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Use of ultrasound to overcome dormancy in *Ochroma pyramidale* seeds

Abstract – The objective of this work was to evaluate the effect of ultrasound waves, in a continuous mode, at different frequencies, intensities, and exposure times on the pre-germination treatment of seeds of *Ochroma pyramidale*, a species commonly known as balsa tree. For the germination and emergence tests in sand, a completely randomized design was adopted, with four replicates of 50 seeds each. Ten treatments were evaluated: control (T1); hot water at 80°C (T2); and ultrasound (T3 to T10) adjusted at different combinations of frequencies (1.0 and 3.0 MHz), intensities (1.0 and 2.0 W cm^{-2}), and exposure times (2 and 3 min). T2 showed the best percentage of germination (84%), followed by T8 (70%). Regarding the germination speed index, T2 and T8 presented the best values of 7.35 and 6.83, respectively. T2 also showed the best emergence speed index of 4.65. Although immersion in hot water at 80°C stands out as the best method, the ultrasound in T8 (at a frequency of 1.0 MHz, intensity of 2.0 W cm^{-2}, and exposure time of 3 min) has a positive effect on overcoming dormancy in seeds of the evaluated species.

Index terms: *Ochroma pyramidale*, emergence, germination, ultrasonic treatment.

Uso de ultrassom para superação de dormência em sementes de *Ochroma pyramidale*

Resumo – O objetivo deste trabalho foi avaliar o efeito de ondas ultrassônicas, em modo contínuo, em diferentes frequências, intensidades e tempos de exposição no tratamento pré-germinativo de sementes de *Ochroma pyramidale*, espécie conhecida comumente como pau-de-balsa. Para os testes de germinação e emergência em areia, adotou-se o delineamento inteiramente casualizado, com quatro repetições de 50 sementes cada uma. Foram avaliados dez tratamentos: controle (T1); água quente a 80°C (T2); e ultrassom (T3 a T10) ajustado em diferentes combinações de frequências (1,0 e 3,0 MHz), intensidades (1,0 e 2,0 W cm^{-2}) e tempos de exposição (2 e 3 min). O T2 apresentou a melhor percentagem de germinação de (84%), seguido pelo T8 (70%). Quanto ao índice de velocidade de germinação, T2 e T8 apresentaram os melhores valores de 7,35 e 6,83, respectivamente. O T2 também apresentou o melhor índice de velocidade de emergência de 4,65. Embora a imersão em água quente a 80°C tenha se destacado como o melhor método, o ultrassom (frequência de 1,0 MHz, intensidade de 2,0 W cm^{-2} e tempo de exposição de 3 min) apresentou efeito positivo na superação da dormência em sementes da espécie avaliada.

Termos para indexação: *Ochroma pyramidale*, emergência, germinação, tratamento ultrassônico.

Ana Paula Falcão Freire(✉)
Universidade Federal do Acre, Rio Branco, AC, Brazil. E-mail: annynha.falcao@gmail.com

Eduardo Pacca Luna Mattar
Universidade Federal do Acre, Rio Branco, AC, Brazil. E-mail: eduardo.mattar@ufac.br

Harley Araújo da Silva
Universidade Federal do Acre, Rio Branco, AC, Brazil. E-mail: harley.silva@ufac.br

Luz Patricia Velásquez Dávila
Universidade Federal do Acre, Rio Branco, AC, Brazil. E-mail: patriciadavila2512@gmail.com

Luis Eduardo Maggi
Universidade Federal do Acre, Rio Branco, AC, Brazil. E-mail: luis.maggi@ufac.br

✉ Corresponding author

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Introduction

The species *Ochroma pyramidale* (Cav. ex Lam.) Urb., commonly known as balsa tree, is a terrestrial tree of the Malvaceae family with a wide natural occurrence in forest areas, especially in those of secondary growth, from Central America to the Amazon (Galos et al., 2022). The wide distribution of this species is associated with its ability to naturally colonize disturbed locations (Levy-Tacher et al., 2015; Charão et al., 2021), in addition to its easy cultivation in plantations (González et al., 2018; Levy-Tacher & Morón-Ríos, 2024).

