



## **Biometric Characterisation and Physiological Quality of Seeds of *Hymenaea courbaril* L.**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JEAI/2018/43824

#### Editor(s):

(1) Dr. Mario Lettieri Teixeira, Professor, Federal Catarinense Institute – Campus Concordia, Brasil.

#### Reviewers:

(1) Egua Maxwell Osaronowen, College of Health Sciences, University of Abuja, Nigeria.

(2) R. Mahalakshmi, India.

(3) A. Kravtsov Alexander, North-Caucasus Federal University, Russia.

Complete Peer review History: <http://www.sciencedomain.org/review-history/26487>

**Original Research Article**

**Received 23 June 2018**  
**Accepted 13 September 2018**  
**Published 01 October 2018**

### **ABSTRACT**

**Aims:** The objective of this work was to verify the influence of seed size on germination and vigour of seeds and seedlings of *Hymenaea courbaril* L.

**Study Design:** The experimental design was completely randomised.

**Place and Duration of Study:** The present work was carried out in the Laboratory of seeds of the State University of the West the Paraná, Brazil, between January to July, 2018.

**Methodology:** The seeds were obtained in the Marialva region, Paraná, classified according to the biometric characteristics, in accordance with the range of values of seed length, in small, medium and large to later be mechanically scarified and immersed in water for 48 hours. The study evaluated the biometric characteristics, moisture content, germination, seed vigour and seedling development.

**Results:** In the present study, the size of the seeds reflected effects on the physiological quality of the seeds, mainly in the parameters related to the performance of seedlings.

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**Conclusion:** The seedlings evaluated from medium and large seeds showed better performance in the majority of parameters. In addition, the use of large seeds is recommended, because they have a higher percentage of seedling emergence and the best quality.

**Keywords:** Forest species; *Hymenaea courbaril* L.; Jatobá; morphology of seeds; physiological potential.

## 1. INTRODUCTION

The jatobá tree (*Hymenaea courbaril* L.), belonging to the family Fabaceae, is a species with great forestry and environmental importance, due to its multiple uses, such as the restoration of forest reserves, feeding of wildlife, medicinal use and potential as a fixative and atmospheric carbon storage, in its constituent assembly on the ground, which may be a sustainable alternative within integrated systems of agricultural production and forestry component [1,2].

The jatobá tree offers several relevant products, the bark, sap, resin and fruit have medicinal properties, the latter being edible, its wood is dense with high durability and workability and can be used in civil construction and fine furniture, increase its value. The species is considered rare, with irregular distribution, due to the harvest of its wood [3,2].

The performance of the seedlings of any plant species is influenced by the physiological quality of their seeds, and this constituted the viability and vigour of the same, another factor that is being evaluated for various species is the size of the seeds as a component of the quality [4]. Within their species, there are individual variations among the trees due to environmental influences during the development of the seed and the genetic variability [5].

Information on the biometric characteristics of the seed can provide subsidies for the selection of seeds, and genetic variables within populations of the same species, and the relationships between this variability and environmental factors, as well as breeding programs genetic [6].

Several studies have been done to demonstrate the importance of knowing the variation in biometric characteristics of different seeds, such as *Sesbania virgata* (Cav.) Pers [7], *Psidium rufum* DC. [8], *Pachira aquatica* Aubl. [9], *Schizolobium amazonicum* Huber ex Ducke [10], *Sterculia striata* A.St.-Hil. & Naudin [11],

*Agonandra brasiliensis* Miersex Benth. & Hook. F. [12], *Annona reticulata* (L.) Vell. [13].

According to Carvalho and Nakagawa [14], the largest and most dense seeds have a greater quantity of substances of reservation, which results in the formation of better embryos, resulting in more vigorous seedlings. According to Oliveira et al. [15], the seeds which are considered small, can manifest less performance than the seeds medium and large, within the same seed lot, and according to Silva et al. [16], to obtain seedlings of similar size and with a uniform emergence, a recommended tactic would be the classification of seed per size and mass.

