

Conservation of Manilkara salzmannii in an Atlantic Forest fragment through the study of fruits and seeds

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ABSTRACT – The objective of the study was to evaluate the biometry of fruits and seeds and the emergence of seedlings on different substrates of *Manilkara salzmannii* (A. DC.) H. J. Lam (massaranduba). We measured 100 fruits and 116 seeds and recorded biometric variables to calculate descriptive statistics, and applied correlation analysis. For seedling emergence, we evaluated four substrate treatments (T1: bovine manure + sand in 2:1 proportion; T2: bovine manure + sand in 1:1 proportion; T3: sand; and T4: sand + clay in 1:1 proportion). The fruits had average length of 16.67 mm, width of 17.10 mm and fresh mass of 3.20 g. The seeds had average length of 10.65 mm, width of 7.13 mm, thickness of 4.68 mm, and fresh mass of 0.22 g. There was a positive correlation between the fruit and seed variables. The lowest seedling emergence percentage was produced with 1:1 sand/clay ratio, which in contrast provided the highest emergence speed index (ESI). The biometric characteristics of fruits and seeds and the identification of the best substrate for seedling emergence will serve as a basis for seedling production and improve understanding of the species' propagation.

Keywords: Biometric Characteristics, Massaranduba, Sapotaceae, Substrates

RESUMO – Conservação de *Manilkara salzmannii* através do estudo de frutos e sementes em um fragmento de Floresta Atlântica. O objetivo do estudo foi avaliar a biometria de frutos e sementes de *Manilkara salzmannii* (A. DC.) H. J. Lam, além da emergência de plântulas em diferentes tipos de substratos. Foram avaliadas variáveis biométricas a partir de 100 frutos e 116 sementes, analisadas por meio da estatística descritiva e correlação. Para a emergência de plântulas foram avaliados os tratamentos T1: adubo + areia (2:1); T2: adubo + areia (1:1); T3: areia; e T4: areia + argila (1:1). Os frutos obtiveram uma média de 16,67 mm de comprimento, 17,10 mm de largura e massa fresca de 3,20 g. As sementes apresentaram média de 10,65 mm de comprimento, 7,13 mm de largura, 4,68 mm de espessura e 0,22 g de massa fresca. Houve correlação positiva entre as variáveis dos frutos e sementes, além de baixa porcentagem de plântulas emergidas, sendo o substrato areia/argila na proporção 1:1 o que proporcionou maior IVE. As características biométricas dos frutos e sementes, bem como o substrato mais adequado para a emergência das mudas, servirão de base para a produção de mudas e compreensão da propagação da espécie.

Palavras-chave: Características Biométricas, Massaranduba, Sapotaceae, Substratos

INTRODUCTION

The Sapotaceae family is represented in the world by 53 genera and 1250 species (Carneiro *et al.* 2009), with the greatest species diversity in tropical and subtropical regions of Asia and South America (Swenson & Anderberg 2005). The species of the family are trees or shrubs with simple leaves that bear fruit of the fleshy berry type that are juicy and generally sweet (Barroso *et al.* 2002), and white latex is often found in cuts of bark, branches, leaves and fruits (Swenson & Anderberg 2005). The trees stand out in the landscape due to their tall stature, and many of them produce good quality wood (Souza & Lorenzi 2012), making the species economically important (Pennington 1990). In Brazil, the family is widely distributed, being present in all phytogeographic domains, with 13 genera

and 246 species (Alves-Araújo *et al.* 2023), of which 33 are considered rare (Carneiro *et al.* 2009).

In the Sapotaceae family, the genus *Manilkara* has 17 species in Brazil (Carneiro 2020), represented in different ecosystems of the Northeast region, 12 of which are native (Almeida Jr. & Zickel 2011), among them *Manilkara salzmannii* (A. DC.) H. J. Lam, popularly known as massaranduba. The species is endemic to Brazil and occurs in the Caatinga and Atlantic Forest phytogeographic domains (Carneiro 2020). *M. salzmannii* is used for reforestation and afforestation, in addition to having economic importance because its wood has high resistance and can be used in civil construction and furniture manufacture (Lorenzi 1998). Its fruits are edible and *in vivo* are globose with smooth orange skin and rounded apex and base (Fabris & Peixoto 2013). Fabris and Peixoto (2013) found *M. salzmannii* in resting areas of non-flooded forest formations, open non-flooded shrub formations, closed non-flooded shrub formations and transitions between closed non-flooded shrub formations and herbaceous flood formations. In turn, Garay and Rizzini (2003) reported that the species is present in Amazonian, Atlantic and Tabuleiro forests, due to its ability to adapt to different types of vegetation and soil types.

