Seed germination in natural populations of *Hancornia speciosa*

Germinação de sementes em populações naturais de *Hancornia speciosa*

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ABSTRACT: The objective of the current study was to assay the germination behavior of seeds from fruits collected from three natural populations of *H. speciosa*, located in remnant areas of Cerrado in the municipality of Porto Nacional, Tocantins state, Brazil. The studied populations were: Canãã, São Judas Tadeu and Providência. Seven genotypes were selected from each population and twenty fruits of each genotype were collected. After ripening, the fruits were manually pulped and seeds of the same genotype pooled. After cleaning, removing adhered pulp and exterior sterilization, seeds were sown in a nursery. The experimental used a Completely Randomized Design (CRD) with three replications. Four seeds of each genotype were sown per polyethylene bag and the germination monitored until the 45th day after sowing. Variables used to assay germination behavior were: Germination (G, %); Mean Time of Germination (MTG, days); Average Speed of Germination (ASG, d⁻¹); Germination Time Variation Coefficient (VCt) and Germination Synchrony Index (Z). For germination data, nonparametric Kruskal-Wallis analysis of variance was performed, followed by Dunn's test at 5% probability. There was a difference between populations for all germination variables, with the Providência population performing the best. The variables MTG, ASG and Z were significantly higher for the Providência population. In absolute terms, the Providência population had the highest percentage of germination (G), but did not differ significantly from the São Judas Tadeu population. The results generated are important for understanding the germination of seeds of different populations of *H. speciosa*. This information is relevant for the *ex situ* spread of the species.

KEYWORDS: Cerrado, mangabeira, conservation.

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INTRODUCTION

_Hancornia speciosa_ Gomes (mangabeira) is a native fruit tree of the Brazilian Cerrado, where it typically occurs on sandy and acidic soils with low fertility (COSTA et al., 2015). This species occurs in the midwest, north, northeast and southeastern regions of Brazil. Its wide distribution, across a wide variety of habitats, indicates a natural ability to adapt to different environments (RODRIGUES et al., 2017). In the Cerrado region the species commonly flowers between August and November, peaking in October, and fruit production from September to November (VIEIRA et al., 2017).

Interest in this species has been growing in recent years, with the fruit (mangaba) as the main commercial focus (ALMEIDA et al., 2018). Mangaba has good digestibility and high nutritional value, with higher protein content than most commercialized fruits (NASCIMENTO et al., 2014). It is rich in ascorbic acid, potassium, iron and zinc (LIMA et al., 2015), and can be consumed fresh or processed as juices, cookies, liqueurs, jams, candies, desserts, cakes, ice cream (OLIVEIRA et al., 2017).

Despite the economic potential, there are few commercial plantations of _H. speciosa_ in Brazil and in most states, the fruits that reach the market and industries come from extractivism (ARRUDA et al., 2016). As well as other fruits of the Cerrado, _H. speciosa_ presents commercial demand far above the supply capacity by means of extractive and this has stimulated a more technified production, encouraging research that seeks new knowledge about this species which is not yet fully domesticated (OLIVEIRA et al., 2018).

Deforestation, real estate speculation, plantations and monocultures have greatly reduced the original area of the ecosystems in which _H. speciosa_ occurs, so that the species is now one of the most endangered native fruits in the Cerrado domain (DA SILVA et al., 2011). It is among the ten species considered CNPq to be of highest conservation priority in the Plants of the Future program organized by World Bank/Global Environment Facility/Ministry of Environment (MMA)/Probio, and coordinated by MMA. It is considered to have the greatest potential for immediate use of any native fruit tree species from the northeast region of Brazil (SANTOS et al., 2011).

Knowledge of seed germination behavior and the factors influencing it are important for effective species propagation (ARAUJO et al., 2016). For _H. speciosa_ such
information can be used to develop mechanisms for the management and conservation of genetic resources and to allow inferences concerning the behavior of the species in environments currently undergoing transformation, such as the Cerrado (PINHEIRO et al., 2018).

Accordingly, the current study aimed to record germination behavior of *H. speciosa* seeds from fruits collected in three natural populations of located in remnant areas of the Cerrado in Porto Nacional, Tocantins State, Brazil.