Therefore, this study is based on the hypothesis that jatobá, as a species with several uses and great potential to explore the size of its seeds, can have a positive influence on the propagation and development of seedlings by the best initial growth until the best germination and force. Thus, the objective of this research work was to verify the influence of seed size on the germination and vigour of seeds and seedlings of jatobá (*Hymenaea courbaril* L.).

## 2. MATERIALS AND METHODS

The fruits of jatobá (*Hymenaea courbaril* L.) were obtained from headquarters located in Marialva, Paraná, Brazil, with the minimum distance between them, 100 meters away. The same was prepared in the Laboratory of Seed Technology, belonging to the State University of Western Parana, (UNIOESTE), Campus Marechal Cândido Rondon, Paraná, Brazil.

The extraction of the seeds was performed manually, consisting in the breaking of the fruit with the aid of a hammer and the endocarp chalky (pulp) removed with the help of a spatula. Subsequently, the tegument of seeds was scarified mechanically with the help of sandpaper iron, on the opposite end of the micropyle, without reaching the cotyledons, then immersed in water for 48 hours [17].

## 2.1 Biometrics Seeds

The seeds of jatobá were characterised biometrically, where they were assessed randomly. Subsequently, of the seed lengths (SL) were classified in small (SL ≤ 24.49 mm), medium (SL > 24.49 mm ≤ 26.49 mm) and large (SL ≥ 26.50 mm).

The following morphometric parameters were determined: length (mm) and diameter (mm) of seeds, measured with the aid of a digital calliper, and the length measured from the base to the apex and the width measured at the midline of the seed, Weight of a thousand seeds was measured, in accordance with the Rules for Seed Analysis (RSA), mass of dry matter and water content, obtained by the oven method 105 ± 3°C [18], for these last 4 repetitions was used with 7 seeds.

For each variable, mean values, maximum, minimum, standard deviation and coefficient of variation were calculated. The data of the length and width of seed were grouped into classes for the better presentation in the histogram of frequency. The data analysis was performed with the aid of an Excel spreadsheet.

## 2.2 Germination Test

The seeds were placed in trays, arranged between layers of fine vermiculite texture and kept in chambers of germination type BOD, with a photoperiod of 12 hours of light and a constant temperature of 25°C. The germinated seeds were counted daily for 35 days until the stabilisation of germination.

With the data computed the in germination test was calculated for germination percentage, germination speed index (GSI), the mean germination time (MGT), the average speed of germination (ASG) and first count of germination test (FCG). The calculations of the percentage of germination, ASG and MGT were performed according to Labouriau [19]:

$$G (\%) = (N/A) \times 100$$

Where, G is the percentage of germination; N is the number of germinated seeds; and A is the total number of seeds germinated.

$$MGT = \frac{\sum nt \cdot ti}{\sum ntotal} \text{ (days)} \quad ASG = \frac{1}{MGT} \text{ (seeds/day)}$$

MGT refers to the average time of germination in days; nt - is the number of germinated seeds in a time interval; ti - is the time interval; ntotal is the total number of germinated seeds; ASG - is the average speed of germination.

The GSI calculations were performed by Maguire [20]:

$$GSI = \frac{G1}{N1} + \frac{G2}{N2} + \dots + \frac{Gn}{Nn}$$

GSI= germination speed index; G= number of germinated seeds; N= number of days from sowing.

## 2.3 Evaluation of Seedlings

Following the germination test, the ratings of seedlings as the length of the main root and aerial part (cm), the diameter of the base of the hypocotyl (mm), the mass of dry matter of the root system (g) and aerial part (g) were measured. The length of the aerial part and root were measured with the aid of a graduated ruler. The hypocotyl diameter and the neck of the aerial part was measured with the aid of a digital calliper. For the evaluations of dry matter mass, the seedlings were separated in the aerial part and root system. Then, were arranged in Kraft paper bags and taken to the greenhouse (air circulation at 65°C for 72 h). Subsequently, the samples were weighed on an analytical balance with a precision of 0.001 g, and the results were expressed in grams.

