Multidisciplinary Sciences Reports

Article

EFFECT OF THE ULTRASOUND APPLICATION IN SEEDS ON THE PERFORMANCE OF SEEDLINGS OF *Hymenaea courbaril* L.

1 Luz Patricia Velásquez D'avila patriciadavila2512@gmail.com

2 Zandra Pilar Vela Navarro zandravela239@gmail.com

3 Isaac de Oliveira Santos isaacoliveira.tk@gmail.com

4 Ronald Antonio Nascimento de Souza souzaronald930@gmail.com

5 Ítalo Felipe Nogueira Ribeiro italoribeiro080@gmail.com

6 William Ferreira Alves william.alves@ufac.br

7 Luis Eduardo Maggi luis.maggi@ufac.br

8 Anselmo Fortunato Ruiz Rodriguez anselmorodriguez73@gmail.com

9 Wagner Coelho de Albuquerque Pereira wcap58@gmail.com

10 Marcelo Castanheira da Silva marcelo.silva@ufac.br

[1,6,8] Nanobiotechnology Laboratory/BIONORTE/Federal University of Acre, Rio Branco – AC, Brazil

[2-5,7,10] Biophysics Laboratory/CCBN/Federal University of Acre, Rio Branco – AC, Brazil

[9] Ultrasound Laboratory/PEB/COOPE/Federal University of Rio de Janeiro, Rio de Janeiro – RJ, Brazil

ABSTRACT

The species *Hymenaea courbaril* L., presents economic potential in the commercialization of its sap and in the wood industry of the Amazon Forest, as well as high importance for popular medicine and civil construction. Therefore, this work evaluated the performance of seedlings after ultrasound application on *Hymenaea courbaril* L seeds. In the experiment, two temperatures of 30 ºC and 35 ºC were used and four groups with four replications of 25 seeds each, being one the control group and three groups with ultrasound application at a frequency of 3 MHz, intensity of 2 W/cm² for 2 minutes (T2), 3 minutes (T3) and 4 minutes (T4). The variables of length and mass of shoots and roots (dry and fresh) were evaluated. According to the results, there was a significant reduction in root fresh mass and a significant increase in shoot fresh mass between the 3- and 4-minutes treatments compared to the control group. However, no significant change was noticed for dry mass. This fact suggested a change in the degree of water absorption, requiring further studies using other parameters.

KEYWORDS: bioacoustics. jatoba. root. stem.

INTRODUCTION

Hymenaea courbaril L., belongs to the leguminous family *(Fabaceae-Caesalpinioideae)* and is popularly known as Jutaí-açu, jatobá do mato, jutaí or jatobá (1). The plant has economic potential in the commercialization of its sap, mainly due to the valuation of the logging company in the Amazon (2). It also has value in folk medicine, civil construction, among others (3), and is widely used in planting seedlings for reforestation and restoration of degraded areas (4).

The average weight of the fruit is 80 g, containing 4 to 8 seeds (3), having a low germination rate due to its integumentary dormancy (1). For commercial production, there is a need to carry out different methods to overcome the dormancy of these seeds, such as mechanical scarification, incisions in the tegument, exposure to high temperatures and chemical scarification using strong acids (5).

In general, the methods used to test the performance of seedlings seek to reproduce the conditions that occur in the field, and the most studied parameters are root length, shoot height, collar diameter, fresh matter mass and dried from shoots and roots (6). Vigor tests based on seedling performance are the most important when it comes to analyzing seed quality, considering that deterioration is related to loss of vigor (7).

For Venâncio (2020) ultrasound applications facilitate the germination process and the initial growth of seedlings (8). The technique of applying ultrasound to seeds and checking the performance of seedlings in several species has been widely investigated. The mechanical effects caused by small oscillations of the medium particles due to the propagation of ultrasound waves through the tissues lead to a probable rupture of the cell wall of the plants, increasing the absorption of water (9) and nutrients (10). On the other hand, frequency, intensity, and time of application must be specific to avoid damage or even death of seedlings (8).

In this sense, more studies on the application of ultrasound in seeds should be carried out evaluating different frequencies and intensities at different times. It should be noted that the application of ultrasound reduces handling and the risk of contamination by impurities when compared to traditional techniques (11). Given the above, this work sought to evaluate the performance of seedlings after the application of ultrasound in seeds of Hymenaea courbaril L.

