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Handroanthus impetiginosus biometry of seed and seedling production after different periods of immersion in water

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Abstract – The objective of this work was to determine the biometric characteristics of *Handroanthus impetiginosus* seed, and to evaluate the effect of different periods of seed immersion in water on the initial growth of seedlings. Seed and embryo characteristics were analyzed for length, width, and thickness. Moisture content, one thousand seed weight, and the number of seed per kilogram were determined. Seed were subjected to 10 immersion treatments in water and to one control without water. The treatments in water consisted of immersion for 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 hours. Lengths of seedling aerial parts and roots were analyzed 30 days after sowing. Water-use efficiency was assessed. The moisture content was 60.03%. The thousand seed weight was 29.860 g, corresponding to about 33,490 seed kg^{-1}. Seed with lateral wings showed 4.486, 0.890, and 0.101 cm for length, width, thickness, respectively. Embryo showed 0.981, 0.655, and 0.076 cm, for length, width, and thickness, respectively. Seed immersion in water for six hours expresses the best results for growth and water-use efficiency, leading to vigorous seedlings of *H. impetiginosus*.

Index terms: Bignoniaceae, seed technology, semiarid zones.

Biometria de sementes de *Handroanthus impetiginosus* e produção de mudas após diferentes períodos de imersão em água

Resumo – O objetivo deste trabalho foi determinar as características biométricas das sementes de *Handroanthus impetiginosus* e avaliar o efeito de diferentes períodos de imersão das sementes em água sobre o crescimento inicial das plântulas. As características das sementes e dos embriões foram analisadas quanto ao comprimento, largura e espessura. O teor de umidade, a massa de mil sementes e o número de sementes por quilograma foram determinados. As sementes foram submetidas a 10 tratamentos com imersão em água e um tratamento-controle sem água. Os tratamentos com água consistiram de períodos de imersão por 6, 12, 18, 24, 30, 36, 42, 48, 54 e 60 horas. Os comprimentos da parte aérea e da raiz das plântulas foram analisados 30 dias após a semeadura. A eficiência de uso da água foi determinada. O teor de umidade foi de 60,03%. A massa de mil sementes foi de 29,860 g, o que corresponde a 33.490 sementes kg^{-1}. As sementes apresentaram comprimento, largura e espessura de 4,486, 0,890 e 0,101 cm, respectivamente. Os embriões apresentaram 0,981, 0,655 e 0,076 cm quanto ao comprimento, largura e espessura, respectivamente. A imersão das sementes em água por seis horas apresenta os melhores resultados de crescimento e eficiência de uso da água para a obtenção de plântulas mais vigorosas de *H. impetiginosus*.

Termos para indexação: Bignoniaceae, tecnologia de sementes, zonas semiáridas.

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Introduction

Handroanthus impetiginosus (Mart. ex DC.) Mattos is a tree species native to Brazil; it can reach from 20 to 35 m height and is popularly known as *ipê-roxo*, *pau-d'arco-roxo*, and *ipê-roxo-da-mata* (Martins et al., 2011; Silva et al., 2015). This species is commonly found in the Brazilian biomes as *Caatinga*, *Cerrado*, Atlantic Forest, *Pantanal*, and Amazon (Maia-Silva et al., 2012). It is part of the upper strata of the forest and has an extended lifespan (Amado et al., 2015).

H. impetiginosus has opposite leaves with five leaflets, long round and pendent fruit ranging from 25 to 30 cm, and flat seed with delicate wings, which favors dispersal by wind. Its wood is characterized as heavy, hard, very resistant but flexible, which allows of the production of bows by indigenous people (Castro & Cavalcante, 2011).

Wood of *H. impetiginosus* is of great economic interest considering its versatility, as it can be used in multiple market categories. It is employed in construction, manufacturing of sporting goods, handles for agricultural tools, turned pieces, and musical instrument production, as well as providing high-quality firewood (Moraes Neto, 2021). However, this has been the reason behind the species' excessive exploitation, highlighting the need for studies aiming at its conservation (Fonseca Filho et al., 2016).

