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SEMINIFEROUS PROPAGATION OF *Cordia oncocalyx* (Allemão) Baill. AND BIOMETRIC CHARACTERIZATION OF DIASPORES AND SEEDS[1]

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ABSTRACT - *Cordia oncocalyx* Allemão Baill., widely known as "pau-branco", is a native species from the Caatinga (Brazilian Savannah) and has socioeconomic and environmental potential; however, there are few silvicultural studies on this species. Therefore, this paper aimed to analyze and compare the biometric characteristics of diaspores and seeds through manual biometrics and by digital image processing, and to evaluate the *in vitro* and *ex vitro* germination of *C. oncocalyx*. In the biometrics evaluation, three hundred diaspores and three hundred seeds were used, applying manual and digital biometrics. Subsequently, *ex vitro* emergence was determined, testing mechanical scarification (in different regions of the diaspore) and chemical scarification (immersion in sulfuric acid for 90 min and 180 min). Finally, *in vitro* germination was tested with different compositions of Murashige & Skoog (M&S) culture medium and sucrose addition. Results showed that digital image processing is a viable and fast technique to obtain the biometric parameters of *C. oncocalyx* fruit and seeds. Chemical and mechanical treatments on diaspores have not influenced seed emergence (0.33%). The composition of the culture medium has influenced the germination percentage, and the maximum value of 96% % was obtained with 6 g/L of sucrose and 0.90 g/L of M&S medium. Thus, the seminiferous propagation of *C. oncocalyx* can be performed successfully when the seeds are germinated *in vitro*, and the digital image processing shows the solidity and applicability aiming to evaluate the quantitative parameters of seed and fruit of this species.

Keywords: Digital biometrics. *In vitro* germination. Germplasm conservation. Seedling production.

PROPAGAÇÃO SEMINÍFERA DE *Cordia oncocalyx* (Allemão) Baill. E CARACTERIZAÇÃO BIOMÉTRICA DE DIÁSPOROS E SEMENTES

RESUMO - *Cordia oncocalyx* Allemão Baill., conhecida popularmente como pau-branco, é uma espécie nativa da caatinga, com potencial socioeconômico e ambiental, entretanto, são escassos estudos silviculturais sobre a espécie. Portanto, objetivou-se analisar no presente trabalho as características biométricas dos diásporos e sementes por meio da biometria manual e pelo processamento digital de imagens, com o intuito de compará-las, bem como avaliar a germinação *in vitro* e emergência *ex vitro* de *C. oncocalyx*. Na avaliação da biometria, foram utilizados 300 diásporos e 300 sementes, aplicando-se a biometria manual e a digital. Posteriormente, foi realizada a emergência *ex vitro,* testando a escarificação mecânica (escarificação em diferentes regiões do diásporo) e química (imersão em ácido sulfúrico por 90 e 180 min). Por fim, testou-se a germinação *in vitro*, utilizando diferentes composições do meio de cultura M&S e adição de sacarose. Os resultados demonstraram que o processamento digital de imagens é uma técnica viável e rápida na obtenção dos parâmetros biométricos de frutos e sementes de *C. oncocalyx*. Os tratamentos químicos e mecânicos nos diásporos não influenciaram na emergência das sementes (0,33%). A composição do meio de cultura influenciou na porcentagem de germinação, sendo o valor máximo observado de 96% com 6 g/L de sacarose e 0,90 g/L de meio M&S. Assim, a propagação seminífera de *C. oncocalyx* pode ser realizada com sucesso quando as sementes são germinadas *in vitro* e o processamento digital de imagem denota solidez e aplicabilidade com vistas à avaliação de parâmetros quantitativos de sementes e frutos da espécie.

Palavras-chave: Biometria digital. Germinação *in vitro*. Conservação de Germoplasma. Produção de mudas.

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INTRODUCTION

Cordia oncocalyx (Allemão) Baill., widely known as "pau-branco", is an endemic species of the Boraginaceae family in the Caatinga region (Brazilian Savannah). The gradual increase in the extraction of its wood and non-wood products and the lack of recommendations regarding the proper propagation methodology have resulted in reduction of *C. oncocalyx* individuals in the species' natural populations, considered a rare species with the possibility of being extinct in the future (MAIA, 2012).

C. oncocalyx propagation is sexual; however, there are limiting factors for the production of seedlings of the species, such as fruit morphology. At the same time, the embryo is damaged by the attack of the Coleoptera larvae of *Pachymerus nucleorum* (Fabricius), which influences reduction in the viability during the storage process (BRITO; ARAÚJO, 2009).