**MATERIALS AND METHODS**

**Study area**

The work was carried out in three natural populations of *H. speciosa* var. *speciosa* occurring in areas of typical cerrado, on private properties located close to the city of Porto Nacional, Tocantins State, Brazil (Fig. 1).

*Figure 1.* Locations of the study area populations: Providência, Canaã and São Judas Tadeu. Porto Nacional Municipality, Tocantins State, Brazil.
The three population were: São Judas Tadeu (10°48'0.6" S and 48° 25'37.3" W; altitude, 260 m), Canaan population (10°40'23.1" S and 48°20'54.3" W; altitude, 280 m), and Providence population (10°33'31.2" S and 48° 24'43.8" W; altitude, 220 m).

The São Judas Tadeu population is located 24 km from Porto Nacional along the TO-070 highway, connecting Porto Nacional to Brejinho de Nazaré. The Canaan population is located approximately 10 km from the urban area of Porto Nacional towards Monte do Carmo on the TO 230 highway, and Providência population is located 18 km from Porto Nacional on the TO 050 highway connecting Porto Nacional to Palmas.

The municipality of Porto Nacional lie at 212 meters altitude, and the climate of the region has two seasons, a dry season (from May to September) and a rainy season (from October to April). The climate is Aw according to the Köppen classification. Average annual temperature is 26.1 °C, with average rainfall around 1667.9 mm (SOUZA; GOMES, 2012).

**Fruit collection**

In November 2017, twenty physiologically mature fruits were collected from seven genotypes of each population (total of 140 fruits per population), georeferenced with the aid of a Global Positioning System (GPS) and numbered with aluminum tags, in order to facilitate locating and identifying them in the field. Determination of the genotypes for sampling was based on earlier studies (FREITAS et al., 2012), which had identified which genotypes were best represented by the phenotypic variability present in each population.

After complete maturation, the fruits were manually pulped and seeds from the same genotype then grouped. Seeds were cleaned in order to remove as much of the adhering pulp as possible without damaging them and subsequently disinfected with sequential immersions, first in 70% alcohol for one minute, then in 2.0% sodium hypochlorite (NaClO) for ten minutes and, finally, in distilled and then autoclaved water for 10 minutes each (PASSOS; PASSOS, 2004).
Germination experiments

Using the disinfected seeds, germination experiments were performed at the Seedling Nursery of the Cerrado Plant Propagation and Development Section of the Environmental Studies Center (NEAMB), based on the Porto Nacional campus of the Federal University of Tocantins (UFT). Due to their recalcitrance, a two days gap between seed cleaning and sowing allowed for (SOARES et al., 2007). The experimental used was a completely randomized design (DIC) with three replications. Each experimental plot consisted of eight 18 x 24 cm polyethylene bags. Each was filled red Cerrado soil, collected in the campus of the Federal University of Tocantins in Porto Nacional, TO.

Nursery shading rate of 50% was used, with seeds sown at a depth of one centimeter (OLIVEIRA et al., 2018) and germination monitored until the forty-fifth day after sowing. Irrigation was performed periodically to maintain ambiente soil moisture. Daily average temperature and daily precipitation data were obtained from the Teaching and Research Meteorological Database (BDMEP) of the INMET (National Institute of Meteorology) station, located in Porto Nacional, Tocantins.

Soil analyses

Three separate soil samples were collected and mixed to obtain a composite sample for later physicochemical analysis. Results of its physicochemical analysis of the soil used as a substrate appear in Tab. 1. In general, it is a nutrient-poor soil with high acidity, physically classified as possessing a medium texture. Such profiles are typical of Cerrado soils.

Data analyses

The variables used to evaluate germination were - Germination (G): calculated via the formula $G = \left( \frac{N}{100} \right) \times 100$, where: $N =$ number of seeds that germinated by the end of the experiment; - Mean Germination Time (MGT) calculated with the formula TMG
SEED GERMINATION IN NATURAL POPULATIONS OF Hancornia speciosa

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\[ \text{MGV} = \left( \frac{1}{\text{TMG}} \right) \]

- Mean Germination Velocity (MGV): the inverse of mean germination time

- Coefficient of Germination Time Variation (CVt) and Index of Germination Synchronization (Z) (SANTANA; RANAL, 2004).