Seed biometry is a relevant tool to detect the genetic variability within populations of the same species, and the relationships of this variability with environmental factors, as well as in breeding programs (Gonçalves et al., 2013). The identification of genetic variability can support the adoption of strategies for the early growth of *H. impetiginosus*, to provide significant advantages for the conservation of this species, promoting the recovery of populations, the efficiency of reforestation programs, and the sustainability of the ecosystems where this tree is native.

Seed characteristics play a relevant functional role because they directly reflect how plants can overcome stress conditions (Patrício & Trovão, 2020). Several studies have reported that larger seed effectively produce larger seedlings, whereas smaller seed produce smaller and lower-quality seedlings (Dranski et al., 2019). Thus, it is necessary to know the biometric characteristics of native forest seeds, in order to propagate vigorous subjects that can support reforestation programs.

Seed quality is one of the factors that directly interfere with seedling formation (Lima Filho et al., 2019). An important qualitative aspect is seed dormancy, which delays germination (Silva et al., 2022). Dormancy occurs when a seed or tuber does not germinate, even under the necessary conditions of temperature, humidity, oxygen, and light (Yildiz et al., 2017). Although dormancy is observed in the field for *H. impetiginosus*, there are no studies on the subject.

In this context, aiming to break the seed dormancy of native plants, different methods were developed to accelerate a process that would occur naturally under favorable conditions (Porto et al., 2019). One of the methods that stands out is the immersion of seed in water, as described by Almeida et al. (2023) when studying seed of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex. S. Moore. This process results in tissue rehydration, which intensifies breathing and other metabolic activities, promoting the supply of energy and nutrients necessary for the growth of the embryonic axis (Carvalho & Nakagawa, 2012).

The objective of this work was to determine the biometric characteristics of *H. impetiginosus* seed, and to evaluate the effect of different periods of seed immersion in water on the initial growth of seedlings.

Materials and Methods

This research was conducted in the municipality of Sumé, located in Cariri Oeste, a microregion of Paraíba state, in northeastern Brazil. The climate in the region is characterized by rainfall concentrated from three to four months, irregularly distributed in time and space, with high means of annual temperature (from 25ºC to 27ºC), mean insolation of 2,800 hours per year, relative air humidity about 50%, and mean evaporation rates about 2,000 mm per year, according to Diniz et al. (2020). In Brazil, this area is known as the Caatinga biome. Hyperxerophilic *caatinga* vegetation predominates in the region, mostly with well-developed Chromic Luvisols and gently undulating topography (Ribeiro et al., 2014).

Ripe fruit of *H. impetiginosus* were collected in December 5, 2017, at the dispersal stage from adult matrices in Sumé (07°40'18"S, 36°52'48"W; at 532 m altitude). After the collection, the fruit were packed in polyethylene bags and taken to the Laboratory of Ecology and Botany of the Universidade Federal

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of Campina Grande (LAEB/UFCG), for manual extraction of seed.

In the present work, the studied seed are winged ones. The wingless seed is the embryo. Physical characterization was performed on seed with lateral wings to allow of the comparison with existing literature. Hydration tests were performed on seed. Seed and embryos were subjected to biometric measurements for length, width, and thickness.

Manual sorting was performed to obtain the biometric data of *H. impetiginosus* seed, discarding those visibly damaged or deformed. Then, a mix was made to select a sample of 100 seed. Using a digital caliper with 0.01 mm accuracy, the following seed dimensions were determined: length from the base to the apex; width between the right and left end; and thickness between the ventral and dorsal part of seed with lateral wings (Figure 1 A) and embryo (Figure 1 B).