Ochroma pyramidale can have several uses, including: pulp, paper, and energy production; source of primary (round) or processed (round) wood, mainly in boat construction; medicinal and landscape applications; and planting for environmental purposes (Carvalho, 2010). Furthermore, the tree not only has chemical properties that are suitable for the production of kraft cellulose (Silva et al., 2022), but also has peculiar wood mechanical properties due to its low average angle of microfibrils and high-crystallinity cellulose (Borrega et al., 2015). Because of its rapid growth, the tree can be used in forest restoration, in pure or mixed forest plantations, as well as in agroforestry systems (Santos et al., 2014).

Despite its fast growth, the germination of *O. pyramidale* seeds is low, ranging from 11 to 20% (Leão et al., 2008). This is caused by seed physical or integumentary dormancy, requiring a pre-germination treatment, such as manual scarification or immersion in hot water for 20 min, which can increase germination in 60 to 89% (Carvalho, 2010; Bao et al., 2016). Santos et al. (2016) evaluated the use of hot water immersion and indicated thermal shock as an efficient method.

The use of ultrasound is another alternative to improve seed germination. In the literature, positive effects have been reported on the seeds of forest species such as *Swietenia macrophylla* King in Hook (Costa et al., 2016), *Senna multijuga* (Rich.) H.S. Irwin & Barneby (Venâncio & Martins, 2019), and *Hymenaea courbaril* L. (Dávila et al., 2021, 2022). This technique is advantageous for two main reasons: it can be applied more easily than other methods, such as sand, acid, and mechanical scarification; and does not involve the use of any chemicals that can contaminate the seeds (Nazari & Eteghadipour, 2017). In addition, ultrasound waves not only can improve germination rate and seedling growth, promote rooting, and increase seedling leaf area, but can also modify the activities of the enzymes superoxide dismutase, catalase, and peroxidase, favoring resistance or tolerance to biotic and abiotic stresses (Wang et al., 2019). However, the use of this method influences gene expression, resulting in biochemical changes (Zhang et al., 2022).

Up to date, there are no known studies characterizing the effect of ultrasonic waves in overcoming dormancy in *O. pyramidale* seeds.

The objective of this work was to evaluate the effect of ultrasound waves, in a continuous mode, at different frequencies, intensities, and exposure times on the pre-germination treatment of seeds of *O. pyramidale*.

Materials and Methods

Fruits and seeds of *O. pyramidale* were collected in the municipality of Senador Guiomard, in the state of Acre, Brazil (67°36'23"W, 10°03'59"S). The fruits were picked with the aid of a pole pruner. After collected, they were packed in dry bags and taken to a laboratory for processing.

During processing, the fruits of *O. pyramidale* were for spontaneous opening. Subsequently, the seeds were manually separated from the surrounding fiber. After processing, the seeds were left to rest in a room, at 22°C, for 48 hours in order to dry. Then, they were stored in a refrigerated chamber, at 20°C, for six months until the experiments were carried out. For the batch of *O. pyramidale* seeds used in the present study, moisture content was 4.68%, the weight of one thousand seeds was 8.765 g, the average number of seeds per kilogram was 114,090, and viability was 85%.

To perform the tests, the *O. pyramidale* seeds were disinfected by immersion in a 70% alcohol solution for 1 min and then in a 2.0% sodium hypochlorite solution for 3 min, being, subsequently, rinsed under running water for 2 min.

For the process of overcoming seed dormancy, the ultrasound was applied based on the methodology adopted by Dávila et al. (2021), using the Sonomed V device (Carci, Vila Mariana, SP, Brazil), adjusted to the frequencies of 1.0 and 3.0 MHz and to the intensities of 1.0 and 2.0 W cm^{-2}. The established exposure times were 2 and 3 min.

The seeds were subjected to ten treatments (T1 to T10), described in Table 1. T1 and T2 were the

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reference treatments, respectively: the control and the recommended to overcome dormancy according to the instructions for the seed analysis of forest species (Brasil, 2013). In T2, the *O. pyramidale* seeds were immersed in water at 80ºC, followed by immersion in 400 mL of water, at room temperature for 30 min, to cool down. Ramos et al. (2006) pointed out that this temperature does not influence the percentage of seed germination.

