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MORPHOLOGY OF FRUITS AND SEEDS AND GERMINATE AND INITIAL DEVELOPMENT ANALYSIS OF *Hancornia speciosa*

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HIGHLIGHTS

Ihe morphological characters of the fruits of *Hancornia speciosa* Brazil's central Cerrado differ from those of other regions.

Germinability expresses the genetic potential of the species and tests in plant-nursery provide said to environmental conservation.

The sowing depth and substrate type test verifying the initial development is important to accelerate seedling production and provide strategies for there covery of altered and degraded areas.

Weibull's cumulative model was used to adjust seed germination over time and the use of its parameters (G, α, β) in the comparison of different treatments.

Keywords:
Mangaba
Germination parameters
Substrate

ABSTRACT

The aim of this study was to analyze the stress effects caused by sowing depth and the amounts and in the substrate; characterize the fruits and seeds of *Hancornia speciosa* (Mangaba); to analyze the germination and the initial development, 150 days after planting. A total of 572 fruits, collected in three localities in the Porto Nacional - TO region, were analyzed for longitudinal and transverse length, fresh mass, number of seeds and seed mass. The results showed values higher than those found in other studies, indicating that the fruits presented larger size and mass, although they presented a higher number of seeds per fruit. Germination potential was analyzed in the laboratory, and the effects of seeding depth and percentage of sand on the substrate, germinability and development were studied in the plant nursery. In the laboratory and plant nursery, respectively, the percentage of germination was 74.4% and 51.5%; mean germination time of 11 and 17 days and synchronization of germination of 2.79 and 2.75. The Weibull cumulative model to express germination behavior of H. speciosa had a good fit of the model to describe the observed data and its parameters (G, α, β) served to compare the effect of germination expression on time. Both germination and early development characters should be best when applied at 1 cm depth and add up to 15% sand to the substrate.

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INTRODUCTION

The mangabeira (*Hancornia speciosa* Gomes), a fruit tree in the family Apocynaceae (APGIV – CHASE et al., 2016) medium size, reaching 5-10 meters in height, native to Brazil is found in natural vegetation in several regions of the country, from the Coastal Plains and Lowland Coastal from the Northeast (Caatinga), where it is most abundant, to the Cerrado as it is known the savannah biome, that includes States of the regions of the Southeast, Center-West and North from the Northeast (Caatinga), where it is most abundant, to the Cerrado as it is known the savannah biome, that includes States of the regions of the Southeast, Center-West and North (Klink; Machado, 2005; Embrapa, 2007).

The native fruit species, such as those of the Cerrado, are generally not cultivated in commercial orchards, being exploited by the local population in an extractive way (FERREIRA, 1999). In Tocantins, a state in the North region, through the initiative of small farmers of the Goianorte county, reated the Instituto Mangaba (Institutional page in social network - https://pt-br.facebook.com/Instituto-Mangaba-de-Goianorte-Tocantins-1453708644882051/) whose main objective is to include the cultivation of *H. speciosa* in the list of species exploited by the family farmer, which is already happening in the region of Balças (MA) where *H. speciosa* is already included in the list of species indicated for the economic exploitation of reserves of rural properties in the Cerrado (Aquino et al., 2007).

H. speciosa is consumed in natura or in the form of sorbets, jams, jellies, liqueurs, soft drinks, wine and vinegar (Lorenzi, 2009) in addition to their medical uses indicated to regulate the blood pressure as it relaxes blood vessels and reduces the pressure; acts as an antioxidant, being rich in vitamin A and C; prevent anemia, it contains good amounts of iron and B vitamins (Lorenzi, Matos, 2002). The widespread occurrence of this species in natural patches facilitates extractive and conservation actions (Lorenzi, 2009).

The exploitation of a native species, a process known as domestication (Homma, 2012), depends on the technical knowledge regarding propagation (germination and planting) and physiological development in relation to environmental variations, which are scarce, especially those related to parameters physiological responses to adverse conditions (Nogueira et al., 2003). *H. speciosa*, as well as other fruits of the Cerrado, presents commercial demand far above the supply capacity by means of extractive (Moura et al., 2003) and this has stimulated a more technified production, encouraging research that seeks new knowledge about this species.

The majority of plant species present sexual reproduction as the main form of propagation, as well as *H. speciosa* (Barros et al., 2006). One of the ways to know the physiological quality of the seeds is the use of germination and vigor tests, and each species requires certain conditions to express its maximum genetic potential (Homma, 2012). When done in the laboratory, under controlled conditions the seeds express the maximum germinative power (Pinã-Rodrigues et al., 2004), but for the seedling emergence test, not only depends on the characteristics of the seed but on the substrate conditions, such as its physical characteristics, structure, aeration, water retention capacity, degree of pathogen infestation (Albuquerque et al. 1998), and of the depth of sowing, being of fundamental importance to know its potential closer to the real as well as the stressful limits.