## 2.4 Emergency Test

This was conducted in a greenhouse, and the seeds were placed in plastic tubes of 120 cm<sup>3</sup>, filled with commercial substrate, and irrigated thrice a day. The emerged seedlings were counted daily until 38<sup>th</sup> day, indicating the stabilisation of the emergence. From the count of emergence, the index of emergence speed [20], the percentage of emergence, the average speed of emergence (ASE) and the mean emergency time (MET) were calculated. The calculations of percentage of emergence, ASE and MET were performed according to the methodology of Labouriau [19].

## 2.5 Experimental Delineament

The experimental design was completely randomised, consisting of 3 seed sizes, 7 replications with 15 seeds. The results obtained

were submitted to tests of normality and homogeneity, and subsequently to the Analysis of Variance by F test at 5% probability of error, and significant when the averages were compared by the Tukey test at 5% probability of error.

### 3. RESULTS AND DISCUSSION

Table 1 shows the biometric characteristics of the jatobá seeds classified into three size classes, small, medium and large. The mean values, the minimum and maximum length of the seeds (SL) were, respectively, 22.55 mm, 24.47 mm and 18.46 mm for small seeds, 25.14 mm, 26.48 mm and 24.50 mm to 27.54 mm and medium seed, 30.48 mm and 26.50 mm for the large seeds.

Cunha-Silva et al. [21], studying the biometry of seeds of *Hymenaea courbaril* var. *Stilbocarpa* found mean values of length and diameter of 25.7 mm and 18.7 mm, respectively, which are values very close to those obtained in this study.

The small seeds presented mean values of the diameter of the seed (DS), the weight of a thousand seeds (WTS), dry mass of seeds (DMS) and water content (WC) equal to 16.31

mm, 3940.06 g, 3.75 g<sup>-1</sup> and 8.93%, respectively. For the seeds classified as medium, the averages were 17.14 mm for DS, 4659.63 g for WTS, 4.67 g<sup>-1</sup> for DMS and 7.74% for the water content. The averages of the physical characteristics of seeds of jatobá classified as large were 17.63 mm, 5295.00 g, 4.92 g<sup>-1</sup> and 8.92% for DS, WTS, DMS and WC, respectively.