MATERIAL AND METHODS

The experiment was carried out between August 2020 and January 2021, at the facilities of the Bionorte Network Laboratory Complex (Nanobiotechnology Laboratory) and the seeds of Hymenaea courbaril L. were collected from four mother trees that vegetate in the Zoobotanic Park (10 °12`14.1`S; 67°42`18.3" W) belonging to the Federal University of Acre - UFAC, in the city of Rio Branco - Acre.

After collection, the seeds were immediately washed with running water to remove the mucilage and, with the help of a magnifying glass with a 4x magnification, the damaged ones were discarded. Then, they were placed to dry for 24 hours on paper towels at a temperature of 25º C on a bench. For the sanitary analysis of the seeds, the test on filter paper (blotter-test) was used, where they were uniformly distributed with the aid of flambéed tweezers, in a group of 20 units on a gerbox-type mini-chamber on two sheets of germitest® paper sterilized at 105 ±3°C for two hours, according to Costa (2015) (1).

Then, they were hydrated with distilled water at 3x the mass of the non-hydrated paper and kept in the B.O.D. (Biochemical Oxygen Demand) with a temperature of 25 ± 3 °C and a photoperiod of 12 hours for 24 hours, to allow analysis of fungal growth. At the end of the period, each gerbox was transferred to 10 °C and remained there for 24 hours. At the end of the procedure, each gerbox was incubated again in the B.O.D. for seven days with the same regime (25 ± 3º C). After that time, the seeds were individually examined under an optical microscope, counting, and separating those with occurrence of fungal fruiting bodies.

Finally, the experimental treatment used was completely randomized (DIC – Completely Random Design) in a 2x4 factorial scheme (two temperatures of 30 ºC and 35 ºC and four groups with four replications of 25 seeds each). The groups were: GC-Control group (intact seeds), and three other groups applying ultrasound at a frequency of 3 MHz, intensity of 2 W/cm² and exposure time for 2 minutes (T2), 3 minutes (T3) and 4 minutes (T4).

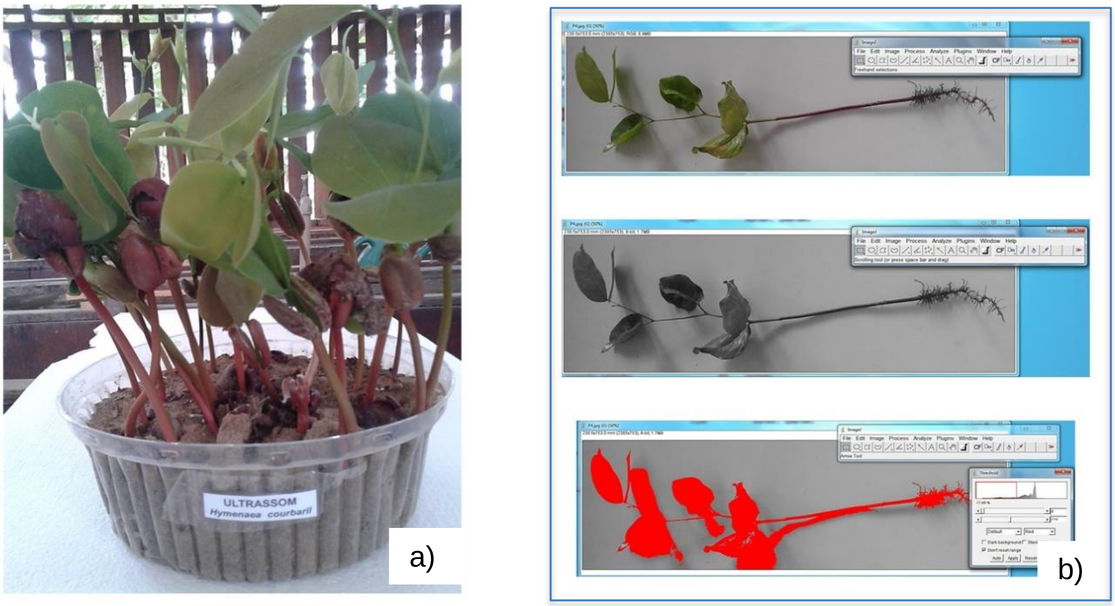
After applying the ultrasound, the seeds of each group were distributed over two sheets of germitest® paper covered with a third sheet and made in the form of a roll moistened with distilled water in a proportion of 2.5 times the mass of the non-hydrated paper. The rolls were placed in plastic bags in an upright position and kept in the B.O.D.

at two temperatures (30°C and 35°C) with a photoperiod of 12 hours (4 daylight fluorescent lamps of 15 W each) and relative humidity of 100% for a period of 60 days.

