Analysis of the effect of ultrasound on *Hymenaea courbaril L.* seeds

Análise do efeito do ultrassom em sementes de *Hymenaea courbaril L*

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ABSTRACT
Hymenaea courbaril L. popularly known as Jatobá is a native species of the Amazon. We selected 100 seeds for the morphometric analysis, for the water content of the seeds, four samples of 5 g each were used, for the germination test, four groups of seeds were evaluated: control group without ultrasound application and three other groups that were submitted to ultrasound application for 2 minutes (U2), 3 minutes (U3) and 4 minutes (U4) at a frequency of 3 MHz and an intensity of 2 W/cm² of exposure. Each group divided into four repetitions of 25 seeds each, were transferred to an incubator (B.O.D.) with a photoperiod of 12 h of light per day and 100% relative humidity, each group being repeated twice and tested at two temperatures (30 °C and 35 °C). The seed has 9.36% fresh mass, 23.62 mm, 14.1 mm, 65.59 mm in length, width, and perimeter respectively and 79.03% purity. The ultrasound waves applied for 3 minutes favored seed germination of 86.12% (30 ºC) and 83.04% (35 ºC) and mean germination time of 21.75 days (30°C) and 21.81 days (35°C) for two and three minutes respectively. Therefore, the ultrasound technique is considered useful and promising tool for breaking tegumentary dormancy in Jatobá seeds.

Keywords: *Hymenaea courbaril* L, ultrasound, seeds, dormancy.

RESUMO
A *Hymenaea courbaril* L. conhecida popularmente como jatobá é uma espécie nativa da Amazônia. Selecionamos 100 sementes para a análise morfométrica, para o teor de água das sementes foram utilizadas quatro amostras de 5 g cada, para o teste de germinação foram avaliadas quatro grupos de sementes: grupo controle sem aplicação de ultrassom e outros três grupos que foram submetidos à aplicação de ultrassom por 2 minutos (U2), 3 minutos (U3) e 4 minutos (U4) a uma frequência de 3 MHz e uma intensidade de 2 W/cm² de exposição, cada grupo dividido em quatro repetições de 25 sementes cada, foi transferido para incubadora (B.O.D.) com fotoperíodo de 12 h de luz por dia e umidade relativa de 100%, repetindo duas vezes cada grupo e testadas a duas temperaturas (30 °C e 35 °C). A semente apresenta 9.36% de massa fresca, 23.62 mm, 14.19 mm, 65.59 mm de comprimento, largura e perímetro respetivamente e 79.03% de pureza. As ondas ultrassônicas de 3 minutos com frequência de 3 MHz e intensidade de 2 W/cm^2 favoreceram a germinação de sementes de 86.12% (30 ºC) e 83.04% (35 ºC) e tempo médio de germinação de 21.75 dias (30 ºC) e 21.81 dias (35 ºC) para

2 e 3 minutos respectivamente. Portanto, a técnica de ultrassom é considerada uma ferramenta útil e promissora para a quebra de dormência tegumentar em sementes de Jatobá.

Keywords: *Hymenaea courbaril* L, ultrassom, sementes, dormência.

1 INTRODUCTION

Jatobá is a semi-heliophytic tree species from the broadleaved semideciduous forest (GONZAGA et al., 2016). It is a very showy tree that can reach up to 50 m in height and a diameter of up to 2 m, known as Jutai-acu (Brazil), being used in the forestry sector as it is a hard and widely traded wood (DA ROCHA et al., 2019). Forest species of the Fabaceae family such as the Jatobá (*Hymenaea courbaril* L.) are being used in nitrogen-deficient environments in favor of the recovery of degraded areas and for the regeneration of devastated natural environments (MARCUZZO et al. 2020).

In the industrial sector, it is used as a vegetable varnish and fuel due to the resin extracted from its roots; in the food sector, it is appreciated by fauna and communities to make flour from the pulp extracted from the fruit, in addition, it is used in folk medicine to alleviate the symptoms of inflammation and pain (SCHWARTZ, 2018).

For Duarte et al. (2016) the Jatobá fruit is an indehiscent, oblong, rounded, or slightly obtuse legume, with a thin endocarp, hard and dark brown epicarp when ripe.

The seed has an ovoid shape, slightly flattened, exalbuminous, with axial embryo and thick cotyledons, dark brown integument, stony consistency, smooth in appearance with a greenish-yellow axial embryo, presenting cutaneous dormancy, a factor that guarantees its longevity, allowing its germination after storage (DUARTE et al., 2016).

