Cuban Journal of Forest Sciences

CFORES

Germinative response of Vismia baccifera to pregerminative treatment with ultrasound

Respuesta germinativa de Vismia baccifera al tratamiento pregerminativo con ultrasonido

Resposta germinativa de Vismia baccifera ao tratamento pré-germinativo com ultrassom

Yamila Lazo Perez[1]* iD , *Arliet Morales Moreno*[2] iD

[1]Universidad Estatal Amazónica, Ecuador.

[2] Palmeras del Ecuador SA Ecuador.

*Corresponding author ylazo@uea.edu.ec

ABSTRACT

Studies on pregerminative treatments to accelerate the germination process of forest species are very important. The *Vismia baciifera* species is very important in reforestation programs, however, it has low germination capacity. The objective of this study was to evaluate the effect of pregerminative treatment with ultrasound on the germination of seeds of *V. baccifera.* A central composite design was carried out with the response surface methodology

using Design Expert version 12.0 software. Thirty seeds with three replicates were used for the experimental condition, with the use of ultrasound with a frequency of 40 KHz. The variables considered were: ultrasonic bath time, 1-10 min, water temperature in the bath, 30-60°C, and germination percentage. The results demonstrated that the pregerminative treatment with ultrasound exerted a positive effect on the germination of seeds of *V. baccifera,* with an increase in germination from 7.7 % to 67.8 % under the experimental conditions of 60°C with 5.9 min. This indicates the use of ultrasound as a pregerminative treatment to increase the germination of *V. baccifera,* which provides new incursion alternatives for forest species with low germination potential.

Keywords: seeds; germination; physical treatment; *Vismia baccifera*

RESUMEN

En la actualidad orientar estudios sobre tratamientos pregerminativos para acelerar el proceso de germinación de semillas forestales es de gran importancia. La especie *Vismia baciifera* es muy importante en los programas de reforestación, sin embargo, posee baja capacidad germinativa. El objetivo de este trabajo fue evaluar el efecto del tratamiento pregerminativo con ultrasonido sobre la germinación de semillas de la especie *V. baccifera.* Se realizó un diseño central compuesto con la metodología de superficie respuesta, mediante el software Design Expert versión 12.0. Se utilizaron 30 semillas con tres réplicas para condición experimental, con el uso de ultrasonido de frecuencia 40 KHz. Las variables consideradas fueron: tiempo del baño ultrasónico (1-10 min), temperatura del agua en el baño (30-60 °C) y porcentaje de germinación. Los resultados demostraron que el tratamiento pregerminativo con ultrasonido ejerció un efecto positivo en la germinación de semillas de *V. baccifera*, con un incremento en la germinación de 7.7 % a 67.8 % bajo las condiciones experimentales de 60 °C y 5.9 min. Esto indica el uso del ultrasonido como tratamiento pregerminativo para el incremento de la germinación de *V. baccifera*, lo que brinda nuevas alternativas de incursión para especies forestales con bajo potencial germinativo.

Palabras clave: semillas; germinación; tratamiento físico; *Vismia baccifera.*

RESUMO

Atualmente, é de grande importância orientar estudos sobre tratamentos pré-germinativos para acelerar o processo de germinação de sementes florestais. A espécie Vismia baciifera é muito importante em programas de reflorestamento, porém apresenta baixa capacidade germinativa. O objetivo deste trabalho foi avaliar o efeito do tratamento pré-germinativo com ultrassom na germinação de sementes da espécie V. baccifera. Foi realizado um planejamento composto central com a metodologia de superfície de resposta, utilizando o software Design Expert versão 12.0. Para a condição experimental foram utilizadas 30 sementes com três repetições, com uso de ultrassom com frequência de 40 KHz. As variáveis consideradas foram: tempo de banho ultrassônico (1-10 min), temperatura da água do banho (30-60 °C) e porcentagem de germinação. Os resultados demonstraram que o tratamento pré-germinativo com ultrassom exerceu efeito positivo na germinação de sementes de V. baccifera, com aumento na germinação de 7,7 % para 67,8 % nas condições experimentais de 60 °C e 5,9 min. Isso indica a utilização do ultrassom como tratamento pré-germinativo para aumentar a germinação de V. baccifera, o que proporciona novas alternativas de incursão para espécies florestais com baixo potencial germinativo.

Palavras-chave: sementes; germinação; tratamento físico; Vísmia baccifera.

INTRODUCTION

Seed germination is one of the most important stages to achieve successful establishment of forest plantations. The seed is responsible for the next generation of plants, maintains the germplasm and species diversity (Sharififar *et al.,* 2015; Rifna *et al.,* 2019; Rehmani *et al.,* 2023). However, the seeds of many tropical species exhibit various dormancy mechanisms that affect germination uniformly (Venâncio and Martins 2019) and delay the germination of viable seeds (Nazari *et al.,* 2015). Therefore, some form of prior seed treatment is necessary in order to accelerate and obtain high germination percentages.

Pregerminative treatments improve germination and optimize the quality and quantity of plants (Abdala *et al.,* 2020). For this reason, selecting a low-cost, fast and highly efficient pregerminative treatment to break seed dormancy (Venâncio and Martins 2019) implies a greater production of seedlings and a substantial increase in their quality (Jiménez *et al.,* 2017).