After germination (primary root protrusion) in the incubator, the seeds were transported to the greenhouse. Plastic trays with moistened sand and sterilized in a vertical autoclave (120 ºC and 1 atm for 1 h) were used to transplant the seeds at a depth of 2 cm, irrigated three times a week. The number of emerged seedlings and the monitoring of their development were computed daily until their stabilization (60 days) (Figure 1a).

The performance test was carried out, randomly selecting 10 (ten) seedlings from each repetition (separated from the cotyledons) and quantifying the shoot (cut with scissors) and the root system (between the mesocotyl and the terminal portion of the main root), evaluating the mass (g) and length (cm) in fresh mass (root and aerial part). Subsequently, the same parameters were evaluated in dry mass of each seedling, placing the seeds individually in properly identified Kraft paper bags and taking them to an oven with forced air circulation at 65°C for 48 hours, until constant mass. The mass results were expressed in grams per seedling according to Carvalho (2000) (6) using a precision scale (0.001 g) and the length results expressed in centimeters per seedling using the ImageJ® software (Figure 1).

The data were submitted to analyzes of normality of the residues by the Shapiro Wilk test of the homogeneity of the variances by the Barlett test (1937) (12), analysis of variance (F Test) and test of comparison of means by the Tukey test ($p \leq 0.05$). All analyzes were performed using the free software RStudio.



Source: Author, UFAC, Rio Branco, AC, 2021.

Figure 1 – Seedling performance analysis process: a) Seedling that received ultrasound treatment in plastic containers and sand as substrate 60 days after sowing. b) Seedling performance evaluation process using ImageJ® software.

RESULTS AND DISCUSSIONS

According to the results, there was no significant difference (p>0.05) between the evaluated treatments for most of the analyzed parameters. The highest value for the Length of Fresh Matter Root (LFMR) can be observed in the control group with 8.09 ± 0.11 cm at 30 °C and for the Length of Dry Matter Root (LDMR) (5, 88 ± 0.06 cm) in the T3 group (3 minutes) at 35 °C (Table 1), however, without significant differences.

The Length of the Aerial Part of the Fresh Mass (LAPFM) presents an average of 17.29 ± 0.08 cm (30 °C) in the control group and the average Length of the Aerial Part of the Dry Mass (LAPDM) showed the highest mean (5.43 ± 0.02 cm) for T4 (4 minutes) at 35 °C, again, without significant differences (Table 2).

Table 1: Mean values for the parameters: Length of Fresh Mass Root (LFMR) and Length of Dry Mass Root (LDMR) as a function of temperature and time of ultrasound application.

Groups	LFMR (cm)		LDMR (cm)	
(Time)	30º C	35º C	30º C	35º C
Control	8.09 ± 0.11 a A	7.18 ± 0.12 a A	5.15 ± 0.13 a A	5.55 ± 0.16 a A
2 minutes	7.42 ± 0.17 a A	7.18 ± 0.06 a A	5.49 ± 0.21 a A	5.71 ± 0.13 a A
3 minutes	7.54 ± 0.08 a A	7.48 ± 0.10 a A	5.69 ± 0.04 a A	5.88 ± 0.06 a A
4 minutes	7.70 ± 0.15 a A	7.73 ± 0.10 a A	5.44 ± 0.16 a A	5.63 ± 0.11 a A

Uppercase letters in rows (for temperature) and lowercase letters in columns (for time) do not differ from each other by Tukey's test at 5% probability of error.

Table 2: Mean values for the parameters Length of the Aerial Part of the Fresh Mass (LAPFM) and Length of the Aerial Part of the Dry Mass (LAPDM) as a function of the temperature and time of ultrasound application.

Groups	LAPFM (cm)		LAPDM (cm)	
(Tempo)	30º C	35º C	30º C	35º C
Control	17.29 ± 0.08 a A	16.21 ± 0.11 a A	4.22 ± 0.15 a A	4.92 ± 0.06 a A
2 minutes	16.56 ± 0.06 a A	15.88 ± 0.09 a A	5.37 ± 0.08 a A	4.46 ± 0.11 a A
3 minutes	17.02 ± 0.13 a A	15.72 ± 0.02 a A	5.08 ± 0.07 a A	4.83 ± 0.06 a A
4 minutes	16.72 ± 0.10 a A	15.95 ± 0.05 a A	4.91 ± 0.13 a A	5.43 ± 0.02 a A

Uppercase letters in rows (for temperature) and lowercase letters in columns (for time) do not differ from each other by Tukey's test at 5% probability of error.