The morphometric characterization of fruits and seeds is an important tool in the differentiation of species of the same genus (Cruz *et al.* 2001). In particular, this can help in the interspecific differentiation of the genus *Manilkara*, which is the fourth largest of the Sapotaceae family (Almeida Jr. *et al.* 2010). Also, the biometry of fruits and seeds reflects the phenotypic variations and associations of the species with environmental and genetic factors, besides being important for identification and taxonomy (Vieira & Carvalho 2009).

Many studies have indicated the importance of seedling characterization for succession and natural regeneration initiatives, but there is still little information related to the seedling stages (Ferreira & Barreto 2015), so there is a need for studies of plant propagation. One of the factors that most influences the germination of seeds and consequently the emergence of seedlings is the substrate, because factors such as structure, aeration, water retention capacity, among others, determine the seed germination success (Albuquerque *et al.* 1998). According to Oliveira (2009), the substrate influences the germination and vigor of the seeds as well as the development of seedlings. Therefore, it is essential to define the most appropriate substrate parameters (nutrients and physical properties) for each species (Lima *et al.* 2006).

Moreover, knowledge of the optimal conditions for germination and seedling emergence is crucial, especially for the species in question, for which there are no studies. Therefore, we evaluated the biometric characteristics of fruits and seeds and the emergence of seedlings of the species *M. salzmannii* in different substrates.

MATERIALS AND METHODS

Study area and fruit acquisition

The collection of *M. salzmannii* fruits took place in a semideciduous seasonal forest fragment located at 5°53'57"S, 35°22'59"W, in the municipality of Macaíba, Rio Grande do Norte state, Brazil (Fig. 1). The study area is a transitional forest formation between the Atlantic Forest and Caatinga biomes, containing approximately 10 hectares (Silva *et al.* 2014). Samples of plant material were deposited in the herbarium of Federal University of Rio Grande do Norte and the results were registered in the Sistema Nacional de Gestão do Patrimônio Genético e do

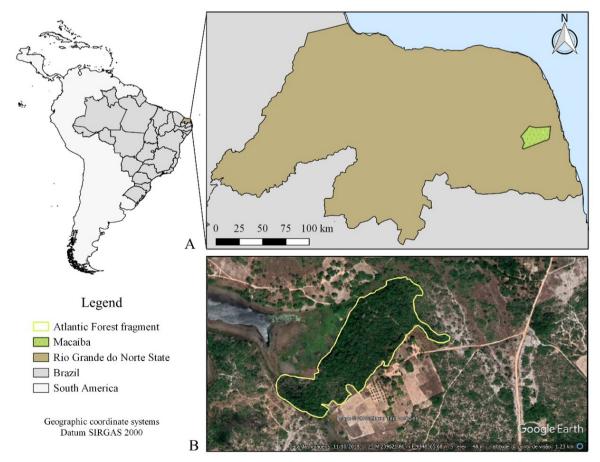


Figure 1. Geographical location of the study area. A. Municipality of Macaíba, RN, Brazil; B. fragment with a population of Manilkara salzmannii.

Conhecimento Tradicional Associado – SISGEN ("National System for Management of Genetic Heritage and Associated Traditional Knowledge") under code ADB6CB0. The local climate is a transition between the As' and BSh' types of the Köppen classification, with high temperatures and average annual precipitation of 903.8 mm (EMPARN 2017).

According to Cestaro (2002), the relative density of the species at the site is 5.51%, and the basal area is 0.26 m^2 , making it one of the most abundant plant species at the site. The fruits were collected from nine matrix trees with height of more than 5 m in March 2016. The trees with fruits were located at the margins of the forest fragment, where they received more sunlight, while the ones inside the fragment were larger, but had no fruit production. The collected fruits were stored in plastic bags for better conservation and taken to the laboratory for measurement.

Biometry of fruits and seeds

All told, 100 fruits were measured (Fig. 2) to evaluate the variables width (mm), length (mm) and fresh mass (g). In turn, 116 seeds were measured, in which the variables width (mm), length (mm), thickness (mm) and fresh mass (g) were evaluated. A precision scale was used to obtain the fresh mass values, and a digital pachymeter for the other variables.