The higher the synchronization index (Z) value, the more temporal overlap occurs in the germination process. Thus, Z = 1 when germination of all seeds occurs at the same time, and Z = 0 there is no recorded temporal overlap, despite multiple seeds being present (RANAL; SANTANA, 2006).

The criterion for “germination” was the emergence of any part of the seedling. The number of seeds meeting such a criterion was counted daily. For germination data, nonparametric Kruskal-Wallis analysis of variance was performed, followed by Dunn’s test at 5% probability.

### Table 1. Physico-chemico analysis of the substrate used in the H. speciosa germination experiments, Porto Nacional, Tocantins state, Brazil, 2017.

| Components                  | Value   |
|------------------------------|---------|
| **Physical analysis**        |         |
| Clay (%)                     | 24      |
| Silt (%)                     | 14      |
| Sand (%)                     | 62      |
| Classification               | Mean    |
| **Chemical analysis**        |         |
| pH CaCl₂                     | 4.02    |
| Ca (meq/100ml)               | 0.2     |
| Mg (meq/100ml)               | 0.18    |
| Al (meq/100ml)               | 0.4     |
| H + Al (meq/100ml)           | 5.69    |
| K (meq/100ml)                | 0.04    |
| P (mg/dm³)                   | 0.8     |
| Org. Mat. (%)                | 1.8     |
| CEC                          | 6.1     |
| V (%)                        | 6.9     |

\[ \text{pH}_{\text{CaCl}_2} = \text{pH as Calcium chlorate; Ca = calcium; Mg = magnesium; Al = aluminium; H + Al = hydrogen + aluminium; K = potassium; P = phosphorous; Org. Mat. = organic material; CEC = Cation Exchange capacity; V = Base saturation.} \]
RESULTS AND DISCUSSION

In the current study, we used emergence of any part of the seedling to define seed germination. Seedling emergence was followed for 45 days after sowing (DAS), but germination started at 16 days and ended 37 DAS. Evaluating *H. speciosa* seed germination patterns from fruits collected in Cerrado remnant areas within the city of Porto Nacional, Tocantins, Oliveira et al. (2018) reported seed germination began 21 DAS. When testing water availability and substrates suitability on *H. speciosa* seedling emergence, Gordin et al. (2016) also reported emergence rates faster than in the present study for some substrates, though lower values (27.2 days) were recorded for the Latosol + Sand substrate.

The Providência population showed the highest average germination percentage, with significant difference for this variable between the Providência and Canaã populations but similar to those for São Judas Tadeu (Tab. 2).

The overall experimental average for the germination variable (G) was 63.99%. This average is not considered low, since the seed germination percentage of the species is often reduced due to recalcitrance, plus the inhibitory action of the pulp conferring (LORENZI, 2009; OLIVEIRA et al., 2014). In the current study, methods were adopted so that these factors did not influence the obtained results. According to Soares et al. (2007) *H. speciosa* seeds should be sown immediately or within 48 hours after removal from the fruit, as from the fourth day germination power drops rapidly.

A possible explanation for the germination rates on the current substrate is that *H. speciosa* grows mostly in soils with low organic matter content, high acidity and low nutrient availability and exchangeable bases (FERREIRA; MARINHO, 2007). Indeed a variety of management regimes and substrates, have reported average germination percentages above 50%. In this sense, Oliveira et al. (2018) identified an average of 51.85%; Soares et al. (2007) of 67%; Nogueira et al. (2003) of 68% and Passos & Passos (2004) of 87.1%.
Table 2. Comparison of the variables used to evaluate the germination of the Canaã, São Judas Tadeu and Providência populations in the municipality of Porto Nacional, Tocantins state, Brazil, 2017.

| Population       | G (%)   | MTG (days) | MGV d⁻¹ | CVt     | Z     |
|------------------|---------|------------|---------|---------|-------|
| Canaã            | 55.03 b | 19.74 a    | 0.4512 b| 11.43   | 0.15 b|
| São Judas Tadeu  | 64.79 ab| 20.09 a    | 0.4970 b| 11.56   | 0.17 b|
| Providência      | 72.17 a | 18.78 b    | 0.6139 a| 13.87   | 0.24 a|
| Overall Mean     | 63.99   | 19.48      | 0.5238  | 12.37   | 0.20  |

* Means followed by the same letter in the column were not statistically different (Kruskal-Wallis test, followed by the Dunn test, at 5% probability). Germination (G, %); Mean Germination Time (MGT, days); Mean Germination Velocity (MGV, d⁻¹); Germination Time Variation Coefficient (CVt); Synchronisation Index (Z).