The scarcity of silvicultural studies makes it difficult to overcome these problems, since this information is not included in the Instructions for Analysis of Forest Species Seeds. Thus, knowledge about fruit biometrics and the adequacy of an efficient propagation protocol are essential for conservation and perpetuation of the species.

Therefore, aiming to increase efficiency and reduce the time spent on biometric evaluation of fruit and seeds, image processing technique has been used in forest and agronomic species, such as *Anadenanthera colubrina* (Vell.) Brenan, *Erythrina velutina* Willd, and *Echinochloa frumentacaea* (L.) (FÉLIX et al., 2018; VENKATESAN; SUJATHA, 2018). In addition to studies related to the biometric characterization of fruit and seeds, it is necessary to standardize the germination test to understand the proper conditions for forest species to start the germination process.

Some methods are used to maximize the germination process in seeds with coats that can inhibit the resumption of embryo development, such as strong acids, abrasions against a solid surface (sandpaper), among others (FREIRE et al., 2019). However, these methods are not always effective in obtaining seedlings in quantity and with quality because seeds from some species cannot germinate under nursery conditions, thus requiring the adoption of other propagation techniques, such as *in vitro* germination.

In vitro seed germination or vegetative embryo rescue is a viable alternative to shorten the reproduction cycle and overcome seed dormancy, and it is also applied when there is deficient embryonic development or when the embryo is immature (KAVERI; RAO, 2015). Therefore, it is a technique that can help in the seedling production and prevent genetic erosion caused by indiscriminate collection, excessive use, and frequent deforestation in areas of natural occurrence of vegetal species.

In view of this scenario, this study aimed to analyze and compare the biometric characteristics of *C. oncocalyx* diaspores and seeds by digital image processing and manual biometrics, and establish a propagation protocol, testing *in vitro* germination and *ex vitro* emergence, so as to identify an efficient methodology in the seminiferous propagation of *C. oncocalyx*.

MATERIAL AND METHODS

Cordia oncocalyx diaspores were collected from eight individuals located at the Wild Animal Multiplication Center (CEMAS, Brazilian acronym) at the Federal Rural University of the Semiarid Region, in the municipality of Mossoró, Rio Grande do Norte State (RN), Brazil. In this phase, a clamp was used (this was the most suitable method for removing seeds, as the diaspores have a rigid endocarp), which was placed in a horizontal position so as not to damage the embryos. Then, the seeds were classified as intact seeds, stunted seeds, and predated seeds (with larvae and insects), and their frequency was calculated to assess the results. Afterwards, the color and shape of the diaspores and seeds were evaluated.

In the biometric description of the diaspores, three replicates of one hundred diaspores were randomly separated to find their length and diameter. The same procedure was done for seeds to find their length, width, and thickness. For diaspores and seeds, length values were found by measuring them from the base to the apex with a digital caliper (0.01 mm precision). The diameter, width, and thickness were measured in the central median region; the diameter of the diaspores and the width of the seeds were measured in the horizontal region, and the thickness of the seed was measured in the region between their 'back' and 'belly'.

Diaspores and seeds from manual biometrics were used for biometrics by digital image processing. A 12 mp lens was used for shooting images at 50 cm distance on an ethylene vinyl acetate (EVA) sheet background, using white sheet for diaspores and blue sheet for seeds, which were marked using a ruler graduated in millimeters as a reference metric. The images were transferred to a computer and processed using ImageJ® software version 1.46, and were selected and defined in millimeter scale, converted to 8-bit format. Finally, a threshold mask was used to contrast the image components for particle analysis (fruit and seeds) using data output in Excel® format (FÉLIX et al., 2020).

Area (mm²), perimeter (mm), circularity (0.0 to 1.0), length (mm), width (mm), roundness (0.0 to 1.0), and solidity (0.0 to 1.0) were the biometric parameters analyzed. Fresh mass was evaluated on a

0.0001 g precision analytical balance (g) for seeds and diaspores.

In the ImageJ® program, the perimeter was calculated from the length of the outer boundary of the seed and diaspore in calibrated square units (mm), and the area was calculated within the polygon defined by the perimeter (mm²) (FERREIRA; RASBAND, 2012).

The biometric characteristics of the diaspores and seeds were analyzed by frequency distribution and descriptive statistics, then the data were subjected to analysis of variance and the method means were compared by Tukey test at a 5% significance level using the BioEstat® software version 5.0.