The experimental design was completely randomized. In all ten treatments, the seeds were tested separately for germination and emergency, in four replicates. The experimental unit was 50 seeds. One set of 2,000 seeds was used for each test. The used substrate was sand.

The germination test was performed in a biochemical oxygen demand germination chamber at 30ºC, with a 12 hour photoperiod (Brasil, 2009). Seeds were distributed on two germination sheets covered with a third sheet, which were then rolled. Each roll of paper was moistened with distilled water at the proportion of 2.5 times the mass of the unhydrated sheet according to the recommendations for seed analysis (Brasil, 2009).

The rolls were packed in plastic bags inside the germination chamber and kept in an upright position. The germination test was completed after 60 days. The seeds counted as germinated were those showing the protrusion of the primary root, with the emission of the main root.

The variables analyzed in the germination test were the following: percentage of germination; germination speed index, proposed by Maguire (1962); and germination speed, proposed by Edmond & Drapala (1958). The used equations were, respectively:

$$GEM = \left(\frac{NG}{SP}\right) \times 100$$

where G is the percentage of germination (%), NG is the total number of seeds germinated, and SP is the total number of seeds placed to germinate.

$$GSI = \frac{NG1}{DS1} + \frac{NG2}{DS2} + \frac{NG3}{DS3} + \cdots + \frac{NGn}{DSn}$$

where GSI is the germination speed index, NGn is the number of seeds germinated in each daily counting, DSn is the number of days passed from sowing to the day of counting, and n is the day of counting.

$$GSD = \frac{\sum NG_i\, IT_i}{\sum NG_i}$$

where GSD is germination speed in days, NG_i is the number of seeds germinated per day, IT_i is the incubation time in days, and i is the day of observation.

The emergence test was conducted in a greenhouse, using polyethylene trays measuring 8.0x30x45 cm, containing washed and sterilized sand. Sterilization was performed in a vertical autoclave at a pressure of 1.0 atm and temperature of 120ºC for 60 min. In the trays, the sand was moistened with distilled water. The amount of water used was calculated according to Brasil (2009). Subsequently, the seeds were sown in the sand at a depth of 1.0 cm. The seedling emergence

Table 1. Method used, frequency, intensity, and time of exposure to the ultrasound in the treatments for overcoming the dormancy of *Ochroma pyramidale* seeds.

Treatment	Method	Frequency (MHz)	Intensity (W cm^{-2})	Time (min)
T1 (control)	No treatment	-	-	-
T2	Hot water at 80°C	-	-	-
T3	Ultrasound	1.0	1.0	2
T4	Ultrasound	1.0	1.0	3
T5	Ultrasound	3.0	1.0	2
T6	Ultrasound	3.0	1.0	3
T7	Ultrasound	1.0	2.0	2
T8	Ultrasound	1.0	2.0	3
T9	Ultrasound	3.0	2.0	2
T10	Ultrasound	3.0	2.0	3

criteria was the elevation of the cotyledons. The test was completed after 60 days.

The variables analyzed in the sand-emergence test were: percentage of emergence; emergence speed index, proposed by Maguire (1962); and emergence speed, proposed by Edmond & Drapala (1958). The used equations were, respectively:

$$EMG = \left(\frac{NE}{SP}\right) \times 100$$

where E is the percentage of emergence (%), NE is the number of seeds emerged, and SP is the total number of seeds placed to germinate.

$$ESI = \frac{NE1}{DS1} + \frac{NE2}{DS2} + \frac{NE3}{DS3} + \cdots + \frac{NEn}{DSn}$$

where ESI is the emergence speed index, NEn is the number of seedlings emerged in each daily counting, DSn is the number of days passed from sowing to the day of counting, and n is the day of counting.

$$ESD = \frac{\sum NE_i \, IT_i}{\sum NE_i}$$

where ESD is the emergence speed in days, NE_i is the number of seeds emerged per day, and IT_i is the incubation time in days.

After 60 days, all seedlings that emerged in each treatment were measured. The height of the aerial part and the length of the main root of each seedling were measured using a caliper. The results were expressed in centimeters.