The exogenous seed dormancy is caused by the difficulty in absorbing water, which prevents it from starting the hydration of the seed tissues (extra-embryonic) (SMÝKAL et al., 2014). It is necessary to break this embryo protection barrier to promote a higher rate of germinated seeds and increase emergence in seedlings (FERRAZ et al., 2019). There are different methods for overcoming seed dormancy, among which the following stand out: mechanical scarification, incisions in the seed coat, exposure to high temperatures, and chemical scarification by strong acids (DE AZEVEDO et al., 2020).

Lately ultrasound waves have been applied as a technique to break seed dormancy, tested in more than fifteen species, and increasing, in most cases, the speed and percentage of germination. The waves impose a mechanical pressure on the cell wall of the seed increasing its porosity and allowing permeability to the entry of oxygen and water (NAZARI; ETEGHADIPOUR, 2017). This fact stimulates cells to increase their

enzymatic activities and change their structure to soften the seed coat and absorb nutrients (HUANG et al., 2020).

Ultrasound waves are mechanical waves that can increase tissue temperature (ZAREDOST et al., 2017). On the other hand, to optimize the conditions for the ultrasound treatment, three levels are considered: sonication time, sonication temperature, and output power that only requires a generator, concluding that, when ultrasound is used, pressure fluctuations cause a violent collapse of microbubbles in liquid sonication causing physical, biological, and chemical effects on seeds (WANG et al., 2012).

There is no defined dose amount for ultrasound, yet and acoustic intensity (in W/cm^2) has become almost universally accepted as the primary exposure (MILLER, 1983).

The present work evaluates the effect of low-intensity ultrasound in Jatobá (*Hymenaea courbaril* L.) seeds to overcome primary integumentary dormancy and its performance.

2 MATERIAL AND METHODS

The *Hymenaea courbaril* L. seeds were collected from four matrix trees of the Zoobotanic Park/UFAC (10° 12' 14.1" S; 67° 42' 18.3" W) in July 2020. The experiment was carried out between August 2020 and January 2021. The seeds selected after processing were dried for 24 h at 25°C. Five groups of 20 seeds each were used in the test (*blotter-test*), uniformly distributed on a *gearbox* type mini-chamber on two sheets of blotter paper sterilized at (105.00 ± 3) °C for 2 h and then hydrated with distilled water at 3 times the mass of unhydrated paper (Figure 1a).

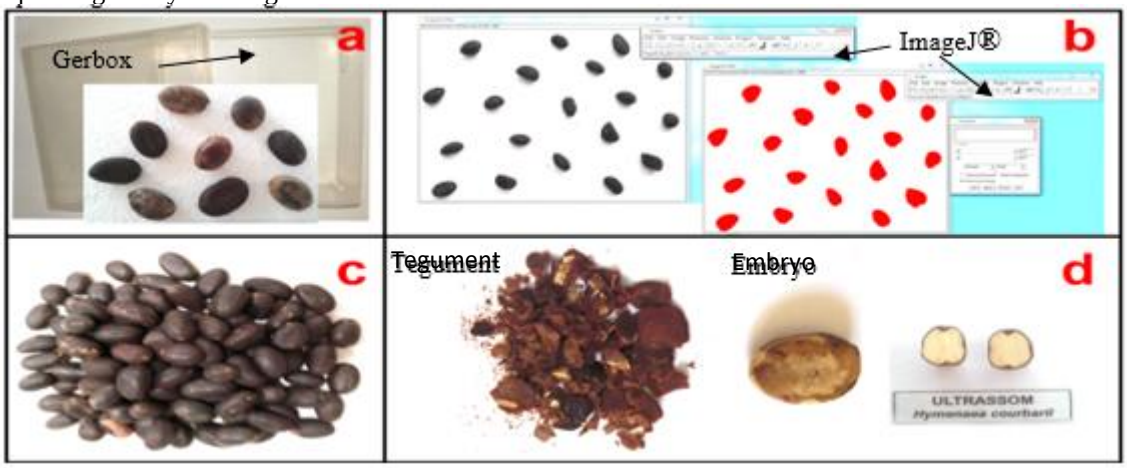
Then, the seeds were placed in a B.O.D. (*Biochemical Oxigen Demand*) with 12 h photoperiod light per day at a temperature of (25.00 ± 3) °C for 24 h, at the end of this period, each group was transferred to an incubator (B.O.D.) at (10.00 ± 3) °C remaining 24 h, finally each *gearbox* was incubated again in the B.O.D. for 7 days (168 h) at a temperature of (25.00 ± 3) °C. Afterward, all seeds were evaluated under an optical microscope and those with fungal growth were separated (NEERGAARD, 1979).