In the process of seedling production, the substrate directly interferes with the quality of the plants, due to the variation of the physical, chemical and biological properties associated with the material used in its composition (Vieira Neto et al., 2002), affecting the germination and the establishment of the seedling at this stage. According to Vieira Neto et al. (2002) *H. speciosa* is found predominantly vegetation in soils poor in organic matter, acids and with low levels of nutrients, characteristics found in soils of the Cerrado.

Santana and Ranal (2004) draws attention to a very common situation in the literature, where different mathematical expressions, with different names that generate equal amounts of mathematical and philosophical point of view; however, the same names are also found for different expressions that generate different information. There are several formulas and values to express the germinative capacity of a species and the results of a germination analysis can be expressed by probability distributions (Carneiro et al., 2000). After the 1990s, the non-linear models to describe the germination occurrences in the time were well explored (Carneiro, et al., 1999), since the accumulated germinative frequencies follow a distribution and the properties of this distribution can become very informative (Silva, 1982).

The aim of this work was to characterize the fruits and seeds of *H. speciosa*, collected in remnant areas of Cerrado, in the region of Porto Nacional, State of Tocantins, evaluating its germinability in laboratory and plant nursery, through final germination parameters and the dynamic aspects of the occurrences germination systems analyzed by the Weibull distribution model, as well as to know the effect of the stresses on plant nursery germination and on the initial development of the plant, caused by the depth of sowing and the increase of the percentage of sand in the substrate.

MATERIAL AND METHODS

Study area

The municipality of Porto Nacional (latitude 10°41'15.94"S, longitude 48°22'55.60"W and 269 m altitude) is located in the central portion of the State of Tocantins, has a tropical climate, being the summer with much more rainfall than the winter. The climate classification is Aw according to Köppen and Geiger, with the average temperature of 26.1°C and the annual average rainfall of 1,622 mm (Climate-Data ORG, 2015).

The seeds used in the experiment were composed of the same proportion of fruits from three localities in the municipality of Porto Nacional: Canaã Farm (10°40'23.1"S and 48°20'54.3"W), located on the right of TO 255, 10 km from the municipality, towards Monte do Carmo; Providência Farm (10°33'31.2"S and 48°24'43.8"W), on the left of TO 070, 18 km from the municipality, towards Palmas; and São Judas Tadeu Farm (10°48'04.80"S and 48°25'38.64"W) on the left of TO 070, 24 km from the municipality towards Brejinho de Nazaré (Figure 1). The three populations are about 20 km apart. 30 matrices were marked, two of which were destroyed by fire. The twenty-eight matrices were georeferenced and detailed in the supplementary material. Matrices are adult and healthy plants, and the fruits were harvested directly from the tree when naturally ripe (second half of October).

The experiments were evaluated in the laboratory and greenhouse, from October 24th, 2014 to March 23rd, 2015, at the Núcleo de Estudos Ambientais of the Universidade Federal do Tocantins (Neamb/UFT), Porto Nacional, State of Tocantins. In the laboratory, biometric measurements were taken and germinability tests were carried out, while in the greenhouse (covered with shade cloth with a reduction of 50% solar luminosity), tests were made for effect of substrates, depth of sowing and initial development.

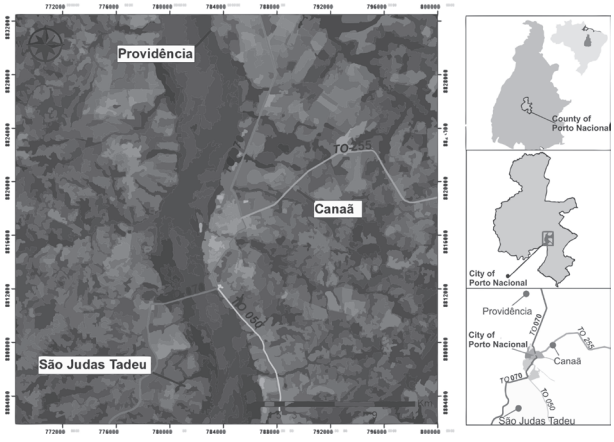

FIGURE 1 Location of Canaã, Providência and São Judas Tadeu Farms, in the county of Porto Nacional, State of Tocantins.

Measures of Fruits and Seeds

In each locality, fruits were collected at the maximum of their development and individually packed in 200mL disposable cups, duly numbered by matrix and taken to the UFT laboratory in Porto Nacional. They were distributed on a bench in a refrigerated room at 25°C until completely ripe for extraction of the seeds.