The diaspores were manually pulped for the emergence test, leaving the endocarp, which was subjected to mechanical and chemical scarification with a grinding engine with sixty sandpaper and concentrated sulfuric acid (density of 1.84% and purity of 95 to 98%), respectively, attempting to reduce the thickness of the diaspore wall, which is a physical barrier to germination, and expose the seeds to better germination conditions, such as humidity.

The treatments consisted of: (T0) the control, in which there was no kind of scarification; (T1) scarification in the peduncular region of the diaspore with 24 h hydration; (T2) scarification in the peduncular region of the diaspore without hydration; (T3) scarification in the opposite region to the peduncle with 24 h hydration; (T4) scarification in the opposite region to the peduncle without hydration; (T5) scarification in the lateral portion of the diaspore with hydration for 24 h; (T6) scarification in the lateral portion of the diaspore without hydration; (T7) acid scarification with immersion in concentrated sulfuric acid for 90 min; and (T8), acid scarification with immersion in concentrated sulfuric acid for 180 min. Diaspores immersed in concentrated sulfuric acid were constantly turned over, aiming to uniform the acid abrasive action. After the pre-established periods, they were washed in running water for 10 min, so that the concentrated sulfuric acid was completely removed.

Randomized block design, with four replicates, twenty-five diaspores, was used in the experiment for each treatment. The sowing was done in plastic polyethylene seedbeds with previously washed, sieved, and sterilized sand. Normal seedlings having the root system and shoot was the criterion used to assess the emergence (BRASIL, 2013), and the results were expressed in percentage.

The evaluations were done for the emergence percentage (E%) and the emergence speed index (ESI), with daily counts of normal seedlings were for ninety days, and the index was calculated according to the formula proposed by Maguire (1962).

Emergence data were subjected to analysis of variance and the means of each treatment were compared to each other by Tukey test at 5% significance level by the BioEstat® software version 5.0.

As for *in vitro* germination, 360 seeds were distributed in M&S culture medium described by Murashige and Skoog (1962). The experiment consisted of four treatments: (a) T1=12 g/L of sucrose and 1.80 g/L of medium; (b) T2=12 g/L of sucrose and 0.90 g/L of medium; (c) T3=6 g/L of sucrose and 1.80 g/L of medium; and (d) T4=6 g/L of sucrose and 0.90 g/L of medium, and all with 3% agar, differing in the concentration of sucrose and culture medium; the pH was adjusted between 5.9 and 6.2.

The culture medium was autoclaved at 120 °C for 30 min, and then placed into Petri dishes, 90 mm x 15 mm dimension. After cooling the culture medium, the seeds were disinfected by immersing them in 70% alcohol (v/v) for 30 s, followed by 2.5% sodium hypochlorite (v/v) for 10 min, then washed three times in deionized water, and placed on the plates and in a Biochemical oxygen demand (BOD) incubator under an average temperature of 25 ºC±2 °C and a photoperiod of 12 h (Figure 1A). The volume of medium used in each plate was 13 mL.

The experimental design was in randomized blocks, with four treatments and fifteen replicates of six seeds per plate. The germination percentage (G%) and the germination speed index (GSI) of the seeds in each treatment were evaluated (MAGUIRE, 1962).

The germination data were subjected to analysis of variance and the means of each treatment were compared to each other by Tukey test at 5% significance level, using the BioEstat® software version 5.0.

Nine days after sowing, the germinated seeds were transferred to plastic cups (50 mL), which were filled with coconut fiber substrate and wrapped with transparent plastic bags to maintain the relative humidity in the environment and consequently the acclimatization of the seedlings (Figure 1B, Figure 1C). These plastic cups were kept at 25 °C. After fifteen days, the seedlings were transferred to the greenhouse under 75% shade net and were kept in 1-L pots, containing washed and sterilized sand as substrate. After seven days, the seedlings were transferred to an area under 50% shading (Figure 1D).

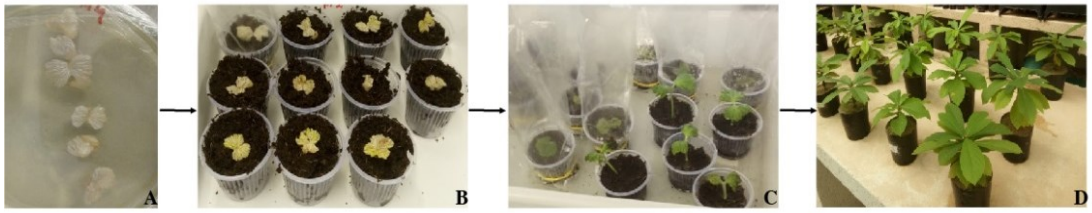

Figure 1. *Cordia oncocalyx* seeds germinated *in vitro* (A); acclimatization process (B); seedlings ready for transplanting (C); seedlings in a greenhouse under 50% shading (D).