In order to determine shoot fresh mass, root fresh mass, shoot dry mass, and root dry mass, seedlings were packed in kraft paper bags, weighed on a semi-analytical balance with a precision of 0.001 g, and dried in an oven with forced-air circulation, at a temperature of 105±3°C, until mass stabilization. After cooling in a desiccator, each sample was weighed again. The results were expressed in grams.

Data were checked for assumptions of normality, independence of residues, and homoscedasticity using the tests of Shapiro-Wilk, Durbin-Watson, and Levene, respectively. Then, the one-way analysis of variance was carried out. The Tukey test was performed at a 5% probability level to identify the difference between treatments. The R, version 4.1.1, software (R Core Team, 2021) was used. Assumptions were met, and no data transformation was necessary.

Results and Discussion

T2 showed the best results for percentage of germination (84%) and of emergence (88%), followed by T8 (70 and 60%, respectively), as shown in Table 2. These results are an indicative that the use of ultrasound on the seeds of the studied species is effective.

In terms of the germination and emergence speed indexes, T8 showed the best results (Table 2). The other treatments did not differ significantly from each other.

In T2, T7, and T8, the best percentage of emergence occurred between the fourth and fifteenth day. In the other treatments, the seeds also started germinating on the fourth day, but not in a uniform way, presenting a low emergence percentage.

In T7 and T8, at the frequency of 1.0 MHz and intensity of 2.0 W cm^{-2}, a significant increase in germination and emergence was observed as the time of exposure of the seeds to the ultrasound waves increased (Table 2). At this frequency and intensity, both of these treatments proved to be superior than the other ones in which the seeds were subjected to ultrasonic waves.

In another study using the same species, Geovo et al. (2021) evaluated the percentages of seed germination using different chemical scarification methods: sodium hypochlorite at the concentrations of 0.5, 1.0, and 2.0% for 15 min; 1.0% hydrochloric acid for 1 min; and hot water at 80°C. In this case, the best germination percentage was 59% using the hot water treatment at 80°C. Bao et al. (2016) also found good results when evaluating this method compared with those of packaging in sulfuric acid at different periods of time. However, Toledo-Gonzáles et al. (2019) observed the best results with the hot water method at 100ºC.

Regarding the development of *O. pyramidale* seedlings after 60 days in the greenhouse, the length of the aerial part did not differ significantly between treatments. In T8, under the frequency of 1.0 MHz and intensity of 2.0 W cm^{-2} for 3 min, root length showed the highest mean of 6.07 cm (Table 3).

The fresh mass of the aerial part presented the best average in T2 (5.09 g), followed by T8 (4.57 g), and T7 (3.52 g). The three best fresh root masses of 3.48, 2.71, and 1.68 g were obtained in T8, T2, and T7, respectively (Table 3).

Regarding the dry mass of the aerial part, T8, T2, and T7 (0.98, 0.88, and 0.70 g, respectively) stood out compared with the other treatments. As to root dry mass, the best values of 1.15, 0.63, and 0.52 g were obtained in T8, T2, and T7, respectively (Table 3).

Fresh mass of the aerial part, fresh root mass, and dry mass of the aerial part did not differ between the reference treatment T2 (using hot water) and T7 and T8 (at a frequency of 1.0 MHz and intensity of 2.0 W cm^{-2}); in addition, the root dry mass of T8 was higher than that of the other treatments. Therefore, it can be inferred that, at that frequency and intensity, the results for the three mentioned variables are similar to those obtained by the hot water method.

According to Wang et al. (2012), the effects of ultrasound in overcoming dormancy occur due to pressure fluctuations on the seeds, which causes the formation, growth, and violent collapse of microbubbles in the sonication liquid; this bubble collapse leads to physical, biological, and chemical changes in the seed. Rifna et al. (2019) attributed the positive results in the seed germination rate through sonication to the mechanical effects produced by the ultrasound, i.e., numerous small holes in the coating and cracks in the pericarp of the seed, noticeably increasing its moisture. In this line, López-Ribera & Vicient (2017) commented on the potential of ultrasound technology to improve the germination process of old seeds without affecting negatively the development of seedlings. However, this observation requires more research related to forest species.