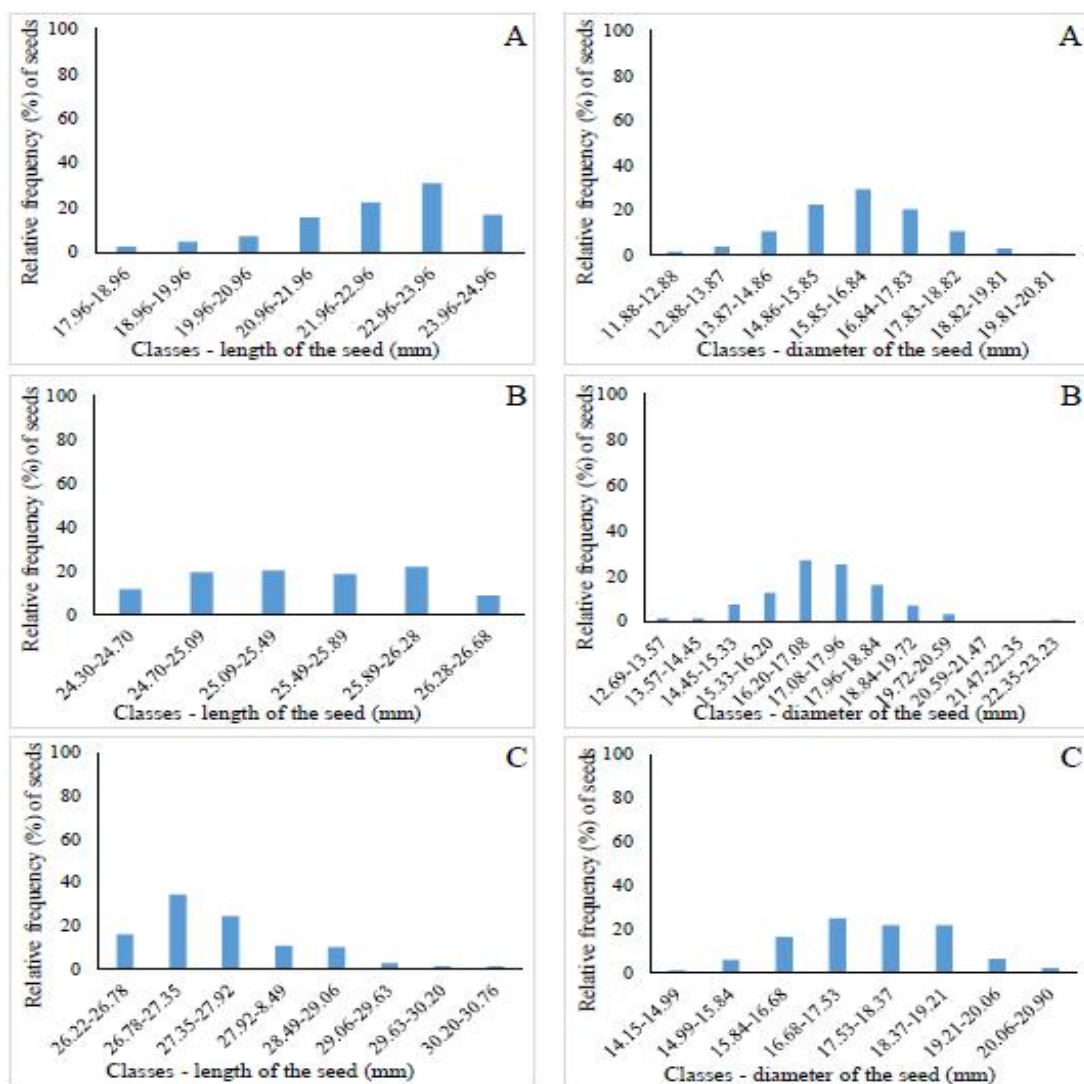
The values measured for length and diameter of the jatobá seed, classified as small seeds, medium-sized and large were separated into classes of distribution and checked the frequency on each one of them. The length of the small seeds (Fig. 1A) were separated into seven classes, ranging from 17.96 mm to 24.96 mm, approximately 80% of the relative frequency of LS these seeds are found in the last 4 classes of distribution, ranging from 20.96 to 24.96 mm, where these, 30% of small seeds have a length ranging from 22.96 to 23.96 mm.

For classified as medium (Fig. 1 B), the lengths of the seeds were distributed into 6 classes with a variation of 24.30 mm to 26.68 mm. The highest relative frequencies of LS were verified in 4 intervals of class average, the intervals of classes of extremities showed lower relative frequencies.

**Table 1. Average, Maximum and minimum standard deviation and coefficient of variation of the physical characteristics of seeds of *H. courbaril* L. The seeds were classified in small (SL ≤ 24.49 mm), medium (SL >24.49 mm ≤ 26.49 mm) and large (SL ≥ 26.50 mm)**

Small seeds					
Determinations	Average	Maximum	Minimum	Standard deviation ( $\sigma$ )	CV (%)
LS (mm)	22.55	24.47	18.46	1.45	6.42
DS (mm)	16.31	28.27	12.38	1.60	9.81
WTS (g)	3940.06	4030.30	3840.80	6.97	1.77
DMS (g <sup>-1</sup> )	3.75	3.96	3.59	0.19	5.03
WC (%)	8.93	10.04	8.36	0.75	8.42
Medium seeds					
Determinations	Average	Maximum	Minimum	Standard deviation ( $\sigma$ )	CV (%)
LS (mm)	25.48	26.48	24.50	0.58	2.29
DS (mm)	17.14	22.79	13.13	1.40	8.15
WTS (g)	4659.63	4769.00	4575.00	65.54	1.41
DMS (g <sup>-1</sup> )	4.67	4.81	4.53	0.14	3.05
WC (%)	7.74	8.49	7.25	0.59	7.57
Large seeds					
Determinations	Average	Maximum	Minimum	Standard deviation ( $\sigma$ )	CV (%)
LS (mm)	27.54	30.48	26.50	0.81	2.96
DS (mm)	17.63	20.48	14.57	1.22	6.89
WTS (g)	5295.00	5358.00	5212.00	47.23	0.89
DMS (g <sup>-1</sup> )	4.92	5.01	4.77	0.11	2.22
WC (%)	8.92	9.26	8.49	0.34	3.79

Note: length of the seed (LS), diameter of the seed (DS), weight of a thousand seeds (WTS), dry mass of seeds (DMS) and water content (WC).