The mean germination time (MGT) has high practical applicability because it provides a robust index with which to evaluate a species occupation speed in a particular ecological niche (FERREIRA et al., 2001). Experimental MGT was 19.48 days, ranging from 16 to 37 DAS, with significant differences between the Providência population and the other two sites. The São Judas Tadeu population had the highest MGT (20.09 DAS), which did not differ significantly from the Canaan population. The lowest MGT occurred in the Providência population (18.78 DAS), indicating that, of the three studied populations, the plants of Providência population, would be the ones to most quickly establish themselves in a particular ecological niche. According to Scalon and Jeromine (2013) such a MGT value is high, and high values are common in native fruits of the Cerrado, where seeds usually take a long time to start the germination process, with representative MGTs being: 52 days for Psidium guineensis Sw., Alibertia edulis Rich. 28 days; 66 days for Eugenia pyriformis Camb., and 103 days for Eugenia dysenterica DC.

The mean germination velocity (MGV) is inversely proportional to MGT, thus, the higher the MGV, the lower the MGT. In the current study, the highest MGV was obtained in the Providência population (0.6139 d⁻¹) and the lowest from the Canaan population (0.4512 d⁻¹). The Canaan and São Judas Tadeu populations showed similar means for this variable, and both differed significantly from the Providência population. The difference in germination velocity between seeds from different populations of the same species may depend, among other factors, on seed size (NOGUEIRA et al., 2003). Several authors recommend that the largest and heaviest seeds be used to standardize seedling emergence and to obtain seedlings of similar size or greatest vigor (DA SILVA...
et al., 2017). According to Carvalho and Nakagawa (2012), the positive effect of large seed size is related to the fact that they are likely to have well-formed embryos and larger amounts of reserves, potentially making for more vigorous seedlings.

Preliminary analysis of fruit and seed morphology from the populations studied showed that the seeds of the Canaan population were smaller in size and mass than those from the Providência and São Judas populations (data not shown). Possibly, the smaller seed size of this population contributed to the lower GVM response. Studies with other Cerrado species have also reported that larger seeds had greater higher emergence velocities. Working with Eugenia dysenterica DC., Nietsche et al. (2004) found that the larger seeds showed higher EVI (Emergence Velocity Index), mean leaf number and plant height. Studying germination and initial growth of cashew (Anacardium microcarpum Ducke), Moreira et al. (2016) concluded that seed size influenced both germination and seedling development, with larger seeds germinating more swiftly and growing more rapidly. In research conducted with jackfruit (Artocarpus heterophyllus Lam.), an exotic fruit tree introduced in Brazil, Silva et al. (2010) found that germination was influenced by seed size, with seeds from small size classes showing a lower germination percentage (70%), and that this differed statistically from treatments with medium, large and extra-large seeds that showed 88%, 96% and 98%, respectively.

The lowest obtained Z analysis value came from the Canaan population (0.15), indicating low germination synchrony. The Providência population had the highest value (0.24) (Tab. 2). The overall mean synchronization value (0.2) is considered low. Santana et al. (2010) studying seed germination and seedling emergence in pau-santo (Kielmeyera coriacea) described Z <0.32, as low and indicative of low synchronization.

Two factors may have influenced the low Z value. According to Dorneles et al. (2013) morphophysiological unevenness in seeds may result in germination asynchrony and this effect is more evident in species with long periods between flowering and seed formation. Since H. speciosa has a period of about 110 days between flowering and fruiting (FERREIRA et al., 2018), this could provide a plausible explanation. Another cause might be associated with the genetic variability generated by species dependence on cross-fertilization, since H. speciosa is allogamous (VIEIRA et al., 2017), which could well give rise to plants with different germination characteristics.