RESULTS AND DISCUSSION

Diaspore of *C. oncocalyx* is dry and has indehiscent nucleus in elliptical shape. The epicarp is plain, glossy, and its color varies from light brown to dark brown, with a stony endocarp. They are surrounded by an increasing chalice in light brown color, which facilitates its spreading by the wind. Diaspores have one to four seeds; however, in this study, the predominance was on average two seeds per diaspore. The seeds are in an elliptical acuminate shape and white color (Figure 2).

The description of the external characteristics of diaspores and seeds of *C. oncocalyx* is in agreement with what was described by Carvalho (2008). Furthermore, for arboreal nuts species, the brown color is associated with the maturation point and, consequently, with better performance of seed germination (LEÃO et al., 2015).

Diaspores have two seeds on average, one intact and one stunted, or both intact. From 800 open diaspores, 1659 seeds were obtained, of which 46% were intact and 38% were stunted, with a prevalence of well-formed seeds compared to the stunted ones. It was found that 16% of the seeds were predated (without the presence of the embryo) and their predator was in larvae or adult form. The larvae that develop inside it emerge and make small holes in the central region of the diaspore, when they reach the adult phase (Figure 3).

Figure 2. Increasing chalice, diaspore, and seed of *Cordia oncocalyx*.

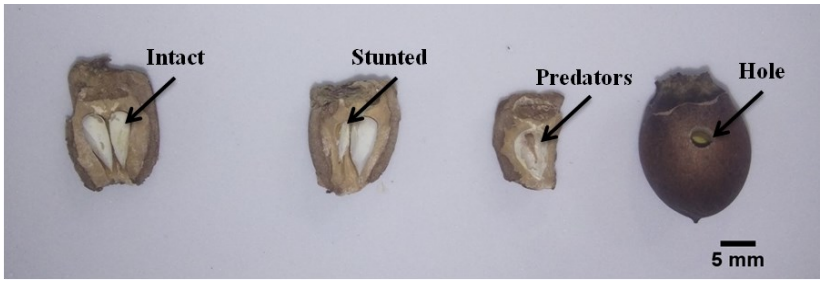

Figure 3. *Cordia oncocalyx* diaspore with intact seeds, intact and stunted seed, predated seed, and holes caused by the insect.

Although it is characteristic of each species, the number of seeds that compose the fruit can be affected by environmental factors (EMERY; OFFORD, 2019). Brito and Araújo (2009) verified that the seeds present in the fruit of *Cordia oncocalyx* are predated by *Pachymerus nucleorum* (Fabricius) larvae (Bruchidae, Coleoptera), and the predation rate reaches 20%, a value close to that found in this study, which recorded 6% of the attacked diaspores. Although the attack by the insect reduces seed viability, this fact can be a strategy that facilitates germination, since the perforations of the *P. nucleorum* coleopteran help water entering into the diaspore, an essential factor for germination when it occurs in soil.

Regarding the manual biometric analysis, the mean values for the length and diameter of the diaspores were 17.85 mm and 13.64 mm, respectively. The fresh mass had an average of 1.11 g. For biometrics by digital image processing, the diaspores had an average length of 19.83 mm (ranging from 16.32 mm to 25.80 mm) and an average diameter of 15.60 mm (ranging from 12.58 mm to 20.64 mm). Circularity was 0.82 mm on average. The area was 233.75 mm² on average. The perimeter was 59.87 mm on average. The roundness was 0.78 mm, and solidity was 0.97 mm (Table 1).

Table 1. Descriptive statistics of manual biometric evaluation and digital image processing biometric evaluation of three hundred *Cordia oncocalyx* diaspores.