The most used method to increase the germination percentage is scarification through chemical treatments, but it is harmful to people's health and causes negative impacts on the environment (Chiu 2015; Pandiselvam *et al.,* 2020; Dziwulska-Hunek *et al.,* 2020). In this context, the scientific community has focused on the search for ecological alternatives that can be used as a pregerminative treatment of seeds. In recent times, a high variety of ecological and environmentally friendly physical treatments have been reported (Liu *et al.,* 2016; Rifna *et al.,* 2019). Among them is the application of ultrasound techniques that allows increasing the percentages of germinated seeds (Mihaylova *et al.,* 2021; Trakselyte-rupsiene *et al.,* 2021; Abd-Elrahman *et al.,* 2023; Nogueira *et al.,* 2024; Liu *et al.,* 2016).

Ultrasound techniques as a pregerminative treatment consist of placing the seeds in a device that emits ultrasonic waves, to which the frequency, power, temperature and sonication time can be adjusted, with the purpose of altering the physiological activity of the seeds. (Chen *et al.,* 2013; Wang *et al.,* 2019). Ultrasonic waves cause fragmentation of the seed coat, which helps improve hydration of dormant seeds (Yaldagard *et al.,* 2008) also modifies the structure of enzyme molecules (Liu *et al.,* 2021), promotes enzyme catalysis and accelerates seed germination (Wang *et al.,* 2019; Yaldagard *et al.,* 2008).

The species *Vismia baccifera* is a tree of the Hypericaceae family of ecological and economic importance, typically from the Amazon rainforest. It is commonly used as a medicinal plant by the indigenous population for the cure of various diseases (Handrini *et al.,* 2018) and its wood is used for carpentry and construction (Guzmán *et al.,* 2023). In recent times it has been widely used by producers in the province of Pastaza for the production of pallets, which implies the indiscriminate felling of its natural populations and causes serious deterioration of the forest. At the same time, it has been observed that it has a low rate of natural regeneration and there are few seeds that have the possibility of completing their

life cycle once the trees have been cut down. There are few or no germination studies of the species and producers report low germination percentages, so the use of pre-germination treatments with ultrasound will allow germination to be stimulated. Hence, the potential of the species and the need for its use in reforestation after pregerminative treatment is recognized. Therefore, the objective of this work is to evaluate the effect of pregerminative treatment with ultrasound on the germination of seeds of the species *V. baccifera*.

MATERIALS AND METHODS

Experiment location and seed collection

The experiment was carried out in the facilities of the Chemistry Laboratory of the main matrix of the Universidad Estatal Amazónica, Puyo parish, canton and province of Pastaza, city of Puyo, Vía Napo Km 2 ½, Paso Lateral S/N. For the development of the research, seeds were collected from trees of the species *V. baccifera*, located in the parish of Fátima, canton and province of Pastaza, Ecuador.

Seed collection was carried out by selecting three trees of the species *V. baccifera*, which were isolated in remnants of natural forests. The selection of individuals was carried out taking as criteria the evaluation of seed trees, considering the phenotypic parameters: straight stem, non-bifurcated trees, dominance of the main axis, angle of insertion of the branches of 90°, shape of the circular crown and diameter average crown of 12 m (Valladolid *et al.*, 2017).

The seeds collected from each tree were mixed into a single sample for later analysis. The seeds were submerged in two 250 ml beakers, in order to physically separate the good quality seeds due to the effect of decantation and flotation of the infertile material (Jiménez *et al.*, 2017).

Experiment design

Design Expert software version 12.0 (Serial number 9847-9696-7992-6750, Stat-Ease Inc., 1300 Godward Strret North, Suite 6400 Minneanopolis, USA). The independent variables

were numerical: ultrasonic time, 1 to 10 min, and water temperature in the bath, 30 to 60°C, and the response variable was the germination percentage (Table 1). The combination of the factors is displayed in Table 2.

Table 1. - Level of experimental variables for pregerminative treatment with ultrasound in V. baccifera seeds

Factor	Name	Unit	Guy	Subtype	Minimum	Maximum	Central point
A	Ultrasonic time	min	Numeric	Continuous	1	10	5
B	Water temperature	°C	Numeric	Continuous	30	60	45

Pregerminative treatment

In an ultrasound (Branson 3800, CPXH series, 130 W, 40 KHz), 30 seeds were immersed with three replicas for each treatment, which were placed on a metal mesh in direct contact with the water of the ultrasonic bath. The experiments were carried out according to each experimental condition established in the design (Table 2). The seeds subjected to each experimental condition as pregerminative treatment (ultrasound) were sown in Petri dishes on sterile cotton moistened with distilled water. Total germination was measured 15 days after sowing and expressed as a percentage.

The significant variables were analyzed in order to choose the optimal levels of the independent variables. The experimental data were fitted using the second-order polynomial equation (Equation 1).

$$y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{ij} X_i X_j \quad \text{(Equation 1)}$$

Where: y represents the predicted response; β_0, β_{ii} and β_{ij} are the regression coefficients for mean, linear, interaction and quadratic terms, calculated based on the experimental results by least squares X_i and X_j.

Likewise, analysis of variance (ANOVA) was applied to evaluate the influence of the independent variables ($P < 0.05$) of the design. The validity of the experimental design was verified by additional experiments with three repetitions, using the predicted conditions.

RESULTS

Influence of pregerminative treatment on V. baccifera seed germination

Table 2 shows the experimental and predicted results for the construction of the predictive model for *V. baccifera* seed germination. The germination percentage under the experimental conditions ranged between 8.9 % and 67.8 %, while without pregerminative treatment it was only 7.7 %.