Factors that influence seedling germination and emergence include temperature, humidity, oxygen availability, soil structure and seed depth (COSTA; DANTAS, 2009). The degree with which these external factors influence germination varies between
species and is determined by the genotype and environmental factors prevailing during the seed germination process (SILVA; CESARINO, 2016).

Temperature interferes with germination rates and percentage success, notably by changing the rate of water absorption and altering the speed of chemical reactions that will mobilize or degrade stored reserves and the rate of synthesis of seedling growth substances (BEWLEY; BLACK, 1994). There is no one uniform effect for all species, as values are species-specific and dependent on the climatic conditions of their regions of origin at the time of natural germination (ALVES et al., 2015). Each species has an optimal germination temperature at which maximum germination occurs in the shortest time, and maximum and minimum temperature limits beyond which germination does not occur (MELO et al., 2008). Most tropical species, for example, germinate at temperatures between 20 and 30 °C (ARAUJO et al., 2016). According to Brancalion et al. (2010) the 25 °C median is considered the most suitable temperature for most species in the Cerrado biome. Graphical analysis of temperature data (Fig. 2) shows that the mean temperature during the experiment was 28 °C, ranging across 25 °C to 32 °C, and so within the optimal temperature range for the species. Consequently, temperature was an unlikely interference factor for seed germination in the current study. Silva et al. (2016) studying the germination of mangabeira seeds as a function of substrate and temperature, and recommended that laboratory tests of germination and GMV be performed using blotting paper as the substrate and temperatures between 25 °C and 30 °C, and paper roll substrate used at a temperature of 30 °C.

The humidity of the substrate on which seeds are sown is one of the essential factors for triggering the germination process (CARVALHO; NAKAGAWA, 2012). During this process, the water absorption is key to promoting coat seed softening and embryo and reserve tissues enlargement, as well as favoring rupture of the testa, gas diffusion and the emergence of the primary root. Water is also important for the protoplasm dilution, allowing the diffusion of hormones and consequently enzymatic system activation. As a result, digestion, translocation and assimilation of reserves develops, resulting in embryo growth (RAMOS et al., 2006). Water deficit reduces cell expansion and may affect the process of cell division and this interferes with plant development. In addition, there is often a decrease in leaf water potential, which induces stomata closure, resulting in reduced gas exchange and consequently biomass production. On the other hand, flooded or excessively moist soils, limitations on oxygen diffusion may also slow down germination rates, largely due to the absence or scarcity of
oxygen, so promoting with-in cell production of ethanol which is toxic to normal metabolism, so causing tissue acidification and death (GORDIN et al., 2016).

Figure 2. Mean temperature and precipitation data for the period during the *H. speciosa* seed germination experiment, Porto Nacional, Tocantins State, Brazil.

During the experiment, water was constantly supplied throughout the germination period, keeping the field capacity of the substrate irrigated in a manner that avoided soil saturation. However, as shown in the rainfall graph (Fig. 2), extensive rainfall naturally occurs during the experimental period, with precipitation averaging around 6.9 mm/day (range 0 to 44.5 mm), which would favor moisture retention for an extended period. In addition, germination peaks occurred during periods with lower precipitation and milder temperature (around 26 °C) (Fig. 2 and 3).

The large volume of rainfall during the experiment, generated briefly (few days) an excessive water accumulation in the substrate, which may have favored the multiplication of rotting microorganisms and decreased survival of *H. speciosa* seeds. According to Lewis and Clements (1999), large amounts of such water can provide suitable habitats for the development of microorganisms that negatively affect seed germination.
The Providência population had a more homogeneous germination process than the other two populations (Tab. 2). Why this was so may be related to the place of location of the population, as it is lies closest to the Luiz Eduardo Magalhães reservoir and so occurs in a region where the water table lies closer to the surface. Since the experimental period was accompanied by a large amount of rain (Fig. 2), the accompanying flooding may have reproduced the environment to which plants of this population are more adapted.

![Graph showing germination process](image)

**Figure 3.** Number of *H. speciosa* seeds germinated per day across the experimental period, Porto Nacional, Tocantins State, Brazil, 2017.