Biometrics characteristics	N	Mean ± SE	Min	Max	SD	CV (%)
Manual biometrics	300					
Length (mm)		17.85 ± 0.08 a	12.49	23.83	1.16	8.50
Diameter (mm)		13.64 ± 0.07 A	10.11	18.92	0.10	7.18
Fresh mass (g)		0.93 ± 0.00	0.55	1.70	0.16	17.50
Digital biometrics	300					
Length (mm)		19.83 ± 0.08 b	16.32	25.80	1.53	7.76
Diameter (mm)		15.60 ± 0.07 B	12.8	20.64	1.25	8.06
Area (mm²)		233.75 ± 1.72	166.85	384.35	29.76	12.73
Perimeter (mm)		59.87 ± 0.24	49.90	78.28	4.07	6.80
Circularity (0.0 – 1.0)		0.82 ± 0.00	0.68	0.88	0.03	4.16
Roundness (0.0 – 1.0)		0.78 ± 0.00	0.60	0.97	0.07	8.53
Solidity (0.0 – 1.0)		0.97 ± 0.00	0.93	0.98	0.01	0.89

N=sample size; Min=minimum; Max=maximum; SE=standard error; SD=standard deviation; CV=coefficient of variation. (Averages for length and diameter in relation to manual and digital biometrics with the same letter do not differ from each other, according to the Tukey test at a 5% significance level).

In manual biometrics and digital image processing, most diaspores had a prevalent length from 16.0 mm to 19.9 mm and a frequency of 86% and 57%, in the proper order (Figure 4A). Observations by manual biometrics, corresponding to 65%, found prevalent diameters distributed into classes from 10.0 mm to 13.9 mm; observations by digital image processing, corresponding to 88.67%, found prevalent diameters distributed into classes from 14.0 mm to 17.9 mm.

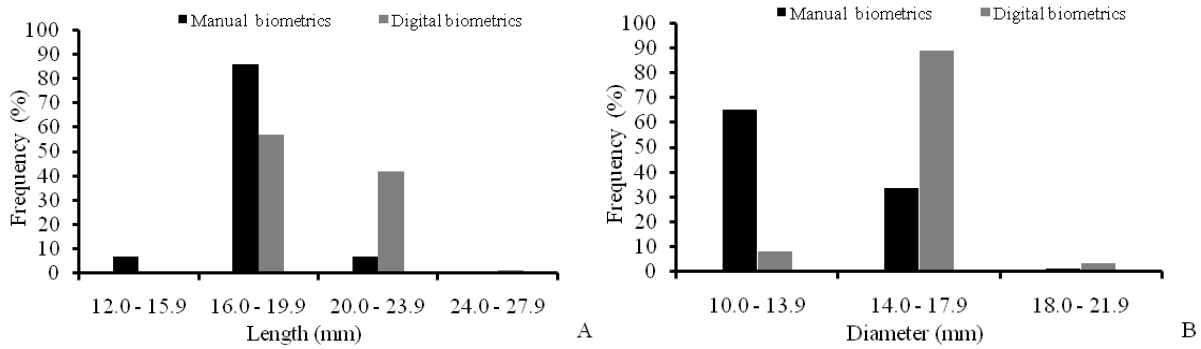

Figure 4. Frequency distribution of length (A) and diameter (B) of *Cordia oncocalyx* diaspores. N=three hundred diaspores.

The variability in the dimensions of fruits from native species can be attributed to genetic variability, biotic and abiotic environments, and genotype-environment interaction (SILVA et al., 2017). In this study, it was found that there was no significant variability for the length and diameter characteristics, and this fact can be related to the proximity of the parent plants in the collection environment, and because they are under the same edaphoclimatic conditions.

For manual biometrics, the seeds had an average length of 7.07 mm (ranging from 3.39 mm to 8.54 mm), width of 4.26 mm (ranging from 3.08 mm to 6.96 mm), thickness of 3.23 mm (ranging from 2.24 mm to 4.39 mm), and the average fresh mass of 0.050 g (ranging from 0.028 g to 0.073 g). In biometrics by digital image processing, the seeds had an average length of 7.07 mm (ranging

from 5.47 mm to 8.91 mm) and an average width of 4.48 mm (ranging from 3.28 mm to 5.78 mm). The circularity for *C. oncocalyx* seeds was 0.75 mm on average. The seed area showed 22.49 mm² on average, 14.48 mm² minimum and 29.64 mm² maximum. For the perimeter, a value of 19.38 mm was found with 15.22 mm minimum and 22.52 mm maximum (Table 2).

Table 2. Descriptive statistics of manual biometric evaluation and digital image processing biometric evaluation of three hundred *Cordia oncocalyx* seeds.