The statistical analysis of the biometric data was performed with the Bioestat 5.0 m program (Ayres *et al.* 2007), to calculate the arithmetic mean, standard deviation, standard error, coefficient of variation (*CV*), asymmetry

 (g_1) and kurtosis (g_2) . As a reference, a positive asymmetric distribution $(g_1 > 0)$ has a right skewed tail and a negative asymmetric distribution $(g_1 < 0)$ has a left-skewed tail (Ayres *et al.* 2007). As for kurtosis, $g_2 > 0$ means a tapered normal (leptokurtic) curve, while $g_2 > 0$ denotes a flat normal (platykurtic) curve. The Lilliefors normality test was used to determine the statistical differences of the data concerning the normal distribution. In cases where data presented a distribution different from normal (P < 0.05), nonparametric tests were applied, with Spearman's correlation coefficient (r_s) determining the degree of association between the variables.

Seedling emergence

The seeds were disinfested in sodium hypochlorite for 5 minutes. The seedling emergence experiment was conducted in a greenhouse with a completely randomized design, with four treatments and seven repetitions containing 10 seeds, totaling 70 seeds per treatment. The seeds were inserted in the different substrates contained in plastic trays, arranged as follows: T1 - bovine manure + sand in the proportion; T3: sand; and T4: sand + clay in the proportion. The number of seedlings emerged daily was evaluated, adopting as criteria the emission of cotyledons. We estimated the percentage of emerged seedlings and the emergence speed index (ESI), proposed by Maguire (1962): ESI = $E_1/N_1 + E_2/N_2 + ... + E_n/N_n$; in which: $E_1, E_2, E_n =$ number of normal seedlings counted and $N_1, N_2, N_n =$ number of days after sowing.

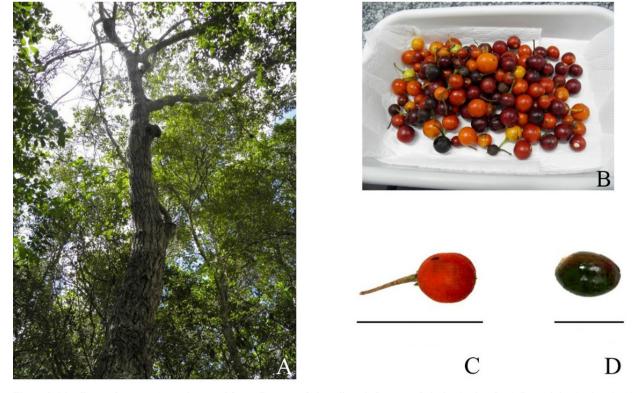


Figura 2. Manilkara salzmannii. A. tree in natural forest; B. mature fruits collected; C. mature fruit size (scale = 3 cm); D. seed size (scale = 1 cm).

The statistical analysis was conducted with the statistical program (Ayres *et al.* 2007). Initially, the normality test was carried out to perform analysis of variance (ANOVA). Since all data had nonparametric distribution, the Kruskal-Wallis test was applied followed by the Dunn test.

RESULTS AND DISCUSSION

The fruits had average length of 16.67 mm in length, width of 17.10 mm fresh mass of 3.20 g, with minimum and maximum values of 12.7 and 20.20 mm in length and 11.90 and 21.40 mm in width (Tab. 1). Regarding seeds, averages of 10.65 mm in length, 7.13 mm in width, 4.68 mm in thickness and 0.20 g in fresh mass were measured. The minimum and maximum values were 8.30 and 13.20 mm in length, 4.80 and 8.50 mm in width, and 3.20 and 5.70 mm in thickness.

Almeida Jr. *et al.* (2010) found minimum and maximum values for fruits of 14.50 to 23.60 mm in length and 16.10 to 28.60 mm in width, as well seed values of 10.60 to 15.40 mm in length, 7.40 to 9.70 mm in width and 4.00 to 5.40 mm in thickness. In turn, Fabris and Peixoto (2013) found values of 15 to 20 mm in length and 10 to 20 mm in width for the fruits and 10 to 20 mm in length, 12 to 13 mm in width and 6 to 7 mm in thickness for seeds. These studies show that despite being in different locations, the trees biometric variables have little variation.