**Fig. 1. Relative frequency of seeds of *H. courbaril* in different classes of length and diameter. The seeds were classified in small seeds (A=  $LS \leq 24.49$  mm), medium (B=  $LS > 24.49$  mm  $\leq 26.49$  mm) and large (C=  $LS \geq 26.50$  mm)**

For the large seeds (Fig. 1C), the lengths of the jatobá seeds were separated into 8 classes with a variation of 26.22 to 30.76 mm. The greater relative frequency of LS was approximately 90%, and was observed in the intervals of classes ranging from 26.22 mm to 29.06 mm. Among this 90%, approximately 55% of the seeds assessed are distributed in the intervals between 26.78 mm and 27.92 mm.

The values of the diameter of small seeds (DS) were divided into 9 classes of distribution (Fig. 1), the greater the relative frequency of seeds were observed in class intervals ranging from

13.87 to 18.82 mm. Seeds averages (Fig. 1 B) were verified into 12 class intervals of seed diameter, where the largest relative frequency, approximately 84%, was found in the intervals between 14.45 and 19.72 mm, while the large seeds (Fig. 1C) had the values of DS separated into 8 intervals of classes, ranging from 14.15 mm to 20.90 mm, where 83% of the seeds observed are distributed at class intervals of diameters between 15.84 and 19.21 mm.

In Table 2 it is possible to check that the large seeds showed a lower mean germination percentage, while small and medium-sized seeds

did not differ statistically. On the GSI, MGT and ASG the treatments did not differ statistically. The medium seeds had the lowest average percentage of first germination count, and the small and large seeds had the same average.

The size of the seed did not influence the germination, because the percentage of germination for larger seeds was lesser and the first count of the germination test of the large seeds did not differ from the small sized seeds. The other parameters also did not differ, This result is different than the other researchers. Carvalho and Nakagawa [14] and Oliveira et al. [15] reported that larger seeds may present better performance and divergence. Silva et al. [16], who worked with different seed sizes of *Artocarpus heterophyllus* Lam., verified that the smaller seeds had a lower percentage of germination.

Pagliarini et al. [22], working with different substrates and sizes of *Hymenaea courbaril* seeds, and found germination percentage similar to the substrate vermiculite, ranging from 77.5% to 87.5% large seed to seed storage medium, with no statistical difference between the classes of seeds.

Unlike found in this study by Pereira et al. [23], studying different sizes of seeds of *Hymenaea*

*stigonocarpa*, found a difference for mean germination time of small seeds, medium-sized seeds and large seeds. They presented respective values of 19.10, 21.03 and 24.92 days, with the large seeds presenting greater MGT than the other sizes, indicating that these take longer to germinate. Also, that the seed size of this kind does not influence the percentage of germination.

The results of the test are available in Table 3. According to the emergence percentage and emergence velocity index, the large seeds obtained the highest average, and small and medium-sized seeds showed no significant difference, while for the parameter MET, with averages of 30.29 days, 29.47 days and 29.46 days, and ASE, with averages of 0.033, 0.034, and 0.034 day<sup>-1</sup>, respectively, for the small, medium and large sized seeds, did not differ statistically among treatments.

Klein et al. [24], working with different seed sizes of pitanga (*Eugenia uniflora* L.) obtained similar results with these for the emergence of seedlings, in which the two largest seed sizes were higher than the smaller. In addition, the study found no significant difference between the sizes, unlike the observed in this study, where larger seeds showed higher mean emergence speed index.