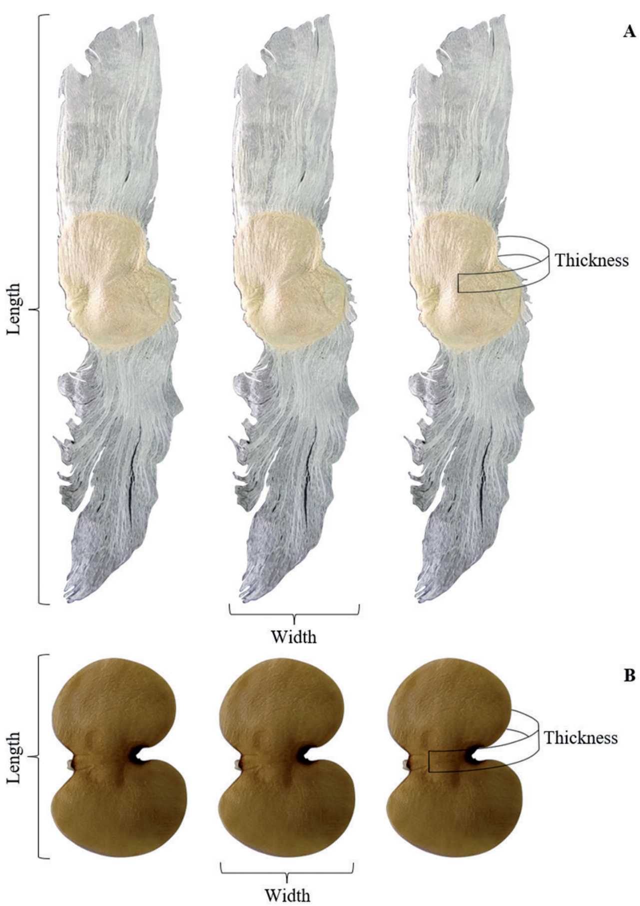

Figure 1. Biometric characteristics of seed (A) and embryos (B) of *H. impetiginosus*.

Biometric data were subjected to descriptive statistical analysis. Histograms were used to analyze the frequency distribution pattern of these data.

For the physical characterization, freshly harvested winged seed of *H. impetiginosus* were used. The tests described below were performed based on rules for seed analysis (Brasil, 2009). Eight replicates of 100 seed were used. The variance (S^2), standard deviation (S), and coefficient of variation (CV%) of the weight values (g) were calculated. The weight of one thousand seed was calculated by multiplying the mean weight of the 100-seed replicates by 10, as in the following equation:

$$\text{Weight of one thousand seed (g)} = X \times 10 \quad (1)$$

where: $\overline{X}$ is the mean weight of 100 seed.

Based on the one thousand seed weight obtained, the number of seed per kilogram was calculated.

Applying the rules for seed analysis (Brasil, 2009), seed moisture content was determined using four replicates of 25 seed, and each one was initially weighed under a wet condition and, then, subjected to drying in an oven at 105 ± 3ºC, for 24 hours, as described in the following equations:

$$\text{Moisture content (\%)} = \frac{100(W - w)}{W - t} \quad (2)$$

where: W is the initial weight (weight of the container with lid plus the wet seed weight); w is the final weight (weight of the container with lid plus the weight of the dry seed); and t is the tare (weight of the container with lid).

A completely randomized design was carried out with 11 treatments and 4 replicates. The experimental unit was a mix of 25 freshly harvested seed (undried). The treatments consisted of one control (no water immersion, that is, 0 hour), and 10 different periods of immersion in water, ranging from six to 60 hours, in steps of 6 hours.

Plant production (subjected to 50% shading) occurred in the laboratory of nursery for native seedlings production of the Universidade Federal de Campina Grande. Direct sowing was performed on December 31, 2017. The treatments consisted of 11 polyethylene trays (46 cm long, 31 cm wide, and 7 cm deep). Each tray was divided into four quadrants using

PVC material and, its base was perforated to allow of excess water drainage of during irrigation.