Another factor that has a major influence on germination is light, with perception and translation of the light stimulus occurring in the embryo is responsible (DOUSSEAU et al., 2008). Seeds have a different degree of sensitivity to light, with species having seeds that are positively or negatively affected (LOPES et al., 2005). In general, light is necessary for the germination of small seeds (positive photoblastic species) (ABREU; GARCIA, 2005). Such species are generally associated with open or anthropized environments (OLIVEIRA; GARCIA, 2005). *Hankornia speciosa* is a species of open environments with high light intensity, consequently it is no surprise that seeds...
are positively affected by the presence of light. Studying the effect of five different sowing depths and light levels on baru (*Dipterix alata* Vog.) seed germination Fonseca et al. (1994) found that a full sun environment with 100% luminosity gave the highest and fastest germination rates. The current experiment was carried out in a nursery covered by a black screen providing 50% shading. In a study on protected environments and substrates for *H. speciosa* seedling production, Arrua et al. (2016) found both the 50% shading black screen and the 50% aluminized heat-reflective screen to be suitable for *H. speciosa* seedling production. Consequently, it is unlikely that luminosity was a factor negatively interfering with seed germination in the current experiment.

Oxygen is necessary for the promotion of a variety of key metabolic reactions in the seed, especially respiration. Although respiration in the early stages of germination is generally anaerobic, it soon becomes oxygen dependent (BORGES; RENA, 1993). According to Melo et al. (2008) the need for oxygen is affected by such factors as humidity and high temperatures. In saturated or excessively moist soils, limitations on oxygen diffusion may also halt germination, probably because oxygen absence or scarcity favors ethanol production in cells, and this is toxic to normal metabolism causing tissue acidification and seed death (GORDIN et al., 2016). As seen in Tab. 1, soil used as the experimental substrate was classified as medium texture, which is unlikely to have hindered seed access to oxygen, since substrates with higher sand percentages favor seed germination via the promotion of better aeration and drainage (SCALON; JEROMINE, 2013).

Substrate directly affects plant quality via a variety of physical, chemical and biological properties associated with the material of which it is composed (VIEIRA NETO et al., 2002). Characteristics of structure, aeration, water retention capacity and degree of pathogen contamination, among others, vary according to the material used in the substrate composition, and can affect germination and seedling establishment, so demonstrating the importance of choosing the most appropriate substrate (NOGUEIRA et al., 2003). A high-quality substrate can be comprised of mineral or organic soil or a mixture of various components, but must generate an appropriate balance between moisture and aeration, be porous enough to allow efficient, free gas exchange, prevent saprophytic or pathogenic microorganisms, and be free from invasive propagules (either seeds or vegetative) (DA SILVA et al., 2011).

The depth at which the seed lies in the soil is another potentially important factor in germination and emergence. Soil, in addition to being a barrier to light
penetration, also exerts physical impediments to seedling growth until they reach the soil surface and no longer depend on cotyledon reserves (TOLEDO et al., 1993). Depth of seed deposition can affect germination, conditioned by temperature, water content, seed peculiarities, soil physical and chemical properties, and climate, among other factors (BOTTEGA et al., 2014). According to Oliveira et al. (2018), the ideal sowing depth guarantees the most homogeneous germination profile, with shorter emergence times and more vigorous seedlings. As pointed out by Koakoski et al. (2007), the greater the depth of planting, the greater the volume of energy consumed while emerging, as well as the greater the damage caused by low temperatures and oxygen levels. On the other hand, at shallower depths the seed has an enhanced possibility of hydric stress. According to Oliveira et al. (2018), *H. speciosa* seeds are influenced by sowing depth and substrate type and should be planted 1 cm deep in cerrado soils that contain up to 15% of washed sand. In the present study, sowing depth was 1 cm, and so probably did not negatively influence seed germination.

**CONCLUSION**

There was a difference between populations for all germination variables, with the Providência population performing the best. Factors such as seed size and environmental conditions may have favored the Providência population.

Average germination time, average germination speed and synchronization index were highest for the Providência population.

In absolute terms the Providência population had the highest germination percentage, but did not differ significantly from the São Judas Tadeu population.

The results generated are important for understanding the germination of seeds of different populations of *H. speciosa*. This information is relevant for the *ex situ* spread of the species.

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