Biometrics characteristics	N	Mean ± SE	Min	Max	SD	CV (%)
Manual biometrics	300					
Length (mm)		7.07 ± 0.04 a	3.39	8.54	0.72	10.23
Width (mm)		4.26 ± 0.03 A	3.08	6.96	0.51	12.00
Thickness (mm)		3.23 ± 0.02	2.24	4.39	0.40	12.32
Fresh mass (g)		0.05 ± 0.00	0.028	0.073	0.01	20.31
Digital biometrics	300					
Length (mm)		7.07 ± 0.04 a	5.47	8.91	0.62	8.62
Width (mm)		4.48 ± 0.03 B	3.28	5.78	0.46	10.29
Area (mm²)		22.49 ± 0.17	14.48	29.64	3.01	13.36
Perimeter (mm)		19.38± 0.08	15.22	22.52	1.34	6.89
Circularity (0.0 – 1.0)		0.75 ± 0.00	0.63	0.87	0.04	5.96
Roundness (0.0 – 1.0)		0.60 ± 0.00	0.44	0.95	0.09	14.27
Solidity (0.0 – 1.0)		0.95 ± 0.00	0.91	0.96	0.01	0.89

N=sample size; Min=minimum; Max=maximum; SE=standard error; SD=standard deviation; CV=coefficient of variation. (Means with the same letter do not differ from each other by the Tukey test at a 5% significance level, comparing the same biometric characteristic for manual and digital biometrics).

In relation to frequency classes, in general, greater representation can be found in the intervals from 5.0 mm to 6.9 mm and from 7.0 mm to 8.9 mm with 41% and 58% and with 30.3% and 69.7% (Figure 5A) for the length of seeds in manual and digital biometrics, respectively. The prevalent width was 3.0 mm to 4.9 mm for both biometrics, with a frequency of 92% for manual biometrics and 84.7% for digital biometrics, respectively. The lowest frequency of seed width occurred from 5.0 mm to 6.9 mm class with 8.0% and 15.3% for manual and digital biometrics, in the proper order (Figure 5B). Regarding thickness, the greatest representation is found in the range of 2.0 mm to 3.9 mm with 97.7% (Figure 5C).

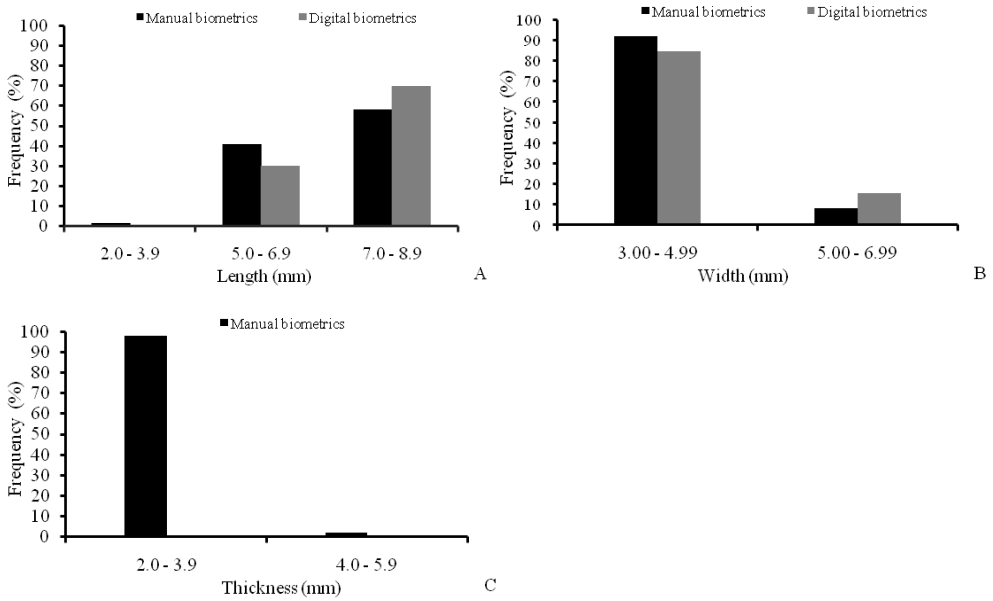

Figure 5. Frequency distribution of length (A), width (B), and (C) thickness of *Cordia oncocalyx* seeds. N=three hundred seeds.

Biometric variations in seeds are influenced by fruit, which are affected by environmental factors such as differences in pollination among inflorescences, strategy in nutrient use, and available water resources. As well as the genotypic diversity of populations, which can result in different phenotypic characteristics, the effect of the environment on seed development is mainly expressed by variations in size, in weight, and in physiological and health potential (BARROS et al., 2020; CORREIA et al., 2019).