Table 2. - *Conditions of the experimental runs of the pregerminative treatment with ultrasound of the species V. baccifera. Experimental results ± standard deviation error and predicted values*

Experiment	Time in ultrasonic bath (min)	Water temperature in the bath (°C)	Experimental germination (%)	Predicted germination (%)
1	10	45	28.8±0.8	26.60
2	10	30	25.5±0.7	24.78
3	5.5	45	63.3±1.5	59.83
4	5.5	45	60.0±0.6	59.83
5	1.0	30	8.9±0.3	8.95
6	5.5	60	67.8±0.7	64.60
7	5.5	30	57.8±0.3	58.26
8	10	60	30.0±0.6	31.62
9	1.0	45	12.2±0.3	10.26
10	5.5	45	56.7±0.5	59.83
11	5.5	45	58.2±0.2	59.83
12	1.0	60	13.3±0.6	14.78
13	5.5	45	59.4±0.7	59.83

V. baccifera germination at 15 days. The distribution of points corroborated the model's ability to cover the entire experimental range. The values of $R^2=0.9920$ and Adj. $R^2=0.9862$ (Table 3) of the regression line indicated a good correspondence between the experimental

and model-predicted values on the experimental data. In Figure 1B it was found that the experimental data met the assumption of normal distribution.
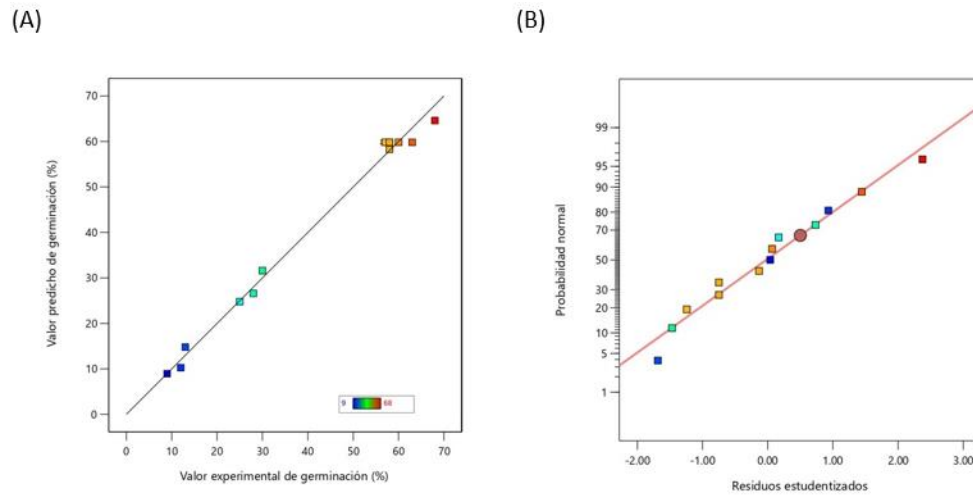

Figure 1. - *(A) Experimental and predicted values of V. baccifera germination. (B) Normal distribution of experimental data*

The results of the ANOVA indicated that the factors immersion time of the seeds in the ultrasonic bath and the water temperature were significant ($P < 0.05$) on the percentage of germinated seeds (Table 3).

Table 3. - Analysis of variance (ANOVA) of the factors ultrasonic time and water temperature on the germination of V. baccifera seeds. P < 0.05 indicate a significant effect

Model	Sum of squares	Degrees of freedom	Mean square	F-value	P-value	
Quadratic	5841.99	5	1168.40	173.12	< 0.0001	Significant
A-Time Ultrasonic	400.17	1	400.17	59.29	0.0001	
B-Water temperature	60.17	1	60.17	8.91	0.0203	
AB	0.2500	1	0.2500	0.0370	0.8528	
A²	4733.01	1	4733.01	701.27	< 0.0001	
B²	7.10	1	7.10	1.05	0.3392	
Residue	47.24	7	6.75			
Lack of fit	24.44	3	8.15	1.43	0.3584	Not significant
Pure error	22.80	4	5.70			
Corrected total	5889.23	12				
R²	0.9920					
Adjusted R²	0.9862					
Predicted R²	0.9602					
Adequate precision	31.5289					

The germination percentage was adjusted to a second-order polynomial equation. This model allowed us to find the relationship between the water temperature in the ultrasound bath, the immersion time and the response to germination (Equation 2).

$$G\ (\%) = -6.1 + 24.1TU - 0.4TA + 0.003AB - 2.04B^2 + 0.007A^2 \quad \text{(Equation 2)}$$

The optimal conditions to achieve a germination percentage of 65% were at a temperature of 60C and a time of 5.9 min (Figure 2). These conditions were validated experimentally, obtaining 66.0 ±1.7 % germination.
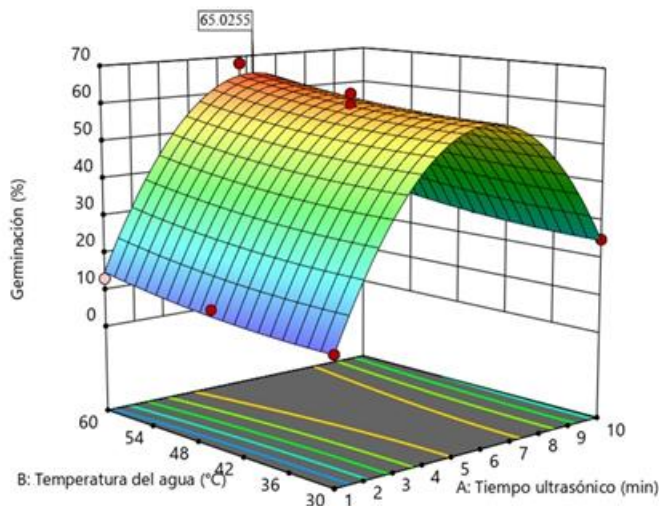
Figure 2. *- Surface response of the effect of ultrasonic seed immersion time and water temperature on the germination percentage of V. baccifera seeds*

DISCUSSION

Ultrasound techniques as a pregerminative seed treatment are a recognized alternative as a highly efficient physical stimulation method (Wang *et al.*, 2019), besides to be ecofriendly (Ding *et al.,* 2018). However, the results of the effectiveness of the treatment are limited with ultrasound techniques (frequency, duration of sonication and water temperature), since they have different degrees of influence on germination (Venâncio and Martins 2019).