The water content of the seeds was estimated with four replications containing approximately 5 g (two seeds) for each, being placed in aluminum capsules with dimensions of 60x40 mm (diameter and height respectively) with a lid and taken to a forced circulation oven at a temperature of (105.00 ± 3) °C for 24 h (BRASIL, 2009). The result was expressed by the arithmetic mean of the repetitions expressed in percentage.

In the calculation of biometrics, length, width, circularity, proportion, roundness, solidity, area, and perimeter were performed on 100 random seeds, using the ImageJ® software (Figure 1b). The mass was evaluated by sampling a thousand seeds divided into subgroups of 100 each (Figure 1c), measured with a precision scale (0.001 g) according to the Seed Analysis Rules procedure (BRASIL, 2009).

For the desiccation tolerance tests, 5 replicates with 10 seeds each were used, wrapped in aluminum foil separately (Figure 1d), drying the embryo and tegument at (105.00 ± 3) °C for 24 h and then calculating the relationship between embryo and tegument expressed in grams with the aid of a precision balance (0.001 g) and using the equation proposed by Daws et al. (2006).

Figure 1: Physical characteristics of seeds: (a) Seeds with the occurrence of fungal fruiting. (b) Assessing seed biometrics using ImageJ® software. (c) Mass of a thousand seeds in groups of 100. (d) Drying test separating embryo and tegument for each seed.



Source: authors' collection.

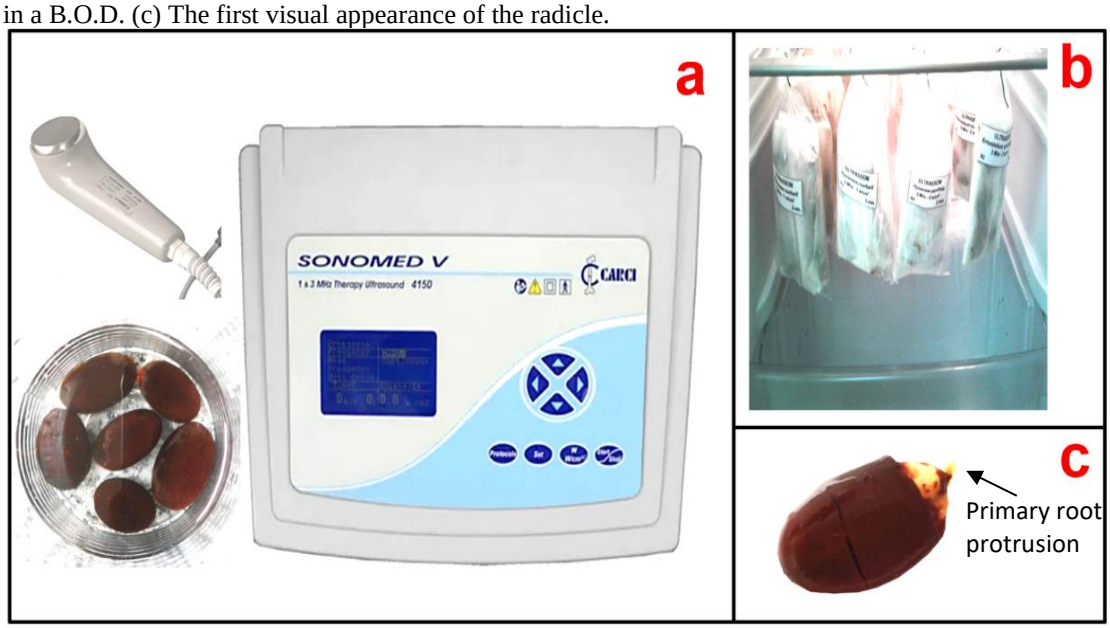
The seeds used for the germination test were superficially disinfected by immersion in 70% alcohol for one minute and then in sodium hypochlorite (2%) for three minutes. Finally, the seeds were washed with running water for one minute.

Four groups of seeds (100 units) were evaluated: the control group without ultrasound application and three other groups that were submitted to ultrasound application, at different times: 2 minutes (U2), 3 minutes (U3), and 4 minutes (U4). Seeds from each group were hydroconditioned for 30 minutes in distilled water, and the groups were subjected to ultrasound, performed in 150 mL disposable plastic cups, with 6 seeds each. After this step, the groups (U2, U3, and U4) were placed on the transducer surface of the ultrasound device (Sonomed V, Carci ®) set at a frequency of 3 MHz and an intensity of 2 W/cm² of exposure (Figure 2a).