The longitudinal and transverse diameter (mm) and the weight of the fruit (g) were first measured. Seeds were manually extracted from the fruits, the pulp residues were removed, the number of seeds per fruit were counted and weighed. An analytical balance (Model BL 3200H) was used to weigh the fruits and seeds and a digital caliper (Starrett 799) was used to determine the diameters. The fruit and seed biometry data were analyzed by means of descriptive statistics, evaluation of the frequency distribution and correlated by Pearson analysis (Zar, 2010)

Germination under Controlled Conditions

Seeds of 28 *H. speciosa* tree genotypes, manually extracted from healthy fruits, were disinfected by immersion in 2% sodium hypochlorite for 10 minutes, then washed twice in deionized and autoclaved water, at the same time, to remove excess sodium hypochlorite and mucilage, decreasing the inhibitory substances in the pulp that could negatively influence seed germination (Passos; Passos, 2004). The procedure was performed in a VECO laminar flow module (model HLFS-12) and, after drying the seed on the bench, on paper towels, the seed was immediately sown, because it is a recalcitrant seed with low viability of conservation.

Seeds (n=540) were collected and stored in Petri dishes containing a double layer of filter paper, previously autoclaved, moistened with 10 mL deionized and autoclaved water, subjected to light exposure, with a 12-hour photoperiod. The germination test was performed in germinators (B.O.D. camera) and the temperature was set at 25°C (± 2°C). The counts were performed daily for 20 days, registering the number of germinated seeds (the one with root length equal to or greater than 2mm), and removed from the test every day - Protocol adapted by the Laboratory of Micropropagation - Propagation

of Cerrado Plants (Neamb/UFT), based on the rules of seed analysis - RAS (Brasil, 2009).

We calculated the germinability (G), which represents the number of seeds in which the germination process took place, under the experimental conditions (Labourian, 1983; Santana; Ranal, 2004), the mean germination time (t) represented by the weighted average of the germination times, using as weights the number of seeds germinated in the established time intervals (Laborourian, 1983; Santana; Ranal, 2004), the germination synchronization index (I) that quantifies the germination variation along time, and the closer to zero the more synchronized germination will be, regardless of the total germinated seeds (Santana; Ranal, 2004). We also constructed a cumulative germination frequency curve that expresses the germination behavior over time. For better distribution of the seeds in the Petri dishes, the repetitions with 45 seeds were divided into three dishes with 15 seeds.

With the aid of PAST 2.17c (Hammer et al., 2001) the germination data in the laboratory were analyzed by means of descriptive statistics and adjustment of statistical distributions of Weibull, of three parameters, being: (G) the maximum germination observed; (α) the time (t_i) necessary to observe 63.21% of this maximum and (β) the dispersion of the data in time (Carneiro et al., 1999), where the value Y_i represents the germination accumulated at time t.

$$Y_i = G \times \left(1 - e^{-\left(t_i/\alpha\right)^{\beta}}\right) \quad [1]$$

Germination of Seeds in the Soil

The experiment was set up in a completely randomized model with three replicates and 24 treatments, combining four sowing depths (1, 2, 3 and 4 cm) and six percentages (0, 15, 30, 45, 60 and 75%) of washed sand (WS) added to the cerrado soil (CS). Initially, nine seeds were planted per pot to estimate the germinability (G) parameters in the soil, the mean germination time (T) and the synchronization index (I). The same experiment was used to analyze the development of the plant after 150 days, leaving 3 seedlings per pot.

The Cerrado soil used in the experiment was collected near the greenhouse, using subsoil material, in order to reduce the incidence of weeds. Physical-chemical analysis resulted in the following chemical characteristics: pH in $CaCl_2$ = 4.80; P = 0.8 mg·dm^{-3}; K = 27.4 mg·dm^{-3}; Ca^{+2}=0.22 cmol$_c$·dm^{-3}; Mg^{+2} = 0.17 cmol$_c$·dm^{-3}; H+Al = 3.71 cmol$_c$·dm^{-3}; OM = 0.4%; Zn = 0.1 mg·dm^{-3}; Cu = 0.5 mg·dm^{-3}; Fe = 4.0 mg·dm^{-3}; Mn = 1.0 mg·dm^{-3}; and physical characteristics: Clay = 23.0%; Silt = 28.0% and Sand = 49%, while the washed sand (AL) was acquired in a building material store presented the physical characteristics: Clay = 10.0%; Silt = 6.0% and Sand = 84%.

No soil acidity correction was performed, since the mangaba tree seedlings present better development in acidic soils, whereas in more neutral soils, the growth is lower (Rosa et al., 2005). In the substrate composition, 20g 4-14-8 NPK formulation was added per pot, avoiding possible losses in the development of plants in the growth analysis experiment, especially in the treatments of higher stress (high depth and high percentage of sand in the substrate). The disinfected seeds, still wet, were placed on paper towel for 24 hours, in the shade, to lose surface moisture. According to Rosa et al. (2005), they must be sown still wet, because in case they dry, the seeds of mangaba loses viability and do not germinate, typical of recalcitrant species. We considered as germinated the seedlings that emerged the eophiles or the cotyledons.

The experiment was conducted in the greenhouse covered with black shade cloth (with 50% reduction of light) located at the Núcleo de Estudos Ambientais of the Universidade Federal do Tocantins (Neamb/UFT), in Porto Nacional, State of Tocantins, beginning in October 2014.