A wide variety of research designs have been reported with different sample sizes and measured variables, in addition to various environmental conditions that show differentiated responses in germination percentages (Awad *et al.,* 2012). Several studies have demonstrated the marked effect of the application of ultrasound techniques as a pregerminative treatment of seeds (Rifna *et al.*, 2019; Trakselyte-rupsiene *et al.*, 2021; Liu *et al.*, 2021; Liu *et al.*, 2016). The application of ultrasound in forest seeds is scarcely reported

in the literature. Recently, Zhang *et al.,* (2023) in a study of a forest species it was found that ultrasound promotes the germination of aged *Pinus tabuliformis* seeds. The ultrasound power could increase the germination rate of aged seeds about three times compared to the control. However, there are various studies on seeds of nutritional interest.

Sharififar *et al.,* (2015) documented the effect of ultrasonic waves on the germination of *Atriplex lentiformis, Cuminum cyminum* and *Zygophyllum eurypterum* seeds*,* where the germination percentage revealed a significant effect at exposures of 1, 3, 5, 7 and 9 minutes. This is because ultrasonic waves break the dormancy of the seeds, causing a positive impact on germination.

On the other hand, studies of species of the Fabaceae family showed that, to achieve a significant percentage of germination in seeds of *Medicago scutellata*, ultrasonic waves were used, under conditions such as: frequency (20 KHz), power (150 W) and exposure time (0 control, 3, 6, 9 and 12 min) where obtained a percentage of 97.25 % at 6 min and 98 % at 9 min (Tajbakhsh *et al.,* 2011). Furthermore, Yang *et al.,* (2015) stated that ultrasound treatment increases the germination rate, shoot length and gamma-aminobutyric acid content in shoots of *Glycine max*.

Ultrasound increases porosity through acoustic cavitation, improves mass transfer. The additional absorbed water reacts freely and easily with the embryo cell, which releases gibberellic acid and leads to metabolic processes in the aleurone cells, and helps mobilize nutrients from the endosperm through cell membrane rupture (Miano *et al.,* 2015).

It has been found that after exposure to 250 W, 350 W and 450 W of ultrasonic waves, for five minutes, a germination percentage of 94 %, 100 % and 97 % respectively can be obtained (Venâncio and Martins, 2019).

Ultrasound can also be used as a microbial control treatment, as demonstrated by Chiu and Sung (2014) who used an ultrasonic bath at a power of 40 KHz for 1 min at 25°C in three varieties of *Pisum sativum*, which increased germination, decreased microbial load and improved shoot growth of *Pisum sativum* up to 97 % (Chiu and Sung, 2014).

Medicago seeds *scutellata* were subjected to the frequency of ultrasonic waves of 42 KHz in different periods of time, 0 min (control), 1, 3, 5, 7 and 9 min, at room temperature (25°C). The 7-min exposure treatment had a positive influence and the nine-minute exposure treatment had a negative impact on the germination percentage (Nazari *et al.,* 2015).

Likewise, ultrasound has substantially improved the sprouting percentage of *Phaseolus vulgaris,* since from an intensity of 360 W and 60 min, and reached 100% (Oforiwaa *et al.,* 2020). On the other hand, Miano *et al.,* (2019) applied two technologies: ionizing radiation (at doses of 2.3 and 3.8 KG and using ÿ rays) and ultrasound (91 WL $^{-1}$ and 25 KHz at 25°C) individually and in combination to evaluate the hydration and germination process of *Phaseolus vulgaris,* revealing that irradiation does not significantly influence the hydration rate and latency time, but it does affect ultrasound because it reduced the processing time by 50%. The effect of the interaction of radiation and ultrasound technology decreases the equilibrium humidity.

Adzuki beans, showed that the use of ultrasound (40 KHz or 80 KHz, at 25°C, in 1 min) improves the hydration and germination of its seeds, as well as the contents of polyphenols, flavonoids, total saponins and even more so the antioxidant activities (FRAP and DPPH) of the sprouts (Chiu, 2021).

In a study on ultrasound-assisted red bean germination to improve the conventional germination process and nutritional quality of red beans, high-intensity ultrasound (HIU) supplemented with hydrogen peroxide was employed as a pre-germination treatment. The results showed that the 350 W-ten-minute treatment produced the highest germination rate (77.09 %), with a shoot length 81.13 % greater than that of the control group. The 350 W for 10 min treatment increased the content of total proteins, soluble proteins and ash, while reducing the content of fats, starch and soluble sugars (Athoi *et al.,* 2024). However, Miano and Duarte (2018) state that the results of ultrasound always depend on the intensity (power and time) with which it is used, because it can enhance or hinder the germination process. In this case, they used ultrasound with a volumetric power of 91 WL $^{-1}$, frequency of 25 KHz, at a constant temperature at 25 ± 1°C for 510 min to incorporate nutrients (iron) in the

hydration of the seeds, consequently, the maximum germination percentage decreased considerably (close to 50 %) due to the high intensity of ultrasound and iron toxicity.