After application of ultrasound, each group was divided into four replicates of 25 seeds each, distributed over two sheets of Germitest® paper covered with a third sheet and, soon after, rolled up moistened with distilled water in the proportion of 2.5 times the mass of the non-hydrated paper (BRASIL, 2009). The rolls were placed in the B.O.D. in plastic bags and kept in an upright position (Figure 2b) with a 12 h photoperiod (4 daylight fluorescent lamps of 15 W each) 12 h light per day and 100% relative humidity, repeating each group twice and tested at two temperatures (30 °C and 35 °C). The test was completed in 60 days.

The primary root protrusion parameter was considered for the criterion for counting germination (Figure 2c). The percentage of germination (G) was evaluated: by the formula $G = (\sum n_i/N/).100$, where: n_i = number of germinated seeds at the end of the test, N = number of seeds ready to germinate. Unit: % (LABOURIAU; AGUDO, 1987); the mean germination time (TMG): calculated by the formula $TMG = (\sum n_i t_i)/\sum n_i)$, where: n_i = number of germinated seeds per day, t_i= incubation time. Unit: days (LABOURIAU; VALADARES, 1976); and the germination speed index (IVG): $IVG = \sum(n_i/t_i)$, where: n_i = number of seeds that germinated in time "i", t_i= time after installation of the test. Unit = seed/day (MANGUIRE, 1962).

Figure 2: Seeds with ultrasound application: (a) Seeds submitted to an ultrasound device immersed in distilled water in a plastic cup. (b) Paper rolls containing 25 seeds distributed in a vertical position and kept in a B.O.D. (c) The first visual appearance of the radicle.



Source: authors' collection.

For statistical analysis purposes, the germination percentage data underwent the arcsine transformation arcsin (%p/100)½, and the average results of germination speed

index and average germination time were individually submitted to residue normality analysis by Shapiro and Wilk (1965) test of homogeneity of variances by Barlett's test (1937). Finally analysis of variance (F test) and mean comparison test by Tukey's test ($p \leq 0.05$) were performed using the statistical program RStudio.

3 RESULTS

According to the data presented in Table 1, the water mass of the seeds was 9.36%, with a desiccation tolerance of 0.45 considered as orthodox, and a purity percentage of 79.03%. Then, when analyzing the biometric data, an average of (23.62 ± 1.03) mm, (14.19 ± 1.21) mm, (258.89 ± 12.02) mm^2 and (65.59 ± 3.16) mm in length, width, area, and perimeter respectively, totaling 5,178.71 g of mass per thousand seeds.

Table 1: Descriptive statistics of biometric evaluation.

Parameters	Mean	Minimum	Maximum	Variance	Standard Deviation	Error Pattern
Length (mm)	23.62	22.07	25.19	1.06	1.03	0.42
Width (mm)	14.19	12.97	15.87	1.45	1.21	0.49
Circularity (0,0 – 1,0)	0.76	0.72	0.79	0	0.03	0.01
Proportion (mm)	1.69	1.52	1.84	0	0.03	0.05
Roundness (0,0 – 1,0)	0.59	0.54	0.66	0	0.05	0.02
Solidity (0,0 – 1,0)	0.98	0.98	0.98	0	0	0
Area (mm^2)	258.89	236.82	290.32	14.92	12.02	3.99
Perimeter (mm)	65.59	61.60	70.81	9.96	3.16	1.29
Desiccation (P)	0.25					
Water content (%)	9.36					
Purity (%)	79.03					
PMS. (g)	5,178.71					

P.M.S.: Weight of a thousand seeds.

The mean germination values at 30 °C differed statistically, providing the highest performance in 3 minutes at 86.12%, followed by 83.04% at a temperature of 35 °C. There is a marked germination drop to 63% for the application of ultrasound (30 °C) for 4 minutes.

Table 2: Analysis of germination percentage (day) as a function of temperature and ultrasound application time.

Treatments (Groups)	G (%)					
	30 °C			35 °C		
Control	78.16 ± 2.08	ab	A	79.31 ± 1.35	a	A
U2	76.03 ± 7.16	ab	A	80.24 ± 3.65	a	A
U3	**86.12 ±** 2.58	a	A	**83.04 ±** 5.26	a	A
U4	63.05 ± 3.78	b	A	70.08 ± 2.58	a	A
CV (%)	11.51					

Uppercase in rows (for temperature) and lowercase in columns (for time), do not differ by Tukey's test at 5% probability. CV is the coefficient of variation.

Accelerating seed germination is extremely important for species used in the recovery of degraded areas, as the faster the seedling develops in the field, the faster the ecosystem will recover (VENÂNCIO; MARTINS, 2019).