Sand was used as substrate, which was initially sieved and then washed. Each tray included 100 seed (four replicates of 25 seed) placed at 1.5 cm depth of. Irrigation was performed daily by providing 250 mL water per replicate, in a controlled manner using a graduated container. Thirty days after sowing, lengths (cm) of aerial part and roots of *H. impetiginosus* seedlings were measured using a graduated ruler.

To obtain the total dry mass (TDM, g), seedlings were placed in identified kraft paper bags and subjected to drying in an oven at 105°C for 24 hours. Afterward, the material was weighed using a digital scale with 0.001 g precision.

The water use efficiency (WUE) was obtained by the quotient between the increase of plant dry mass (primary productivity) and the total evapotranspiration water, which corresponded to the volume of water used to irrigate the plants (adapted from Hatfield et al., 2001), as described in the equation below:

$$\mathrm{WUE} = \frac{\mathrm{TDM}}{\mathrm{ETW}} \quad (3)$$

where: WUE is the water use efficiency (g L^{-1}); TDM is the total dry mass (g); and ETW is evapotranspiration water (L).

The data were subjected to the analysis of variance using the F-test (α=0.05). The normality assumption of the residuals was tested using the Shapiro-Wilk's test, while the homoscedasticity was evaluated with the Levene's test. The results indicated that both assumptions were met. Additionally, the independence of errors was confirmed through the inspection of the residuals. When there was a significant effect, the means were compared by the Scott-Knott's post-hoc test (α=0.05). The analyses were performed using Excel 2019, the R software package version 4.4.1 (R Core Team, 2024) with RStudio IDE, and the SISVAR version 5.8 (Ferreira, 2011).

Results and Discussion

The following paragraphs are mostly descriptions of the results obtained. The discussion was constrained by the limited literature on the biometrics and seed dormancy of *H. impetiginosus*. This study represents a significant initial contribution to the field, seeking to establish a base knowledge to support future researches.

H. impetiginosus seed with lateral wings showed 60.03% water content of. The weight of one thousand winged seed was 29.860 g, with 0.146 variance, 0.382 standard deviation, and coefficient of variation of 12.783%, corresponding to approximately 33,490 seed kg^{-1}.

Moisture content analysis assesses the seed quality of forest species. According to Sarmento et al. (2015), this variable directly influences several aspects of the physiological quality of seed, making its determination a process for quality tests of seed lots.

It is worth mentioning the relevance of seed weight, which for some species can be considered as an indicator of seed physiological quality. Lighter seed usually have a lower performance than heavier ones (Menezes et al., 2018). The number of seed per kilogram was within the expected range for the species, which varies from 8,000 to 35,000 (Brasil, 2013).

The mean values for length, width, and seed thickness were 4.486±0.035, 0.890±0.005, and 0.101±0.001 cm, respectively. All characteristics of the assessed seed had low-standard error values. Regarding standard deviation, the parameter length had the highest sample variance (0.352). As to the coefficient of variation, thickness stood out with the greatest variation (13.904%) compared to the mean of the other seed characteristics.

Embryo had mean length, width, and thickness of 0.981±0.009, 0.655±0.005, and 0.076±0.001 cm, respectively. Standard error values were also low, accurately describing the sample. In the standard deviation analysis, length had the highest sample variance (0.088). Regarding the coefficient of variation, thickness stood out with the greatest variation (12.037%).

When studying *H. impetiginosus*, Felix et al. (2018) found mean values for length, width, and thickness of seed with lateral wings of 3.9 x 1.2 x 0.2 cm, and embryo of 1.6 x 1.1 x 0.2 cm, respectively. These values are higher than those found in our research, except for the length of seed with lateral wings, which was higher than that found by the aforementioned authors. There are also biometric differences within the same family. Oliveira et al. (2006) obtained mean values for length, width, and thickness of *T. aurea* seed with lateral wings of 5.78 x 2.06 x 0.31 cm, and embryo of 1.73 x 1.33 x 0.17 cm, respectively.