The results indicated that only the fresh mass of the fruits presented a positive coefficient of asymmetry (g_1) (right asymmetric distribution), indicating that fruits with lower fresh mass predominated in the analyzed sample, with a value close to zero, indicating a normal distribution (Fig. 3). The other variables presented negative asymmetry (left asymmetrical distribution), indicating that the smaller dimensions were predominant. According to the kurtosis coefficient ($g_2 < 0$), all the biometric variables of the fruits and the fresh mass of the seeds showed platykurtic distribution, in which the frequency of the analyzed variables has a flatter normal curve, that is, it has greater amplitude of data distribution. The length, width and thickness of the seeds showed leptokurtic distribution ($g_2 > 0$), where the values are concentrated around the mean.

The fresh mass of fruits was the variable with the highest coefficient of variation (CV). Some studies have found that higher variation can be influenced by environmental factors possibly related to the anthropization of the site and soil and climatic factors, such as the availability of water, which is essential for the formation of fruits and seeds (Gonçalves et al. 2013, Gusmão et al. 2006, Zuffo et al. 2014). Genetic variability is also one of the factors that influences fruit and seed variation, generally being greater in native trees and smaller in cultivated trees (Vasconcelos et al. 2015). Also, variables such as fresh seed mass are important factors for each plant species and may be related to seed formation and quality (Rocha et al. 2014). Spearman's correlation coefficient (rs) indicated a positive and significant association between fruit and seed variables. This shows that fruit and seed dimensions have shared influences, with the highlight being the correlation of fruit width and fresh mass (rs = 0.9685), followed by length and fresh mass (Table 2).

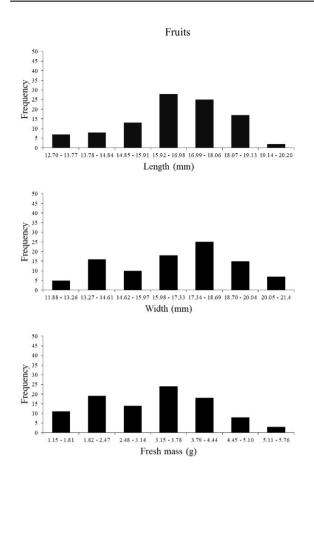
Gonçalves et al. (2013), studying the correlation between the variables of Hancornia speciosa, observed that the fresh mass of the fruits was correlated with all other variables analyzed. Fresh mass is one of the variables that most positively influenced the size of fruits and seeds. This direct correlation between the fresh mass of fruits and seeds and the other biometric variables was identified for the species Sterculia foetida, Butia capitata and Byrsonima verbascifolia by Araújo et al. (2015), Gusmão et al. (2006) and Moura et al. (2010), respectively. It is important to emphasize that fresh mass depends directly on climate variables, especially precipitation. In this sense, studies describing this relationship are necessary to characterize the best conditions for the formation of fruit and seeds. Selecting vigorous seeds can optimize the methods of sexual propagation of the species, mainly to produce seedlings for projects to recover degraded areas.

The seedlings started to emerge 39 days after sowing and ceased after 98 days. The highest percentage of *M. salzmannii* seedlings occurred in the substrate composed of sand and clay in the proportion of 1:1 (Table 3), while the lowest was with the bovine manure/sand substrate in the 2:1

Biometric characteristics	n	Min	Max	$Mean \pm SE$	CV (%)	g_1	g ₂
Fruits							
Fresh mass (g)	100	1.15	5.76	3.20 ± 0.11	33.58	0.05	-0.75
Length (mm)	100	12.70	20.20	16.57 ± 0.16	9.72	-0.45	-0.07
Width (mm)	100	11.90	21.40	17.10 ± 0.22	12.82	-0.27	-0.68
Seeds							
Fresh mass (g)	116	0.03	0.31	0.20 ± 0.01	27.59	-0.49	-0.09
Length (mm)	116	8.30	13.20	$10.65{\pm}~0.08$	8.14	-0.51	0.86
Width (mm)	116	4.80	8.50	7.13 ± 0.06	9.81	-0.60	0.41
Thickness (mm)	116	3.20	5.70	4.68 ± 0.06	13.22	-3.85	27.78

Table 1. Biometric characteristics of the fruits and seeds of Manilkara salzmannii.

n: sample size, Min: minimum, Max: maximum, SE: standard error, CV: coefficient of variation, g1: asymmetry, g2: kurtosis.