In general, manual biometrics and digital image processing biometrics for diaspores and seeds differed statistically from each other, and these differences may be related to measurement errors when using the caliper. Biometrics by digital image processing had resulted in lower values of standard error (SE), standard deviation (SD), and coefficient of variation (CV) for length, diameter, and width, so it is a more viable and faster method for the biometry of diaspores and seeds of *C. oncocalyx*, when compared to manual biometrics. Coefficient of variation below 20% shows the precision of the experiment (MENEGATTI et al., 2017). The only exceptions were for the diaspore area, in which the standard deviation was higher, indicating a high dispersion of data in relation to the mean sample, which may have occurred due to irregularities in the diaspore shape and in the fresh mass of the seeds, as the coefficient of variation was above 20%.

In this study, the circularity in diaspores and seeds was not close to one; the closer the circularity is to one , the closer the seed is to a circle (FÉLIX et al., 2020), which was expected due to the elliptical shape of seed and diaspores. Thus, the use of digital image analysis helps in the development of many indices that allow finding seed shape, which can be used in comparative taxonomy, genetics, and biochemistry (CERVANTES; MANTÍN; SAADAOUI, 2016).

Considering the results presented, digital image analysis confirms its efficiency in obtaining biometric characteristics of *C. oncocalyx* seeds and diaspores, characterizing itself, therefore, as an easy, fast, and accurate tool of low operational and economic costs. The efficiency and precision of the analyses are associated with the biometric method used and the high sample quantity adopted, which is only feasible using computerized image analysis (FÉLIX et al., 2020).

Regarding seedling emergence, it was found that there was no emergence over ninety days of evaluation in treatments T0, T1, T2, T3, T4, T5 and T7, with no significant difference. However, there was an exception in the treatment in which the diaspore was scarified in the lateral region without hydration (T6), in which an emergence percentage of 0.33% was found, resulting in a GSI of 0.12 (Table 3).

Table 3. Emergence percentage (E%), emergence speed index (ESI) of *Cordia oncocalyx* diaspores cultured *ex vitro*.

Treatments	E%	ESI
T0	0 b	0 b
T1	0 b	0 b
T2	0 b	0 b
T3	0 b	0 b
T4	0 b	0 b
T5	0 b	0 b
T6	0.33 a	0.12 a
T7	0 b	0 b
T8	0 b	0 b

T0=Control (intact diaspore); T1=scarification in the peduncular region of the diaspore with 24-h hydration; T2=scarification in the peduncular region of the diaspore without hydration; T3=scarification in the opposite region to the peduncle of the diaspore with 24-h hydration; T4- scarification in the opposite region to the peduncle of the diaspore without hydration; T5=scarification in the lateral portion of the diaspore with hydration for 24 h; T6=scarification in the lateral portion of the diaspore without hydration; T7=acid scarification with immersion in concentrated sulfuric acid for 90 min; T8=acid scarification with immersion in concentrated sulfuric acid for 180 min. (Means with the same letter do not differ from each other by Tukey test at 5% probability).

The seeds of *C. oncocalyx* under natural conditions, that is, in intact fruit, take 70 to 120 days to emerge, because the fruit has a rigid endocarp (CARVALHO, 2008), which was also found in this study, that is, there was no seedling emergence at ninety days in intact diaspores.

The non-emergence in treatments with scarification and imbibition of the diaspores in water for 24 h in this study may be related to the absence of O_2 and the place of the scarification. Extended

periods of imbibition reduce the amount of O_2 molecules in the seed intercellular spaces, reducing efficiency rates in energy production, resulting in the delay and stoppage of the germination process, or even the death of the embryo (MARCOS FILHO, 2015). The relevance of the scarification place is probably due to the variations that the different openings can promote in the water absorption (BEWLEY; BLACK, 1994).

Due to the above-mentioned factors, the treatment with lateral scarification and without soaking could have provided greater water permeability, also it did not damage the seed structure. Although scarification with sulfuric acid is very efficient to break the dormancy of forest seeds with waterproof integuments such as *Peltophorum dubium* (Spreng.) Taub. (DUTRA et al., 2012), *C. oncocalyx* diaspores have not responded positively to this treatment.

The lower emergence percentage and consequently the lower emergence speed index can be associated with mechanical dormancy, which results from the diaspore impermeability and prevents water to enter, and which explains the lower emergence percentage of the sown diaspores. In addition, it may be related to a physiological or morphophysiological dormancy of the seeds, since the seeds had not emerged in any treatment, even with imbibition.

In addition, another factor that may have promoted the lower emergence percentage and emergence speed index is the viability of seeds that are attacked by insects because the conditions presented cannot be seen when sown inside the diaspore. The shortest time for germination to occur is relevant when producing seedlings, because seeds can undergo rapid deterioration when spending a long time exposed to environmental conditions without germinating (BEZERRA et al., 2014). In addition, the cost of the seedling can be higher due to the longer period for production, greater use of inputs, and manpower involved.