There was a statistical difference between the Fresh Mass of Root (FMR) with 0.89 ± 05 g for the control treatment at 30 °C in relation to the treatments, but there was not for the Dry Mass of Root (DMR) despite the higher raw value have been for T3 (3 minutes) at 30 °C of 0.21 ± 0.07 g (Table 3).

There was a significant difference in the Fresh Mass of Air Part parameter (FMAP) for treatments T3 (3 minutes) with 4.38 ± 0.11 g and T4 (4 minutes) with 4.42 ± 0.06 g at 30 °C (Table 4). As for the Dry Mass of the Aerial Part (DMAP), there was no significant difference between the treatments, with the T2 group (2 minutes) at 30 °C having the highest gross value (1.48 ± 0.06 g) (Table 4).

Mult. Sci. Rep. 2022; v. 2 n. 1 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v2i1.18
Submetido: 25 março, 2022 – Aceito: 10 abril, 2022

Table 3: Mean values for Fresh Mass of Root (FMR) and Dry Mass of Root (DMR) parameters as a function of temperature and time of ultrasound application.

Groups	**FMR (g)**		**DMR (g)**	
(Tempo)	**30º C**	**35º C**	**30º C**	**35º C**
Control	0.89 ± 0.05 a A	0.68 ± 0.06 a B	0.18 ± 0.14 a A	0.19 ± 0.11 a A
2 minutes	0.65 ± 0.08 b A	0.66 ± 0.05 a A	0.20 ± 0.07 a A	0.18 ± 0.08 a A
3 minutes	0.67 ± 0.08 ab A	0.76 ± 0.08 a A	0.21 ± 0.07 a A	0.19 ± 0.03 a A
4 minutes	0.70 ± 0.13 ab A	0.65 ± 0.05 a A	0.18 ± 0.11 a A	0.18 ± 0.11 a A

Uppercase letters in rows (for temperature) and lowercase letters in columns (for time) do not differ from each other by Tukey's test at 5% probability of error.

Table 4: Mean values referring to the parameters Fresh Mass of the Aerial Part (FMAP) and Dry Mass of the Aerial Part (DMAP) as a function of the temperature and time of ultrasound application.

Groups	**FMAP (g)**		**DMAP (g)**	
(Tempo)	**30º C**	**35º C**	**30º C**	**35º C**
Control	4,15 ± 0,06 a A	4,37 ± 0,11 a B	1,42 ± 0,12 a A	1,39 ± 0,11 a A
2 minutes	4,24 ± 0,06 a A	4,47 ± 0,06 a A	1,48 ± 0,06 a A	1,29 ± 0,14 a A
3 minutes	4,38 ± 0,11 ab A	3,82 ± 0,08 a A	1,42 ± 0,10 a A	1,27 ± 0,11 a A
4 minutes	4,42 ± 0,06 ab A	4,01 ± 0,10 a A	1,38 ± 0,08 a A	1,47 ± 0,08 a A

Uppercase letters in rows (for temperature) and lowercase letters in columns (for time) do not differ from each other according to the Tukey test at 5% probability of error.

CONCLUSION

No significant differences were found between the evaluated treatments and the control group for the parameter length, root, or aerial part, which allows making an initial inference that the ultrasound technique applied to seeds of Hymenaea courbaril L. at the frequency of 3 MHz with an intensity of 2 W/cm² in the application times employed, did not favor the growth of seedlings in 60 days. However, there was a significant reduction in root fresh mass and an increase in shoot fresh mass between treatments of 3 and 4 minutes in relation to the control group. However, no significant change was noticed for dry mass. This fact suggests a change in the degree of water absorption, and further studies are needed to confirm this result.

REFERENCES

1. Costa CB. BOAS PRÁTICAS DE MANEJO PARA O EXTRATIVISMO SUSTENTÁVEL DO JATOBÁ. Brasilia DF; 2015.

2. Costa DL da, Silva Ribeiro RB da, Vieira DDS, Santos MF dos, Gama JRV, Lima B de A. Multipropósito de *Hymenaea courbaril* L. em uma área de manejo florestal comunitário na Amazônia. Advances in Forestry Science. 2019 Jul 24;6(2):691–7.

3. dos Santos JCC, Ribeiro Silva DM, Costa RN, Santos SA, dos Santos Silva LK, Silva JV. Biometry of fruits and seeds and pre-germination treatments of *Hymenaea courbaril* seeds. Revista em Agronegocio e Meio Ambiente. 2019;12(3):957–79.