Readings were performed daily up to 50 days after sowing, counting the number of seedlings emerged from the substrate. Periodic irrigation and manual weeding were carried out whenever necessary. Soil germination analysis data were presented by means of descriptive statistics and adjusted according to the Weibull model, per treatment, analyzing the effect of sowing depth and substrate type, separately.

Analysis of Seedling Growth – after 150 days

From the previous experiment, where three seedlings were left per pot, with nine initial replicates (nine pots), analyzed in a 6x4 factorial arrangement (percentage of washed sand added to the substrate x depth of sowing), pots with no plants at the end of the 150 days of experiment were eliminated, reducing the number of replicates of that treatment, that is the average of each treatment was 5 - 9 replicates. Every 50 days, topdressing fertilization was done with 50mL liquid 8-6-6 NPK formulation was diluted in the ratio of 1:200 (5 ml.l^{-1} of water) according to the manufacturer's instructions. At 150 days after planting, we evaluated the height of the plant in centimeter (HEIG) using a ruler,

from the lap of the plant to the apical bud; the collar diameter, in millimeters (DIAM), using a digital caliper and measured at ground level; number of leaves, in units (NL); and the ratios, height/diameter ($cm.mm^{-1}$) and leaf number/height ($unit \cdot cm^{-1}$).

The data were analyzed by analysis of variance (ANOVA) and presented on response surface, considering the axes: sowing depth (x), percentage of increased sand (y) and analyzed variable (z). To obtain the parametric assumptions, the homogeneity was tested by Levene and normality by Shapiro-Wilk, being transformed according to Box-Cox.

RESULTS AND DISCUSSION

Measures of Fruits and Seeds

Fruit characterization is important for knowing the variability presented in the analyzed populations. For the fresh fruit market, the heaviest fruits, and consequently larger, are more attractive to consumers, according to Chitarra and Chitarra (2005), in addition to having fewer seeds.

One way to determine fruit size is through its longitudinal (DL) and transverse (DT) diameters. In this study, mangaba fruit presented mean ± standard deviation of 39.67 ± 5.86 and 38.12 ± 5.57 mm, respectively, and an approximately normal distribution (Table 1 and Figure 2A and 2B). The mean values for these variables were higher than those found by Nascimento et al. (2014) in mangaba in the State of Bahia (32.34 and 31.87 mm); Ganga *et al*. (2010) in natural populations of the Cerrado of Mato Grosso, Mato Grosso do Sul, Goiás, Tocantins and Bahia (37.30 and 34.00 mm); Souza et al. (2007) in fruits of different mangaba tree clones of the Active Germplasm Bank in João Pessoa, State of Paraíba (38.27 and 34.66 mm); Guilherme *et al*. (2007) in fruit from northern Minas Gerais (37.59 ± 10.66 mm and 34.53 ± 8.83); Soares et al. (2008), 32 ± 9 mm and 28 ± 8 mm; Campos et al. (2011), studying storage of mangabas, found values of 34.29 ± 2.07 mm and 35.22 ± 1.44 mm, being this low variation due to the selection of the fruits for the storage experiment. The values observed in this survey were lower than that found in the eastern region of Mato Grosso by Gonçalves et al. (2013) with DL and DT of 44.57 ± 5.60 mm and 41.59 ± 5.25 mm, respectively.

Mangaba's fruit is berry fruit, of varied size, shape and colors, usually ellipsoidal or rounded, yellowish or greenish with red pigmentation or without pigmentation (PEREIRA et al., 2010), the genotypes analyzed in this work presented characteristics similar to those described. Of the 572 fruits analyzed, it was observed that the DL/DT ratio presented the lowest variation among the characters analyzed (10.46%) with mean ± standard deviation of 1.05 ± 0.11, predominantly round fruits (Table 1 and Figure 2D). 73.8% fruits showed DL/DT ratio, between 0.9 and 1.1, suggesting a rounded shape; 1.9% of flat fruits; and 24.3% of elongated fruits.

The data showed that fruit weight varied from 10.36 to 99.85 g, with a positive asymmetric distribution and positive kurtosis, indicating a concentration of fruit weight in the lowest values, with mean and standard deviation of 31.94 ± 13.84 g (Table 1 and Figure 2C), higher than the mean values found by Nascimento et al.

TABLE 1 Minimum values, mean and maximum values of the longitudinal diameter (DL), transverse diameter (DT), ratio between diameters (DL/DT), fruit weight (FW), number of seeds per fruit (NS), total seed weight (TSW) and seed weight (SW); n: sample size, coefficients of variation, asymmetry and kurtosis.