The length of seeds with lateral wings had a negative asymmetrical distribution, evidenced by the greater distance from the median to the minimum point (Figure 2 A), whereas the width and thickness showed a positive asymmetric behavior (Figure 2 B and 2 C), influenced by the greater distance from the median to the maximum point (Table 1). The thickness showed less data dispersion, evidenced by the amplitude that was equivalent to 0.071 cm.

Embryo length and thickness had a negative asymmetrical distribution (Figure 2 D and 2 F), influenced by the greater distance from the median to the minimum point (Table 1), whereas the width was very close to symmetry (Figure 2 E), with a slight asymmetry to the right, due to the greater distance from the median to the maximum point (Table 1).

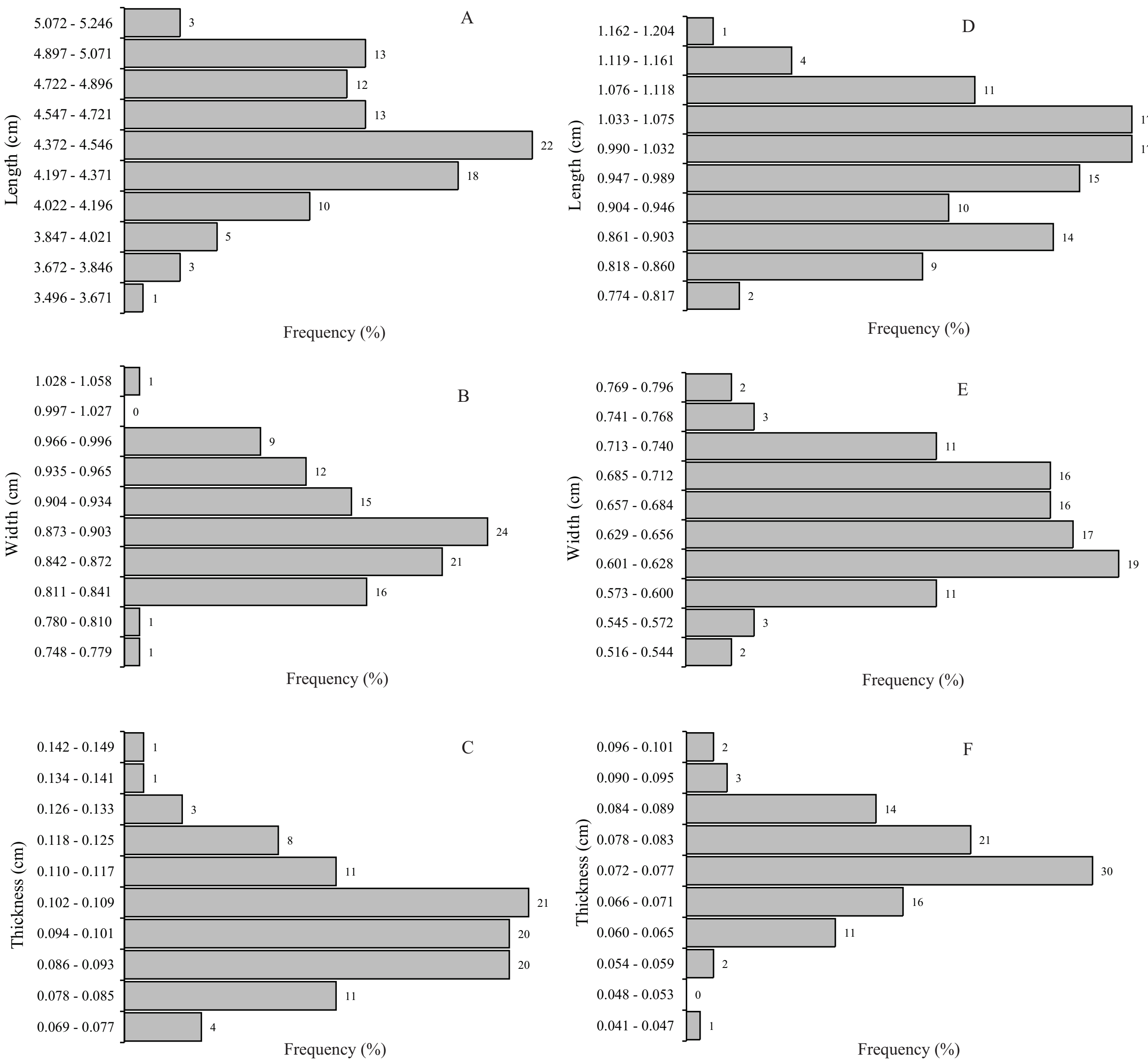

Figure 2. Frequency distribution of seed and embryos characteristics of *H. impetiginosus*: A, seed length; B, seed width; C, seed thickness; D, embryo length; E, embryo width (E); and F, embryo thickness.

Thickness had less dispersion of data, evidenced by the amplitude, corresponding to 0.054 cm (Table 1).

Regarding the five-point summary (Table 1), about 50% of the values of length, width, and thickness were below the median value for seed with lateral wings (4.425, 0.882, and 0.100 cm, respectively) and embryo (0.989, 0.653 and 0.075 cm, respectively).

Seed size is important because it can influence the germination. Size variation indicates a greater or lesser concentration of nutrient reserve (Pinheiro et al., 2017). Seed size has a positive correlation with the initial growth rate of seedlings. It results in vigorous subjects (Carvalho & Nakagawa, 2012).

The frequency distribution of *H. impetiginosus* seed with lateral wings showed that their length concentrated between 4.197 and 4.546 cm, with an accumulated frequency of 40% of seed (Figure 2 A). Seed width concentrated in the interval 0.811–0.903 cm, with 61% accumulated frequency of seed (Figure 2 B). Seed thickness was mainly in the interval 0.086–0.109 cm, with 61% of seed (Figure 2 C).

