilidade, *significativo a 5% de probabilidade e **significativo a 1% de probabilidade.

4 Conclusion

According to these findings, it is concluded that distroferic red latosol, latosol+sand (1:1 ratio) and latosol+bioplant® (1:1 ratio) can be recommended for the emergence and initial

growth of *Hancornia speciosa* seedlings in water availability of 75 to 100%.

The substrates compounded by poultry litter and water availabilities of 25 to 50% undermined the growth and quality of *Hancornia speciosa* seedlings.

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