Thus, *C. oncocalyx* seeds were removed from the diaspores and cultivated *in vitro* to overcome the inconveniences related to their conventional germination. It is important to highlight that in ninety days of experimentation, *ex vitro* emergence provided three seedlings, unlike *in vitro* germination, in which seeds have started to germinate on the fifth day after sowing, with 97% germination in the T4 treatment (Table 4), showing itself to be an efficient alternative for this species' spreading.

The method to be used to obtain seedlings of forest species varies from species to species, since each individual has specific needs to start its germination process, such as nutrition, temperature, and asepsis, factors that can be reached under controlled environments, such as *in vitro* germination.

In treatments T4 and T3, the seeds started the germination process on the fifth day after sowing, stabilizing on the seventh day with a germination percentage of 97% and 84%, respectively. Although T4 does not differ statistically from T3, it becomes economically viable, since it used only half of the culture medium. As for T1 and T2, lower germination percentages were found with 34% and 64%, in the proper order; both treatments started the germination process on the sixth day after sowing (Table 4).

The same occurred in the GSI for T3 and T4, in which they did not differ statistically from each other. The lowest GSI was found in seeds submitted to T1 and T2 with 5 and 7.22, respectively. The higher the GSI, the higher the seed germination speed, which indicates greater seed vigor (OLIVEIRA et al., 2009).

Table 4. Germination percentage (G%) and germination speed index (GSI) of *Cordia oncocalyx* seeds cultivated *in vitro*.

Treatments	G%	GSI
TI	34 c	5.00 c
T2	64 b	7.22 b
T3	84 a	12.80 a
T4	97 a	15.05 a

T1=12.0 g sucrose and 1.80 g MS; T2=12.0 g of sucrose and 0.90 of MS; T3= 6.0 g sucrose and 1.80 MS; T4=6.0 g sucrose and 0.90 MS. (Means with the same letter in the column do not differ from each other by Tukey test at 5% probability).

M&S is among the most used *in vitro* culture media, which is highly enriched with macronutrients, micronutrients, and inorganic salts, necessary for the beginning of the germination and growth process of seedlings cultivated *in vitro* (KONÉ et al., 2015).

However, plants grown in environments with a high concentration of salts and/or sugars have low osmotic and water potential, resulting in metabolic changes and accumulation of amino acids at high levels in response to water deficit, which can

influence germination and root formation, since the increase in the concentration of salts and sugars in the culture medium causes decrease in water absorption (LEMES et al., 2016), which was noticeable in T1 and T2 sown at higher concentrations of sucrose.

Studies have shown that 50% reduction in M&S medium and sucrose promoted better results regarding germination, *in vitro* growth, and seedling quality of *Muntingia calabura* L., *Handroanthus impetiginosus* (Mart. ex DC.) Mattos, *Jacaranda brasiliana* (Lam.) Pers, and *Vigna subterranea* (L.) Verdc, resulting in a germination percentage of up to 90% (ARENCIBIA et al., 2018; PIERINE; GIANINI; MORAES, 2019; KONÉ et al., 2015; SOUZA et al., 2020). The suppression or reduction of sucrose and the *in vitro* culture medium allows plants to modulate carbohydrate metabolism and photosynthesize easily during acclimatization, reducing seedling production costs (LEMBRECHTS et al., 2017).

Considering the results found, the protocol developed in this study can be used to obtain *C. oncocalyx* seedlings, in addition to serving as a tool for the species conservation. *In vitro* germination becomes an ideal alternative to the classic spreading methods of native forest species, as it increases the multiplication rate (PEREIRA; NAVROSKI; REINIGER, 2015). The possibility of cultivating plants in glass allows the *in vitro* germination to be a technique that requires less space and labor compared to the conventional propagation method (LIU et al., 2016).

CONCLUSIONS

Biometrics by digital image processing becomes applicable, since it is a reliable, fast, and accurate tool for the biometric characterization of *C. oncocalyx* diaspores and seeds, contributing to optimize process and reduce operational errors. The *in vitro* germination protocol developed in this study promoted positive germination results for the species *C. oncocalyx*, proving to be an efficient technique for obtaining large-scale seedlings of this species. Although *in vitro* germination has promoted positive results in terms of *C. oncocalyx* seed germination, studies related to vegetative spreading are still needed, which can support new seedling production strategies for *C. oncocalyx*.

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