The frequency distribution analysis of seed embryos of *H. impetiginosus* showed that their length ranged mainly from 0.947 to 1.075 cm, corresponding to 49% of the total sample (Figure 2 D). Embryo width concentrated in the interval 0.601–0.712 cm, resulting in an accumulation of 68% of the samples (Figure 2 E). Embryo thickness concentrated mainly in the interval 0.072–0.083 cm, in 51% of the samples (Figure 2 F).

From the biometric frequency distribution of the species, it is possible to select the appropriate type of sieve to separate seed. This separation can be performed in several ways, such as the classification by sieves based on morphometric characteristics, considering parameters to better homogenize the size and/or mass of seed (Hanzen & Dranski, 2020).

Regarding the aerial part length of *H. impetiginosus* seedlings (Table 2), the immersion of seed in water for 6 hours resulted in the highest mean (3.9 cm), differing significantly from the other treatments. There was no significant difference between the control treatment and the immersion for 12 hours. In addition, the seed immersion in water for periods between 18 and 60 hours resulted in the lowest mean values of aerial part length.

Regarding the root length of *H. impetiginosus* seedlings (Table 2), there was no difference between the means in the different periods of seed immersion in water, which resulted in values ranging from 6.8 to 8.6 cm.

Concerning the WUE in the production of *H. impetiginosus* seedlings (Table 2), the immersion of seed in water for 0 hour (11.60 g L^{-1}), 6 hours (11.55 g L^{-1}), 12 hours (10.90 g L^{-1}), and 30 hours (9.97 g L^{-1}) resulted in the highest means, not differing significantly from each other. The seed immersion in water for 42 hours had the lowest WUE mean value.

The WUE mean in the 6-hour treatment agree with the values found by Chaves et al. (2018), who assessed the seed physiological quality and germination under different substrates and levels of light restriction; they found 3.91 cm aerial part height in a soil under full sun condition.