**Table 2. Germination test of seeds of *H. courbaril* L. of different sizes**

Seed Size	G (%)	GSI	MGT (days)	ASG (days <sup>-1</sup> )	FCG (%)
Small	79.05 a	0.427 a	28.23 a	0.0353 a	4.76 a
Medium	74.29 a	0.398 a	28.39 a	0.0353 a	2.85 b
Large	66.67 b	0.362 a	28.18 a	0.0354 a	4.76 a
CV (%)	6.29	12.89	4.14	5.36	14.83

Medium followed by the same letter in column did not differ statistically among themselves by Tukey test ( $p \leq 0.05$ ).

Note: germination (G), germination speed index (GSI), mean germination time (MGT), average speed of germination (ASG), first count of germination test (FCG).

**Table 3. Emergency test of seeds of *H. courbaril* L. of different sizes**

Seed Size	E (%)	ESI	MET (days)	ASE (days <sup>-1</sup> )
Small	42.86 b	0.216 b	30.29 a	0.033 a
Medium	44.76 b	0.232 b	29.47 a	0.034 a
Large	52.39 a	0.271 a	29.46 a	0.034 a
CV (%)	5.89	6.21	3.27	3.56

Medium followed by the same letter in column did not differ statistically among themselves by Tukey test ( $p \leq 0.05$ ).

Note: emergence (E), emergence speed index (ESI), mean emergency time (MET), average speed of emergence (ASE).

**Table 4. Performance of seedlings of *H. courbaril* L. originated from seeds of different sizes**

Seed Size	LH (cm)	RL (cm)	HD (mm)	DMAP (g)	DMR (g)
Small	24.08 b	11.07 a	4.58 b	9.08 b	1.73 b
Medium	26.09 a	11.81 a	4.94 a	11.03 a	2.05 a
Large	26.14 a	12.34 a	5.12 a	10.41 ab	2.12 a
CV (%)	5.11	8.20	5.17	9.73	14.59

Medium followed by the same letter in column did not differ statistically among themselves by Tukey test ( $p \leq 0.05$ ).

Note: length of hypocotyl (LH), root length (RL), the hypocotyl diameter (HD), mass of dry matter of aerial part (DMAP), dry mass of the root (DMR).

The findings of the evaluation of seedlings after germination are displayed in Table 4. The seedlings from the small seeds showed an average of the dry mass of the root and hypocotyl length and diameter of the inferior to other treatments. Pagliarini et al. [22], also worked with different sizes of seeds of *Hymenaea courbaril* L., who found no difference in the diameter of the hypocotyl.

Silva et al. [25], met with results similar to the present study while studying different dimensions of seed of field bean (*Clitoria fairchildiana* R. A. Howard.), where the results of their research suggested that the germination was not affected by the size of the seed, but larger seeds showed higher mean weight of root dry matter.

Klein et al. [24], analysed seeds of pitanga (*Eugenia uniflora* L.) with different sizes, corroborated by the results of the length of the shoot, root and hypocotyl diameter. Whereupon the averages for shoot length and diameter of the hypocotyl were smaller for the smaller seeds, and the averages for root length did not differ.

Searching for jackfruit seeds (*Artocarpus heterophyllus* Lam.), Silva et al. [16], observed that for the mass of dry matter of the root system, the hypocotyl diameter and length of the aerial part, the smaller seeds presented lower averages.

The size of the seed is a variable that demonstrates enough influence in the vigour of seed and seedling performance, especially those obtained from native species. Since during the whole process of formation of the seed, the climatic components, which the mother plants are subjected exert a great deal of interference in the characteristics of the fruit and, consequently, of the seed. In the present study,

the size of the seeds reflected effects on the physiological quality of the seeds, mainly in the parameters related to the performance of seedlings.

#### 4. CONCLUSION

For this species, it was possible to verify that the size of seeds did not influence the germination of the same, but when evaluated in greenhouse conditions (not controlled) larger seeds showed better performance.

The seedlings of medium and large seeds showed better performance in the majority of parameters, in addition, when available in sufficient quantity, the use of large seeds is recommended, because they have a higher percentage of seedling emergence and the best quality.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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