The information has shown greater results with ultrasound effects for food or grass species, for example, Miano *et al.,* (2015) determined the effect of ultrasound on the germination and vigor of four lots of *Hordeum vulgare* seeds, resulting in batch 1 with the highest germination rate (89 %) and batch 4 (43 %) with the lowest, based on the conditions: frequency (20 KHz), volumetric power (0.028 Wm^{-3}) and time (4h), demonstrating that ultrasound technology can improve the hydration process and induce physiological changes that increase seed vigor. Another study achieved a germination rate of 99.4 % at 30°C, frequency of 20 KHz, constant power of 460 W and at an intensity of 100 % for 15 min (Yaldagard *et al.,* 2008). However, in the same period of time (15 min) and temperature 30°C, but at a higher frequency (42 KHz) Pour *et al.,* 2018), obtained a germination rate of 100 %, thus showing that the power was not significant for the germination of *Hordeum vulgare.*

Oforiwaa and Ngadi (2020) reported that paddy rice seeds subjected to ultrasound increased their germination percentage since ultrasonic waves transfer energy to cytoplasmic cells. Similarly, Xia *et al.,* (2020) examined the stimulation of ultrasonic waves (28 KHz, 17.83 W) on *Oryza sativa* seeds, finding a significant acceleration in the germination process within 5 to 30 min of exposure. Ultrasonic treatment of seeds with a mixed frequency of 20 to 40 KHz for 1.5 minutes had a positive impact on germination and growth of aromatic rice due to modulation of morphological and physiological attributes of seeds.

Ultrasonic treatment improves germination potential and increased soluble protein in leaves together with photosynthetic efficiency, which contributed to improving the biomass and grain yield of rice (Huang *et al.,* 2024).

Ultrasonic treatments had positive effects on the germination percentage of aged *Festuca arundinacea* and *Psathyrostaehys juncea Nevski* seeds by 79 % and 89.3% respectively, at a sonication time of 15 minutes, a sonication temperature of 45°C and an output power of 350W (Liu *et al.,* 2016).

Wheat seeds were subjected to ultrasonication (model, KQ-200VDV; frequency, 45 KHz; power, 160 W) throughout different doses of ultrasonic vibration (0, 5, 10, 15, and 20 min, respectively). Ultrasonic vibration in 5 and 10 minutes caused an increase in seed germination rate, after 20 h of ultrasonic exposure, in chlorophyll and protein concentration, in root and shoot length, and in fresh weight (Chen *et al.,* 2013). In another study, wheat grains germinated at 86 % (Guimarães *et al.,* 2020).

Another experiment under the same frequency (40 KHz) and temperature (15 or 20C), but at different times (5, 10, 15, 30, 45 or 60 min) showed that the germination rate in *Citrullus lantanus , Triticum durum* and *Cicer arietinum* increased by 133% (at 45 minutes), 95% (30 min), and 45% (five minutes), respectively (Goussous *et al.,* 2010).

Ultrasound has also been applied to the study of some genera of orchids, in which their seeds are difficult to germinate. Chiu (2015) achieved 54% germination of *Paphiopedilum SCBG* seeds, when he treated them with ultrasound at 40 KHz, within 8 minutes at room temperature. Likewise, Lee *et al.,* (2007) managed to germinate 34.8 % of *Calanthe tricarinata* seeds under the conditions of 200 W, 44 kV, 40 KHz, in 45 min.

Pulsatile ultrasound fields are presented in scientific literature as ways to break dormancy and the thick seed coat (Singureanu *et al.,* 2015). Therefore, Pascual *et al.,* (2004) carried out seven treatments to eliminate hardness and four to break the physiological dormancy of *Capparis spinosa* seeds, where the combination of mechanical scarification with ultrasound (Selecta Ultrasons, model 513, 150 w, 40 KHz) and room temperature for 30 minutes, obtained results greater than 70%. In the same way, Rinaldelli (2000) subjected *Capparis spinosa* seeds to the effect of ultrasonic waves in exposure times from 10 minutes to 6 h, with a temperature between 25 and 30°C, with the difference that they were previously subjected to scarification with sulfuric acid, the seeds responded positively to this combination. Although, the highest percentage of germination, greater than 50%, was obtained when ultrasonic treatment was carried out in the presence of gibberellic acid (400 mgL $^{-1}$).

Ultrasonic treatments were carried out on artificially aged and unaged seeds of the *Arabidopsis thaliana* species, using three sonication times (30 s, 1 and 2 min) frequency of 45 KHz and constant temperature of 24°C, aged seeds showed no significant effects on germination, although artificially aged seeds increased 10 % in germination from brief ultrasound treatments (30 and 60 s) (López-Ribera and Vicient, 2017).

Ultrasonic waves improve germination of aged *Ricinus communis* seeds under drought and saline stress conditions (Babaei *et al.,* 2023). In *Chenopodium album* seeds, ultrasound was applied with a frequency of 35 KHz, throughout 0 (control), 5, 10, 15 and 30 minutes. Germination percentages of 83.25 % were achieved after 15 minutes of exposure. It should be noted that if the exposure time is extended (30 minutes), it can be harmful to the seed (Babaei-Ghaghelestany *et al.,* 2020).