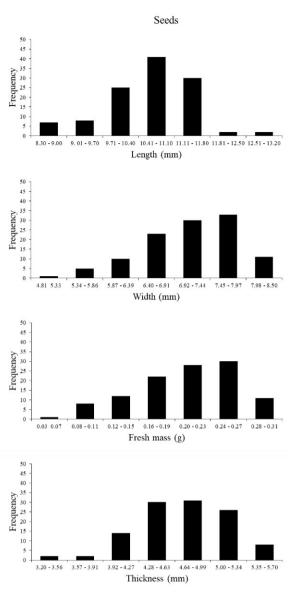


Figure 3. Absolute frequency distribution of the biometric characteristics of the fruits and seeds of Manilkara salzmannii.

proportion. However, the averages did not differ statistically, so all substrates had the same seed germination success.

The ESI was also greatest with the substrate composed of sand and clay in 1:1 proportions (Table 3). In contrast, the lowest ESI was observed in treatments 1 and 2, where the substrates contained bovine manure. Thus, the use of organic bovine manure as a substrate probably reduces the emergence and the ESI. This shows that the species in its initial development, mainly in seedling production, does not adapt well to substrates with large amounts of bovine manure. Probably the seeds of the species have greater germination capacity in places with sandy-clayey soils, such as the forest fragment where the parent trees are located. The soil of the area studied is classified as an abruptic plinthosol, formed by the accumulation of clay

Table 2. Spearman correlation (r_x) between the biometric variables of the fruits and seeds of *Manilkara salzmannii*.

Correlations	r _s		
Fruits			
Length × Width	0.7925 *		
Length × Fresh mass	0.8519 *		
Width × Fresh mass	0.9685 *		
Seeds			
Length \times Width	0.4399 *		
Length × Thickness	0.3437 *		
Length × Fresh mass	0.6677 *		
Width × Thickness	0.2838 *		
Width × Fresh mass	0.6413 *		
Thickness × Fresh mass	0.4495 *		

(* = P < 0.05)

Table 3. Percentage of Manilkara salzmannii seedling emergence (E), emergence speed index (ESI) and Kruskal-Wallis (H) variance.

Treatments	E (%)	ESI	Н
T1	23	0.10 c	81.48*
Τ2	24	0.11 bc	92.88*
Т3	26	0.13 b	119.96*
T4	37	0.24 a	187.69*

T1: bovine manure + sand in the proportion 2:1; T2: bovine manure + sand in the proportion 1:1; T3: sand; and T4: sand + clay in the proportion 1:1. Values followed by the same letter in the column do not differ statistically by Dunn's test (P > 0.05). (*) = P < 0.05; H, Kruskal-Wallis test.

with iron and aluminum oxides, with fertility ranging from poor to very poor, but with good water retention due to the silt and clay contents, formed by the accumulation of clay with iron and aluminum oxides, with fertility ranging from low to very low, but with good water retention due to the silt and clay contents (Cestaro 2002).

There was a low percentage of seedling emergence, suggesting that the rigidity of the seed coat prevented the rapid absorption of water and expansion of seeds, as occurs in the species *Manilkara subsericea* (Barbosa 2002), which has similar characteristics of fruits and seeds to *M. salzmannii* (Fabris & Peixoto 2013). Lorenzi (1998) suggested that for seedling production, seeds should be sown in sandy substrate, which will germinate in 40 to 60 days, despite low germination, as occurred in this study. Almeida Jr. *et al.* (2010) also reported that the low germination rate occurs due to the low production of seeds per fruit, as well as because the fruit contains latex, which can hinder the propagation of seedlings.

Almeida Jr. et al. (2012), in a study of conservation of species of the genus Manilkara, described that although M. salzmannii is not classified as an endangered species, it still will face future risks if no conservation measures are taken. Our study shows the importance of knowing the characteristics of the species for the production of seedlings to be used in forest restoration, including identifying the most suitable substrate, because this will influence the vigor and germination of seeds and the development of seedlings (Oliveira et al. 2009). Moreover, the species occurs in the highly fragmented Atlantic Forest biome, which is considered one of the world's hotspots (Martinelli & Moraes 2013). Further research is necessary, such as analyzing the acceleration of seed germination via studies of dormancy breakage. Freire et al. (2019), in a study of Protium heptaphyllum in the same fragment, observed low genetic diversity of the population. This suggests the need for further investigations and effective conservation actions in fragmented landscapes, where ecological relationships and genetic resources must be maintained.

Given the low germination rate and fruit production by the species, we conclude that analysis of the biometric characteristics of fruits and seeds and choice of the most suitable substrate for seedling emergence are necessary to optimize seedling production for conservation of the species, along with studies to promote an increase in the rate of seed germination.

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