4. Galdino OP da S. SOBREVIVÊNCIA E CRESCIMENTO DE 36 ESPÉCIES NATIVAS PLANTADAS VIA SEMEADURA DIRETA EM ÁREAS DEGRADADAS NO DF, APÓS 3 ANOS. [Planaltina DF]; 2018.

5. Azevedo GA de, Melo AAR de, Moreira LDK, Silva R dos S, Sánchez DEJ, Ribeiro CB, et al. Effect of using ultrasound to break dormancy and germination of xylopia emarginata mart seeds. Research, Society and Development. 2020 Sep 7;9(9):e789997840.

6. Carvalho NM de, Nakagawa J. SEMENTES: Ciência, tecnologia e produção. Funep. Jaboticabal; 2000. 1–588 p.

7. Cherobini EAI, Muniz MFB, Blume E. Avaliação da Qualidade de Sementes e Mudas de Cedro. Ciencia Floresta, Santa Maria. 2008;18(1):65–73.

8. Venancio RS da S. ESTUDO DA INFLUÊNCIA DO ULTRASSOM DE BAIXA FREQUÊNCIA SOBRE A GERMINAÇÃO DE SENNA MULTIJUGA (RICH.) H. S. IRWIN & BARNEBY. [SOROCABA SP]; 2020.

9. Nazari M, Eteghadipour M. Impacts of Ultrasonic Waves on Seeds: A Mini-Review. Agricultural Research & Technology: Open Access Journal. 2017 Apr 28;6(3):1–6.

10. Huang S, Jia Y, Liu P, Dong H, Tang X. Effect of ultrasonic seed treatment on rice seedlings under waterlogging stress. Chilean Journal of Agricultural Research. 2020 Oct 1;80(4):561–71.

11. Dávila LPV, Maggi LE, Rodriguez AFR, Lacerda R de F, Pereira WC de A, da Silva MC, et al. ANALYSIS OF THE EFFECT OF ULTRASOUND ON *HYMENAEA COURBARIL* L. SEEDS. BRAZILIAN JOURNAL OF DEVELOPMENT. 2021 Nov 1;7(11):105910–21.

12. Bartlett MS. Properties of Sufficiency and Statistical Tests [Internet]. Bracknell, Berks; 1937. Available from: https://royalsocietypublishing.org/

13. Costa CHM da, Diaris KB, Guimareas TM. MÉTODOS DE ESCARIFICAÇÃO PARA SUPERAÇÃO DE DORMÊNCIA DE SEMENTES DE JATOBÁ. Revista Científica Eletrõnica de Engenharia Florestal. 2017;30(1):1–9.

14. Pagliarini MK, Moreira ER, Nasser FA de CM, Mendonça VZ, Castilho RMM de. NÍVEIS DE SOMBREAMENTO NO DESENVOLVIMENTO DE MUDAS DE *Hymenaea courbaril* var. Stilbocarpa. Cultura Agronomica, Ilha solteira. 2017;26(3):330–46.

15. Oliveira HFE de, Felix D v., Souza CL, Fernandes LS, Xavier PS, Alves LM. DESENVOLVIMENTO INICIAL DO JATOBÁ (H. COUBARIL L.)EM FUNÇÃO DE DIFERENTES SUBSTRATOS E CINCO LÂMINAS DEIRRIGAÇÃO. In: XXV CONIRD – Congresso Nacional de Irrigação e Drenagem. São Cristovão SE: UFS; 2015. p. 1–6.

16. Silva EM da. PRODUÇÃO DE MUDAS DE ESPÉCIES FLORESTAIS DA CAATINGA EM DIFERENTES SUBSTRATOS. [Pombal - PB]; 2013.

17. Larson LC dos SR, Boliani AC, Santo TL do E, Teodoro PE, Costa E. Substrates, Emergence and Seedling Quality of Hymenaea stigonocarpa Mart. (Jatoba) in Protected Cultivation. Biosci Journal. 2018;34(3):615–22.

18. Duboc E, Ventorim N, do Vale FR, Davide AC. Nutrição de Plantas: a chave para alta produção com qualidade [Internet]. NUTRIÇÃO DO JATOBÁ: *Hymenaea courbaril* L. var. stilbocarpa (Hayne) Lee et Lang. 2006 [cited

Mult. Sci. Rep. 2022; v. 2 n. 1 / ISSN: 2764-0388
DOI: https://doi.org/10.54038/ms.v2i1.18
Submetido: 25 março, 2022 – Aceito: 10 abril, 2022

2022 Feb 26]. p. 1–12. Available from: http://www.nutricaodeplantas.agr.br/site/downloads/unesp_jaboticabal/omissao_jatoba1.pdf.