Studies were carried out on *Dioscorea fandra* species, consisting of subjecting seeds to cooling (5°C for two weeks), and then placing them for immersion in an ultrasonic bath (Bransonic 3210R) with 47 KHz, 130 W, at 10 min and 20 min, at 25°C. Under these conditions, the species obtained the highest germination percentage (90%) once the seeds were exposed to ten minutes (Andriamparany and Buerkert, 2019).

Studies have also been carried out on the germination behavior of sesame seeds through an ultrasonic bath, varying two factors, the duration of the treatment (10, 20 and 30 minutes), the germination temperature (15, 20 and 25°C) and frequency (20 KHz) as a constant value, obtaining a maximum of 22 % germination, at an optimal temperature of 15°C for 20 minutes (Shekari *et al.,* 2015).

In the present study, the ultrasound treatment obtained 67.8% germination under the conditions: temperature (60°C), time (5.9 minutes), this being a novel result for forest species with the use of ultrasound. This showed that although there are limited reports in the literature for forest species, the effect of ultrasound to increase germination capacity is corroborated.

CONCLUSIONS

Pregerminative treatment with ultrasound had a positive effect on the germination of *V. baccifera* seeds with an increase in germination from 7.7% to 67.8% under the experimental conditions of 60°C and 5.9 minutes. This result indicates the use of ultrasound as a pregerminative treatment to increase the germination of *V. baccifera,* which provides new incursion alternatives for forest species with low germination potential.

REFERENCES

ABD-ELRAHMAN, H., EL-BIALEE, N., MAHMOUD, M., MORSY, N. y ELSSAWY, W., 2023. Responding Oil Seeds Germination Indices to Ultrasonic Waves. *Journal of Soil Sciences and Agricultural Engineering* [en línea], vol. 14, no. 11, [consulta: 7 agosto 2024]. ISSN 2090-3685. DOI 10.21608/jssae.2023.235550.1187. Disponible en: https://jssae.journals.ekb.eg/article_324485.html.

AMPOFO, J.O. y NGADI, M., 2020. Ultrasonic assisted phenolic elicitation and antioxidant potential of common bean (*Phaseolus vulgaris*) sprouts. *Ultrasonics Sonochemistry* [en línea], vol. 64, [consulta: 7 agosto 2024]. ISSN 1350-4177. DOI 10.1016/j.ultsonch.2020.104974. Disponible en: https://www.sciencedirect.com/science/article/pii/S1350417719315949.

ANDRIAMPARANY, J.N. y BUERKERT, A., 2019. Effect of ultrasonic dormancy breaking on seed germination and seedling growth of three wild yam species (Dioscorea spp.) from SW-Madagascar. *Genetic Resources and Crop Evolution* [en línea], vol. 66, no. 6, [consulta: 7 agosto 2024]. ISSN 1573-5109. DOI 10.1007/s10722-019-00779-5. Disponible en: https://doi.org/10.1007/s10722-019-00779-5.

ATHOI, W., MARBOH, V. y MAHANTA, C.L., 2024. Effect of ultrasonication, germination, and steaming on the properties of a black rice variety. *Food Chemistry Advances* [en línea], vol. 4, [consulta: 7 agosto 2024]. ISSN 2772-753X. Disponible en: http://www.sciencedirect.com/science/article/pii/S2772753X24000777.

AWAD, T.S., MOHARRAM, H.A., SHALTOUT, O.E., ASKER, D. y YOUSSEF, M.M., 2012. Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International* [en línea], vol. 48, no. 2, [consulta: 7 agosto 2024]. ISSN 0963-9969. DOI 10.1016/j.foodres.2012.05.004. Disponible en: https://www.sciencedirect.com/science/article/pii/S096399691200141X.

BABAEI, M., PIRDASHTI, H. y BAKHSHANDEH, E., 2023. Ultrasonic waves improve aged seed germination of castor bean (Ricinus communis L.) under drought and salt stresses. *Acta Physiologiae Plantarum* [en línea], vol. 45, no. 7, [consulta: 7 agosto 2024]. ISSN 1861-1664. DOI 10.1007/s11738-023-03563-2. Disponible en: https://doi.org/10.1007/s11738-023-03563-2.

BABAEI-GHAGHELESTANY, A., ALEBRAHIM, M.T., MACGREGOR, D.R., KHATAMI, S.A. y HASANI NASAB FARZANEH, R., 2020. Evaluation of ultrasound technology to break seed dormancy of common lambsquarters (Chenopodium album). *Food Science & Nutrition* [en línea], vol. 8, no. 6, ISSN 2048-7177. DOI 10.1002/fsn3.1547. Disponible en: https://pubmed.ncbi.nlm.nih.gov/32566183/.

CHEN, Y., LIU, Q., YUE, X., MENG, Z. y LIANG, J., 2013. Ultrasonic vibration seeds showed improved resistance to cadmium and lead in wheat seedling. *Environmental Science and Pollution Research International* [en línea], vol. 20, no. 7, ISSN 1614-7499. DOI 10.1007/s11356-012-1411-1. Disponible en: https://pubmed.ncbi.nlm.nih.gov/23296973/.

CHIU, K. y SUNG, J., 2013. Use of ultrasonication to enhance pea seed germination and microbial quality of pea sprouts. *International Journal of Food Science & Technology* [en línea], vol. 49, no. 7, DOI 10.1111/ijfs.12476. Disponible en: https://www.researchgate.net/publication/259549807_Use_of_ultrasonication_to_enhance_pea_seed_germination_and_microbial_quality_of_pea_sprouts.