Biometric character	n	Minimum	Mean ± stantard deviation	Maximum	Coefficient of variation (%)	Asymmetry	Kurtosis
			Fruit				
DL (mm)	572	24.90	39.67±5.86	58.12	14.78	0.4858	0.0763
DT (mm)	572	26.12	38.12±5.57	57.10	14.61	0.4313	0.2333
DL/DT	572	0.80	1.05±0.11	1.53	10.46	1.3610	2.3265
FW (g)	572	10.36	31.94±13.84	99.85	43.33	1.2913	2.5022
			Seeds				
NS (unit)	529	3	17.88±10.19	57	57.01	1.0869	1.0475
TSW (g)	529	0.92	6.96±3.95	22.87	56.69	1.3535	2.2712
SW (g)	529	0.11	0.41±0.11	1.19	26.08	0.8361	5.0074

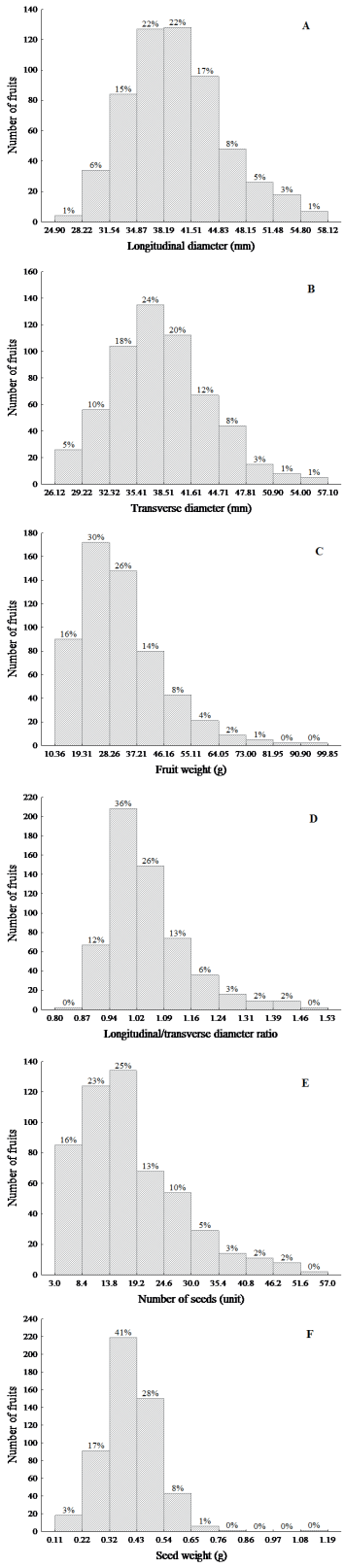

FIGURE 2 Frequency distribution of the variables: longitudinal diameter – mm (A); transverse diameter – mm (B): fruit weight– g (C); longitudinal/transverse diameter ratio (D); number of seeds per fruit (E) and seed mass – g (F).

(2014), Ganga et al. (2010) and Souza et al. (2007), which were 17.17; 27.88 and 25.74 g, respectively, and lower than that found by Gonçalves et al. (2013), which was 46.49 ± 17.47g. Silva et al. (2001) presents a variation of 30 - 260g for the weight of mangaba found in the cerrado, which agrees with the values found herein. Possibly, the genetic variability associated to environmental variation contributed to the great variation of fruit weight, as reported by Vieira and Gusmão (2008) and Santos et al. (2009).

The number of seeds per fruit ranged from a minimum of three to a maximum of 57 units, with mean ± standard deviation of 17.88±10.19 (Table 1 and Figure 2E). This was higher than that found by Nascimento et al. (2014), which was from 1 to 29; than the mean reported by Souza et al. (2007) and Ganga et al. (2010) of 14.80 and 13.40 seeds, respectively and lower than the mean of Gonçalves et al. (2013) which was 22.00±9.10.

The mean seed mass per fruit was 6.96±3.95g, with asymmetric distribution and kurtosis greater than zero (leptokurtic) (Table 1, Figure 2F) resulting in a mean unit mass of seed of 0.41±0.11 g. The seed mass per fruit of the populations studied in this study had a higher value than that observed by Nascimento et al. (2014), Ganga et al. (2010) and Gonçalves et al. (2013), respectively, 2.40; 3.88 and 6.33g. The presence of a higher number of seeds per fruit is an undesirable character for the consumption of fresh fruit.

In general, the several characteristics of economic importance are correlated with each other, in varied magnitude and direction (CRUZ et al., 2012), and the variability is indispensable in a future breeding program, since it conditions gains with the selection of superior genotypes. Analyzing the correlations between the different characters evaluated, we observed: DL x FW ($r = 0.8722$, $p < 0.0001$, $n = 572$); DT x FW ($r = 0.9660$, $p < 0.0001$, $n = 572$); (FW x NS, $r = 0.5509$, $p < 0.0001$, $n = 529$) and (FW x TSW, $r = 0.7162$, $p < 0.0001$, $n = 529$), indicating that larger fruits are heavier, have more seeds and consequently a higher total weight of the seeds/fruit. Also, to a lesser extent, the increase in the number of seeds in the fruit results in seeds with lower weight ($r = -0.3260$; $p < 0.0001$, $n = 529$), that is, small seeds.