However, the highest WUE mean found in the present research is lower than the finding by Costa et al. (2021), who observed an improvement of the nutritional conditions of degraded soil treated with sewage irrigation, when producing *H. impetiginosus* seedlings. The seedling mean height of aerial part was

Table 1. Five-point summary of the descriptive statistical analysis of the biometric characteristics of seed and embryos of *H. impetiginosus*.

Biometric characteristic	Five-point summary of the boxplot				
	Minimum	First quartile	Median	Third quartile	Maximum
			Seed (cm)		
Length	3.583	4.269	4.425	4.793	5.158
Width	0.764	0.851	0.882	0.929	1.045
Thickness	0.073	0.091	0.100	0.109	0.144
			Embryo (cm)		
Length	0.795	0.907	0.989	1.045	1.178
Width	0.530	0.614	0.653	0.692	0.780
Thickness	0.044	0.070	0.075	0.082	0.098

6.0 cm, which may be related to the age (60-day-old) of seedlings in that study.

The highest mean of root length in the present study was higher than those reported in the literature. Bassegio et al. (2017) assessed the development of the species as a function of culture media (WPM and MS) and BAP doses, during the in vitro multiplication stage, and they found 8.12 cm root length in the WPM, and 4 mg L^{-1} BAP concentration.

Analyzing the influence of different substrates on the emergence and initial growth of *H. impetiginosus* seedlings, Oliveira (2021) recorded 8.47 cm root length in vermiculite and sand (1:1) substrate. In this sense, the treatments contributed to seedling root growth in the species, promoting greater productive and quality.

The initial periods of immersion of *H. impetiginosus* seed in water provided the highest WUE. This result may be related to the immersion treatments, since they resulted in higher means for the analyzed growth characteristics.

In a study on *H. impetiginosus*, Souza et al. (2018) found that *H. impetiginosus* was one of those with the highest WUE, which can be justified by the strict stomatal control combined with the development and leaf abscission that is peculiar to this deciduous species. It should be noted that studies involving WUE in the production of seedlings of any species are scarce, as also addressed by Bezerra et al. (2019).

Table 2. Mean and standard deviation of the aerial part length, root length, and water-use efficiency (WUE) of *H. impetiginosus* seedlings for each period of water immersion (treatment)[(1)].

Treatment (hours)	Aerial part length (cm)	Root length (cm)	Water use efficiency (g L^{-1})
0 (control)	3.2 ± 0.2b	7.4 ± 0.2a	11.60 ± 2.87a
6	3.9 ± 0.2a	7.3 ± 0.9a	11.55 ± 1.07a
12	3.5 ± 0.3b	8.2 ± 0.7a	10.90 ± 0.47a
18	2.8 ± 0.1c	8.4 ± 1.0a	8.37 ± 0.61b
24	2.9 ± 0.2c	7.6 ± 0.4a	8.48 ± 0.78b
30	2.7 ± 0.2c	8.6 ± 1.0a	9.97 ± 0.71a
36	2.9 ± 0.4c	7.5 ± 0.6a	8.11 ± 0.69b
42	2.1 ± 0.3d	6.8 ± 0.7a	4.79 ± 0.64c
48	2.8 ± 0.4c	6.8 ± 1.2a	6.69 ± 1.32b
54	2.9 ± 0.3c	7.8 ± 1.5a	8.02 ± 0.80b
60	2.9 ± 0.2c	7.0 ± 0.7a	7.53 ± 0.16b

[(1)]Means followed by equal letters, in the columns, do not differ significantly from each other by Scott-Knott's test, at 5% probability.

Conclusions

1. Seed of *Handroanthus impetiginosus* with lateral wings exhibit physical characteristics in accordance with what is expected for the species.
2. The biometric analysis indicates the possibility of use of sieves for the classification of *H. impetiginosus*.
3. The immersion of *H. impetiginosus* seed in water for 6 hours results in more vigorous seedlings.
4. The data shows genetic variability and provide information on the biometric characteristics of the species in the Semiarid region of Brazil.

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