The shorter the Mean Germination Time (TMG), the more efficient the treatment. The interaction between temperature and exposure time determined the occurrence of lower TMG compared to the Control group, resulting in values of 21.75 (U2) and 21.81 (U3) for 30 °C and 35 °C, respectively (Table 3).

Table 3: Analysis of Mean Germination Time (day) as a function of temperature and ultrasound application time.

Treatments (Groups)	TMG (day)					
	30 °C			35 °C		
Control	44.11 ± 0.22	b	A	44.73 ± 0.73	b	A
U2	**21.75 ±** 0.33	a	A	23.17 ± 0.94	a	A
U3	23.45 ± 0.31	a	A	**21.81 ±** 1.07	a	A
U4	24.18 ± 0.48	a	A	25.51 ± 0.96	a	A
CV (%)	6.73					

Uppercase in rows (for temperature) and lowercase in columns (for time), do not differ by Tukey's test at 5% probability. CV is the coefficient of variation.

According to Table 4, the IVG mean values differ between the groups, registering the highest index of 1.14 seeds/day for a temperature of 35 °C, and 0.95 seeds/day for a temperature of 30 °C with a 3 minutes ultrasound time for both temperatures.

Table 4: Analysis of the Germination Speed Index (seeds/day) as a function of temperature and ultrasound application time.

Treatments (Groups)	IVG (seeds/day)					
	30 °C			35 °C		
Control	0.48 ± 0.11	b	A	0.45 ± 0.04	b	A
U2	0.76 ± 0.21	ab	A	0.95 ± 0.08	a	A
U3	**0.95** ± 0.03	a	A	**1.14** ± 0.01	a	A
U4	0.71 ± 0.25	ab	A	0.85 ± 0.06	a	A
CV (%)	21.97					

Uppercase in rows (for temperature) and lowercase in columns (for time), do not differ by Tukey's test at 5% probability. CV is the coefficient of variation.

4 DISCUSSION

For Duarte et al. (2016), the water content of the seeds was 13.1% collected between December 2014 and January 2015 from fallen fruits on the ground from a total of eleven trees 300 m apart, The amount was higher when compared to the one recorded in the present work (9.36%). A for the mass of a thousand seeds the result was 3,102 g (DUARTE et al., 2016) which was lower compared to our values (5,1782 g).

Regarding seed length, the mean of our result (23.62 mm) was higher compared to those studied by Ristau et al. (2018) with 22.55 mm in small seeds. As for its width, the results of Duarte et al. (2016) were on average 17.7 mm higher than our result (14.19 mm).

The average germination percentage for Duarte et al. (2016) was 89%, using as a pre-germinative treatment the sterilization of seeds with sodium hypochlorite solution (2%) for five minutes and then laterally sanded and placed to soak for a period of 24 h and to germinate in substrate sand at a controlled temperature of 30 °C and constant light, our work when we applied ultrasound had slightly lower values (86% and 83% for 30 °C and 35 °C, respectively).

For Duarte et al. (2016), the best average germination time occurred in the combination of a substrate in the sand at a controlled temperature of 30 °C and constant light, followed by the substrate in vermiculite and 30 °C of controlled temperature and constant light, with results of 7.74 and 8.51 days respectively, being lower than our ultrasound results.

The longer the treatment time, the higher the final temperature of the medium that received the ultrasound application, causing physical or chemical damage to the seed (WANG et al., 2012).

For Sharififar et al. (2015), the time of exposure to ultrasound, with treatments longer than seven minutes for three seeds of important plants (*Atriplex lentiformis*,

Cuminum cyminum, and *Zygophyllum eurypterum*), had a destructive effect on cell appearance and negatively affected the percentage of seed germination. However, the author used a bath ultrasound device (Elma Elmasonic E30H; Germany), whose average output power tends to be higher than that used in this research.

Unfortunately, there are no major researches in the literature on the seed of *Hymenaea courbaril* L. for dormancy breaking by ultrasonic waver.

5 CONCLUSION

The ultrasound technique proved to be a positive tool in breaking dormancy in *Hymenaea courbaril* L. seeds. However, exposure time must be considered as an important factor in germination.

The mechanical scarification presented satisfactory results, corroborated with the scientific literature, however, it should be emphasized that the application of ultrasound eliminates impurities and handling in the seeds.

The ultrasound application technique is more practical, safer, and does not generate chemical residues when compared to other physical and chemical methods to break seed dormancy.

Further research needs to be done in the field under natural environmental conditions to check various factors affecting the dormancy and germination of the seeds.

The biochemical analysis of seeds must be further investigated to prove that their properties do not change after the application of ultrasound.

More studies should be carried out evaluating different frequencies and intensities at different times.

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