CHIU, K.Y., 2015. Ultrasonication-enhanced seed germination and microbial safety of sprouts produced from selected crop species. *Journal of Applied Botany and Food Quality* [en línea], vol. 88, [consulta: 7 agosto 2024]. ISSN 1439-040X. DOI 10.5073/JABFQ.2015.088.017. Disponible en: https://ojs.openagrar.de/index.php/JABFQ/article/view/3177.

CHIU, K.-Y., 2021. Changes in Microstructure, Germination, Sprout Growth, Phytochemical and Microbial Quality of Ultrasonication Treated Adzuki Bean Seeds. *Agronomy* [en línea], vol. 11, no. 6, DOI 10.3390/agronomy11061093. Disponible en: https://www.researchgate.net/publication/351963088_Changes_in_Microstructure _Germination_Sprout_Growth_Phytochemical_and_Microbial_Quality_of_Ultrasoni cation_Treated_Adzuki_Bean_Seeds.

DEWI, A.H. y ANA, I.D., 2018. The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation, sinus augmentation, and periodontal bone defect: A systematic review. *Heliyon* [en línea], vol. 4, no. 10, ISSN 2405-8440. DOI 10.1016/j.heliyon.2018.e00884. Disponible en: https://pubmed.ncbi.nlm.nih.gov/30417149/.

DING, J., HOU, G.G., DONG, M., XIONG, S., ZHAO, S. y FENG, H., 2018. Physicochemical properties of germinated dehulled rice flour and energy requirement in germination as affected by ultrasound treatment. *Ultrasonics Sonochemistry* [en línea], vol. 41, [consulta: 7 agosto 2024]. ISSN 1350-4177. DOI 10.1016/j.ultsonch.2017.10.010. Disponible en: https://www.sciencedirect.com/science/article/pii/S1350417717304728.

DZIWULSKA-HUNEK, A., SZYMANEK, M. y STADNIK, J., 2020. Impact of Pre-Sowing Red Light Treatment of Sweet Corn Seeds on the Quality and Quantity of Yield. *Agriculture* [en línea], vol. 10, no. 5, [consulta: 7 agosto 2024]. ISSN 2077-0472. DOI 10.3390/agriculture10050165. Disponible en: https://www.mdpi.com/2077-0472/10/5/165.

GOUSSOUS, S.J., SAMARAH, N.H., ALQUDAH, A.M. y OTHMAN, M.O., 2010. ENHANCING SEED GERMINATION OF FOUR CROP SPECIES USING AN ULTRASONIC TECHNIQUE. *Experimental Agriculture* [en línea], vol. 46, no. 2, [consulta: 7 agosto 2024]. ISSN 1469-4441, 0014-4797. DOI 10.1017/S0014479709991062. Disponible en: https://www.cambridge.org/core/journals/experimental - agriculture/article/abs/enhancing-seed-germination-of-four-crop-species-using -an-ultrasonic-technique/CD6DC32BA53B0B0128455E17A0EB989B#.

GUIMARÃES, B., POLACHINI, T.C., AUGUSTO, P.E.D. y TELIS-ROMERO, J., 2020. Ultrasound-assisted hydration of wheat grains at different temperatures and power applied: Effect on acoustic field, water absorption and germination. *Chemical Engineering and Processing - Process Intensification* [en línea], vol. 155, [consulta: 7 agosto 2024]. ISSN 0255-2701. DOI 10.1016/j.cep.2020.108045. Disponible en: https://www.sciencedirect.com/science/article/pii/S0255270120305067.

GUZMÁN, M.N.N., BELTRÁN, L.C., RODRIGUEZ, C.H. y ROA-FUENTES, L.L., 2023. Functional seed traits as predictors of germination and seedling growth for species with potential for restoration in Caquetá, Colombia. *Trees* [en línea], vol. 37, no. 3, [consulta: 7 agosto 2024]. ISSN 1432-2285. DOI 10.1007/s00468-023-02396-3. Disponible en: https://doi.org/10.1007/s00468-023-02396-3.

HUANG, S., ASHRAF, U., DUAN, M., REN, Y., XING, P., YAN, Z. y TANG, X., 2024. Ultrasonic seed treatment improved seed germination, growth, and yield of rice by modulating associated physio-biochemical mechanisms. *Ultrasonics Sonochemistry* [en línea], vol. 104, [consulta: 7 agosto 2024]. ISSN 1350-4177. DOI 10.1016/j.ultsonch.2024.106821. Disponible en: https://www.sciencedirect.com/science/article/pii/S1350417724000695.

JIMÉNEZ ROMERO, E., GARCÍAS FRANCO, L., CARRANZA PATIÑO, M., CARRANZA PATIÑO, H.M., MORANTE CARRIEL, J., MARTÍNEZ CHÉVEZ, M. y CUÁSQUER FUEL, J., 2017. Germinación y crecimiento de Ochroma pyramidale (Cav. ex Lam.)

Urb. en Ecuador. *Scientia Agropecuaria* [en línea], vol. 8, no. 3, [consulta: 7 agosto 2024]. ISSN 2077-9917. DOI 10.17268/sci.agropecu.2017.03.07. Disponible en: http://www.scielo.org.pe/scielo.php?script=sci_abstract&pid=S2077 - 99172017000300007&lng=es&nrm=iso&tlng=es.

LEE, Y.-I., LU, C.-F., CHUNG, M.-C., YEUNG, E.C. y LEE, N., 2007. Developmental Changes in Endogenous Abscisic Acid Concentrations and Asymbiotic Seed Germination of a Terrestrial Orchid, Calanthe tricarinata Lindl. *Journal of the American Society for Horticultural Science* [en línea], vol. 132, no. 2, [consulta: 7 agosto 2024]. ISSN 2327-9788, 0003-1062. DOI 10.21273/JASHS.132.2.246. Disponible en: https://journals.ashs.org/jashs/view/journals/jashs/132/2/article-p246.xml.