Germination in the Laboratory

Seeds began to germinate after six to 20 days from the start of the experiment. Of the 540 seeds allowed to germinate, 402 germinated. Table 2 lists the minimum, maximum, mean, standard deviation, coefficient of variation (CV), asymmetry and kurtosis for the 12 replicates with 45 seeds each. The germinability of mangaba seeds ranged from 60.0 to 91.1% (mean

± standard deviation, 74.4 ± 12.0%), with a mean germination time between 8.0 and 13.9 days (10.6 ± 2.0 days) and the germination synchronization index between 2.03 and 3.23 (2.79 ± 0.47), with values closer to zero indicating greater synchronization.

Germination experiments conducted in the laboratory presented relatively low value coefficients of variation, due to the greater local control, which was not observed in this experiment. The variabilities found in the analyzed characters: germinability (G), mean germination time (t) and synchronization index (I) showed coefficients of variation between 15 and 20% (Table 2). This variation is due to the genetic differences between matrices in the populations, corroborated by the ripening degree of the fruits at harvest. Ripe fruits produce a shorter mean time to germinate and better germination synchronization. Similar results were observed in the studies of Parente and Machado (1986) and Passos and Passos (2004).

Cumulative germination frequencies, evaluated at one-day intervals, were combined with the Weibull cumulative distribution, where the germination percentage of mangaba (G) was 74.44%, requiring 11.3576 days (α) to reach 63.21% of this value, with a time dispersion over time (β) of 3.6699. The Weibull model fitted well to describe the total cumulative frequency of germinated seeds in the twenty days of the experiment, with a coefficient of determination (R^2) of 0.9329 (Figure 3).

The period around the mean germination time (10.6 ± 2.0 days) is the section with the highest germination rate. Before eight days and after 13 days, the number of seeds that germinated is low (Figure 3).

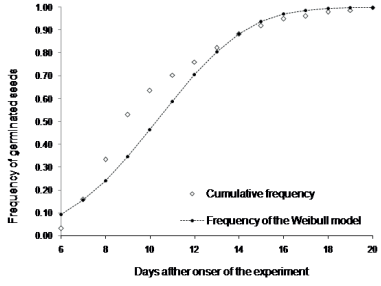

FIGURE 3 Germination probabilities of mangaba seeds, in germinators.

Germination in the soil – Emergency of seedling

An essential step for the production of seedlings is the selection of the appropriate substrate. Other factors, such as water availability and light intensity are also usually associated with germination and growth responses of seedlings (Gordin 2011).

Table 3 presents the germination start and end values (in days), the germinability at each depth and substrate, the mean germination time and the germination synchronization index.

As for the seedling emergence for 50 days after planting (DAP), germination began after 21 days and ended at 42 days, agreeing with Vieira Neto (2001), who indicates the emergence of plants at 21 days after planting. the sowing, extending for another 30 days; and if using fruit with uniform degree of ripening, the germination and the development of the seedlings will be more uniform. Fonseca et al. (1994) stated that germination begins in the third week and stabilizes in the sixth week after sowing.

The mean germination of the experiment was 51.85%±5.33% (mean±standard deviation), with a mean germination time of 26.92±1.2 days and a germination synchronization index of 2.76±0.06, indicating low synchronization. The mean germinability in the field test compared to the laboratory tests, performed in Petri dish, was 45.6% lower, with a longer germination time (60.6%) and lower synchrony (26.9%), which was expected since in the laboratory the environmental conditions are more controlled.

The same table presents the parameters of the Weibull model (G, α and β) and the coefficient of determination. The mean germination percentage (G) of mangaba in the greenhouse at the four depths was 51.85%, requiring 29.1334 days (α) to reach 63.21% of this value, with a dispersion of data in time (β) of 6.3796 (Table 3) and coefficient of determination (R^2) of 0.9131 (Figure 4A).

The ideal sowing depth is the one that guarantees more homogenous germination, in shorter emergence time and more vigorous seedlings. In the experiment, the values α and β increased as sowing depth increased

TABLE 2 Minimum, mean ± standard deviation and maximum values of germinability (G) mean germination time (t) and germination synchronization index (I); number of repetitions, coefficients of variation (CV), asymmetry and kurtosis.

Biometric character	n	Minimum	Mean ± stantard deviation	Maximum	Coefficient of variation (%)	Asymmetry	Kurtosis
G(%)	12	60.00	74.44±12.00	91.11	16.13	0.1285	-1.6257
t (dias)	12	8.05	10.61±2.00	13.86	18.89	0.1552	-1.4692
I	12	2.03	2.79±0.47	3.23	16.90	-0.6371	-1.4681

TABLE 3 Mean values of germination start and end, Weibull model parameters, coefficient of determination (R^2), maximum germinability (G), mean germination time (mean t), germination synchronization index (I), for depths of 1 to 4 cm and different combinations of Cerrado soil (CS), percentage of washed sand added (% AL) and standard deviation (SD).