Like other methods, the use of ultrasound for overcoming seed dormancy has pros and cons. The main advantage of this technique is its easy application compared with that of methods such as acid or

Table 2. Results of mean and mean tests for germination percentage (G), germination speed index (GSI), germination speed (GS), emergence percentage (E), emergence speed index (ESI), and emergence speed (ES) of *Ochroma pyramidale* seeds in the evaluated treatments[1].

Treatment	G (%)	GSI	GS (days)	E (%)	ESI	ES (days)
T1	0d	0.00c	0.00a	2d	0.08c	10.88abc
T2	84a	7.35a	9.39a	88a	4.65a	10.35abc
T3	2d	0.13c	8.13a	7d	0.22c	17.73ab
T4	8d	0.87c	4.78a	8d	0.85c	5.43c
T5	8d	0.87c	5.31a	4d	0.23c	7.79bc
T6	4d	0.26c	11.13a	4d	0.12c	18.79a
T7	41c	4.01b	7.83a	44c	2.68b	10.18abc
T8	70b	6.83a	7.75a	60b	3.25b	11.45abc
T9	11d	1.26c	4.31a	4d	0.27c	7.71bc
T10	11d	1.04c	9.65a	11d	0.50c	14.3 abc

[1]Means followed by distinct letters differ from each other by the Tukey test, at a significance level of 5%.

Table 3. Results of mean and mean tests for aerial part length (AL), root length (RL), fresh shoot mass (FSM), fresh root mass (FRM), shoot dry mass (SDM), and root dry mass (RDM) of *Ochroma pyramidale* in the evaluated treatments[1].

Treatment	AL (cm)	RL (cm)	FSM (g)	FRM (g)	SDM (g)	RDM (g)
T1	4.20a	1.99b	0.17c	0.03c	0.03b	0.02d
T2	5.66a	5.20ab	5.09a	2.71ab	0.88a	0.63b
T3	5.45a	4.11ab	0.71c	0.18c	0.12b	0.04d
T4	6.06a	5.30ab	0.71c	0.20c	0.14b	0.05d
T5	3.01a	2.82ab	0.32c	0.08c	0.09b	0.04d
T6	4.36a	4.82ab	0.30c	0.10c	0.06b	0.03d
T7	5.54a	5.01ab	3.52b	1.68b	0.70a	0.52bc
T8	4.88a	6.07a	4.57ab	3.48a	0.98a	1.15a
T9	3.85a	4.58ab	0.43c	0.15c	0.10b	0.19cd
T10	4.86a	5.33ab	1.08c	0.44c	0.22b	0.13d

[1]Means followed by distinct letters differ from each other by the Tukey test, at a significance level of 5%.

mechanical scarification, while the disadvantage lies in the small size of the apparatus available for the application of ultrasonic waves in the seed industry (Nazari & Eteghadipour, 2017). Despite the positive results reported for that technique, the method with hot water at 80°C was approximately 16% more efficient in seed germination than the best ultrasound treatment. Therefore, hot water is still the most recommended for overcoming dormancy of the *O. pyramidale* species, especially when considering the cost and practicality of the method.

Conclusions

1. The hot water at 80°C method is the most effective for overcoming the dormancy of *Ochroma pyramidale* seeds.
2. The T8 ultrasound treatment at frequency of 1.0 MHz, intensity of 2.0 W cm^{-2}, and exposure time of 3 min has a positive effect on overcoming seed dormancy.
3. Although the use of the ultrasound has potential to overcome dormancy in *O. pyramidale*, it is not recommended due to its costs and low practicality.

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Author contributions

Ana Paula Falcão Freire: conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, writing – original draft, writing – review & editing; **Eduardo Pacca Luna Mattar**: conceptualization, methodology, project administration, supervision, writing - review & editing; **Harley Araújo da Silva**: conceptualization, data curation, methodology, validation, writing – original draft; **Luz Patricia Velásquez Dávila**: conceptualization, methodology, writing – review & editing; **Luis Eduardo Maggi**: resources, writing – review & editing.

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No generative artificial intelligence (AI) was used in this study.

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The authors declare no conflicts of interest.