Character	Treatment	Start	End	G	α	β	R^2	t	I
Sowing depth (cm)	1	21	41	54.3210	27.7088	5.3555	0.8223	25.6037	2.7572
	2	21	41	44.4444	29.4284	4.9816	0.9124	26.6207	2.6787
	3	21	42	56.7901	31.1433	10.7424	0.5016	27.0945	2.8106
	4	21	42	51.8519	31.7914	16.4868	0.6132	28.3742	2.7855
	Mean	21.5	41.5	51.8519	29.1334	6.3796	0.9131	26.9233	2.7580
	SD	0.6	0.6	5.3340				1.1500	0.0572
Combination of substrates	CS + 00%	21	33	52.7778	26.5005	7.1469	0.8876	24.9135	2.5476
	CS + 15%	21	34	57.4074	26.1481	6.6556	0.8911	24.8587	2.6168
	CS + 30%	21	41	49.0741	29.9460	7.1610	0.8985	27.5372	2.6117
	CS + 45%	21	38	58.3333	29.7861	8.4706	0.9085	28.6717	2.8207
	CS + 60%	21	42	51.8519	31.0073	6.8766	0.9187	28.6717	2.9910
	CS + 75%	21	41	41.6667	29.9703	6.7971	0.9607	27.9682	2.9602
	Mean	21.0	38.2	51.8519	29.1755	6.2699	0.9108	27.1035	2.7580
	SD	0.0	3.9	6.0858				1.7714	0.1922

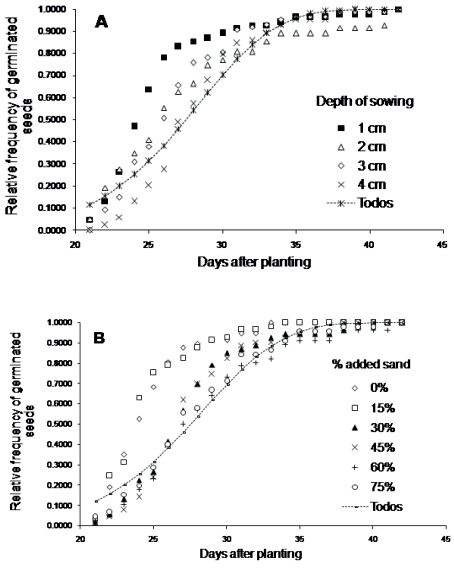

FIGURE 4 Germination probabilities of mangaba seeds per depths of sowing (A) and percentage of sand added to the substrate (B), in greenhouse.

(Table 4), indicating that although the final germination was practically the same, the more superficial planting (1 or 2 cm) produced a higher percentage of germination in a shorter time and lower data dispersion (Figure 4A), presenting more adjusted models, with coefficient of determination of 0.8223 and 0.9124, respectively. These results corroborate the values of mean germination time and synchronization index. Vieira Neto (2001) attributed the depth of 1 cm as the ideal for sowing mangaba.

The ideal porosity of the substrate is the one that allows the movement of water and air, favoring the germination. For this to occur, seeds do not require nutrients, but only hydration and aeration, so that the reactions that induce the formation of the hypocotyls and the radicle occur (Villagomez et al., 1979). In the experiment, this factor was not limiting, since periodic irrigation was carried out, always maintaining water availability and the substrate provided adequate aeration. Seeds cannot be moistened in excess to prevent the water film from completely enveloping the seed, restricting the entry and absorption of oxygen (Villagomez et al., 1979).

The mean germination curve expressing the germination in the different substrates presented a germination mean (G) of 51.85%, requiring 29.1755 days (α) to reach 63.21% of this value, with a dispersion of data in time (β) of 6.2269 and coefficient of determination (R^2) of 0.9109 (Figure 4B).

When analyzing the germinability of seeds in the substrate treatments, it was observed that the treatment (CS + 45%WS) produced the largest number of seedlings, that is, 58.3% of the seeds germinated. Analyzing the individual curves for each treatment of substrate types, it can be concluded that those which received less sand in the composition (CS + 0%WS) and (CS + 15%WS) reached the comparison value (P50) in a shorter time, indicating that the cerrado soil used in the experiment is a good substrate for the production of mangaba seedlings (Table 4), as it had 49% sand in its original physical composition. Nogueira et al. (2003) obtained a germinability of 68% in the sand, probably because it has necessary characteristics of a good substrate for germination, such as porosity and sterility, but did not differ from the natural soil substrate. Parenteand Machado (1986) investigated the germination of mangaba seeds from fruits harvested at different ripening degrees and observed that the percentage of germination for natural soil and sand substrates were similar.

Growth analysis – 150 days

The growth analysis allows evaluating the final growth of the plant as a whole and the contribution of the

different organs for the total growth. From the data, it is possible to infer physiological activity, that is, to estimate, quite accurately, the causes of variations of growth between genetically different plants or between plants growing in different environments (Peixoto et al., 2011).

The results of the analysis of variance evidenced no significant effect for the interaction between the sowing depth and the amount of sand added to the substrate. Thus, the effects were interpreted separately. In the 150 days after planting, there was no significant difference ($p > 0.05$) for both sowing depth and amount of sand in the substrate, for the number of plants (NP), indicating that all treatments did not differ as to the number of plants analyzed; ratio of height to collar diameter (HEIG/DIAM) and leaf number to plant height ratio (NL/HEIG), indicating a proportional growth among characters (Table 4).

For plant height (HEIG), collar diameter a mean height of 6.35 cm, a value proportionally lower than those found in this experiment.

It can be observed that the variables HEIG(t), DIAM(t) and NL(t) presented a decreasing response surface (Figure 4) with increasing sand percentage in the substrate and sowing depth. The models determined for each variable, presented at the bottom of each Figure (Figure 5A, 5B and 5C), presented a highly significant ($p < 0.01$) coefficient of determination (R^2), with the following magnitudes: 0.8133, 0.8022 and 0.4284. Although significant the R^2 for the NL(t) was of smaller value, this should the influence of the variable be of the discrete type. The models indicate that the increase in the percentage of sand added to the substrate and the increase in sowing depth significantly reduced plant height, diameter and leaf number at 150 days of experiment.

TABLE 4 Summary of analysis of variance showing the degrees of freedom, F-test and respective probability, for the characters: number of plants per pot (NP); plant height (HEIG); collar diameter (DIAM); number of leaves (NL) and the ratios, height/diameter (HEIG/DIAM) and leaf number/height (NL/HEIG), 150 days after planting. (t) in front of the variable means that the data used in the analysis were Box-Cox transformed. Bold values represent the significant effects ($p < 0.05$).

Variables		Source of variation		
		Depth (D)	Substrate (S)	Interation D x S
Degrees of freedom		3	5	15
NP	F	1.2941	1.5190	0.7482
	p-value	0.2784	0.1868	0.7323
HEIG (t)	F	3.4933	2.2980	0.1680
	p-value	0.0171	0.0477	0.9998
DIAM (t)	F	9.8279	4.7248	0.5420
	p-value	0.0000	0.0005	0.2750
NL (t)	F	4.4125	3.1864	1.2026
	p-value	0.0052	0.0091	0.2750
HEIG/DIAM (t)	F	2.5569	0.8663	0.5349
	p-value	0.0572	0.5052	0.9178
NL /HEIG (t)	F	0.2094	0.4048	0.6286
	p-value	0.8898	0.8449	0.8482

Dias et al. 2009 reported a lower development in height in mangaba seedlings with increasing proportion of coconut fiber in the substrate, once the coconut fiber, due to low fertility, in high proportions in the substrate, affected the growth of the seedlings. In the present study, sand may have functioned as a material of low fertility, hardly retains nutrients and does not retain irrigation water.

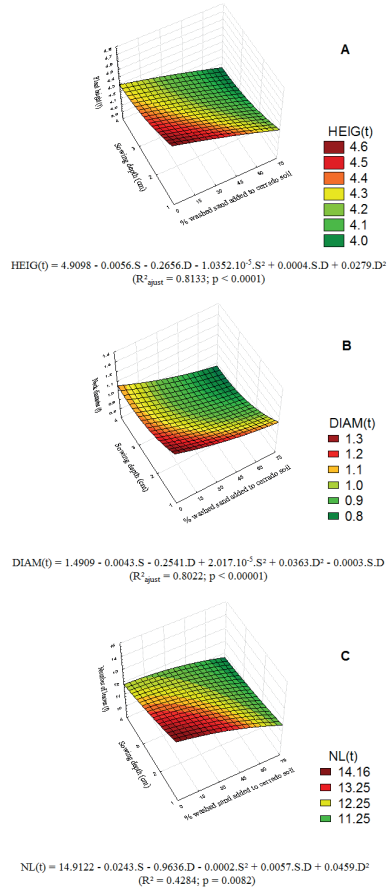

FIGURE 5 Response surface for plant height (A), neck diameter (B) and number of leaves (C) as a function of substrate (S) - % washed sand plus cerrado soil and seeding depth (P) – cm, after of 150 days of planting. Below each graph are presented the polynomial equations and their coefficient of determination.The symbol (t) in front of the variable indicates that the data used in the analysis were transformed by Box-Cox.

CONCLUSION

Mangaba seeds are influenced by sowing depth and substrate type, and should be planted at 1 cm depth in cerrado soil with up to 15% washed sand.

The use of cumulative germination frequency curve is a better alternative to express germination behavior over time than the germinability coefficients that express only the final moments of the test, especially when analyzing stress effects.

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