LIU, H., LI, Z., ZHANG, X., LIU, Y., HU, J., YANG, C. y ZHAO, X., 2021. The effects of ultrasound on the growth, nutritional quality and microbiological quality of sprouts. *Trends in Food Science & Technology* [en línea], vol. 111, [consulta: 7 agosto 2024]. ISSN 0924-2244. DOI 10.1016/j.tifs.2021.02.065. Disponible en: https://www.sciencedirect.com/science/article/pii/S0924224421001722.

LIU, J., WANG, Q., KARAGIÆ, Đ., LIU, X., CUI, J., GUI, J., GU, M. y GAO, W., 2016. Effects of ultrasonication on increased germination and improved seedling growth of aged grass seeds of tall fescue and Russian wildrye. *Scientific Reports* [en línea], vol. 6, no. 1, [consulta: 7 agosto 2024]. ISSN 2045-2322. DOI 10.1038/srep22403. Disponible en: https://www.nature.com/articles/srep22403.

LÓPEZ-RIBERA, I. y VICIENT, C.M., 2017. Use of ultrasonication to increase germination rates of Arabidopsis seeds. *Plant Methods*, vol. 13, no. 1, ISSN 1746-4811. DOI 10.1186/s13007-017-0182-6.

MIANO, A.C. y AUGUSTO, P.E.D., 2018. The ultrasound assisted hydration as an opportunity to incorporate nutrients into grains. *Food Research International* [en línea], vol. 106, [consulta: 7 agosto 2024]. ISSN 0963-9969. DOI 10.1016/j.foodres.2018.02.006. Disponible en: https://www.sciencedirect.com/science/article/pii/S096399691830098X.

MIANO, A.C., SABADOTI, V.D. y AUGUSTO, P.E.D., 2019. Combining Ionizing Irradiation and Ultrasound Technologies: Effect on Beans Hydration and Germination. *Journal of Food Science* [en línea], vol. 84, no. 11, [consulta: 7 agosto 2024]. ISSN 1750-3841. DOI 10.1111/1750-3841.14819. Disponible en: https://onlinelibrary.wiley.com/doi/abs/10.1111/1750-3841.14819.

MIANO PASTOR, A., FORTI, V., ABUD, H., GOMES-JUNIOR, F., CICERO, S. y AUGUSTO, P., 2015. Effect of ultrasound technology on barley seed germination and vigour. *Seed Science and Technology* [en línea], vol. 43, no. 2, DOI 10.15258/sst.2015.43.2.10. Disponible en: https://www.researchgate.net/publication/279223611_Effect_of_ultrasound_techn ology_on_barley_seed_germination_and_vigour.

MIHAYLOVA, E. y PERUHOV, M.M. and N., 2021. Ultrasound seed treatment for organic farming. *Bulgarian Journal of Agricultural Science* [en línea], [consulta: 7 agosto 2024]. ISSN 2534-983X. Disponible en: https://journal.agrojournal.org/page/en/details.php?article_id=3521.

NAZARI, M., SHARIFIFAR, A. y ASGHARI, H., 2014. Medicago Scutellata Seed Dormancy Breaking by Ultrasonic Waves. *Plant Breeding and Seed Science* [en línea], vol. 69, no. 1, DOI 10.1515/plass-2015-0002. Disponible en: https://www.researchgate.net/publication/273064533_Medicago_Scutellata_Seed_ Dormancy_Breaking_by_Ultrasonic_Waves.

NOGUEIRA, A., PUGA, H., GERÓS, H. y TEIXEIRA, A., 2024. Seed germination and seedling development assisted by ultrasound: gaps and future research directions. *Journal of the Science of Food and Agriculture* [en línea], vol. 104, no. 2, [consulta: 7 agosto 2024]. ISSN 1097-0010. DOI 10.1002/jsfa.12994. Disponible en: https://onlinelibrary.wiley.com/doi/abs/10.1002/jsfa.12994.

PANDISELVAM, R., MAYOOKHA, V.P., KOTHAKOTA, A., SHARMILA, L., RAMESH, S.V., BHARATHI, C.P., GOMATHY, K. y SRIKANTH, V., 2020. Impact of Ozone Treatment on Seed Germination A Systematic Review. *Ozone: Science & Engineering* [en línea], vol. 42, no. 4, [consulta: 7 agosto 2024]. ISSN 0191-9512. DOI 10.1080/01919512.2019.1673697. Disponible en: https://doi.org/10.1080/01919512.2019.1673697.

PASCUAL, B., BAUTISTA, A., IMBERNÓN, A., LÓPEZ-GALARZA, S., ALAGARDA, J. y MAROTO, J.V., 2004. Seed treatments for improved germination of caper (Capparis spinosa). *Seed Science and Technology* [en línea], vol. 32, no. 2, DOI 10.15258/sst.2004.32.2.33. Disponible en: https://riunet.upv.es/handle/10251/99486.

POUR, M., HOBBI, M., GHASEMI, H. y NAZARI, M., 2016. Plausible Mechanisms by Which Ultrasonic Waves Affect Seeds. *Plant Breeding and Seed Science* [en línea], vol. 74, no. 1, DOI 10.1515/plass-2016-0017. Disponible en: http://ojs.ihar.edu.pl/index.php/pbss/article/view/225.

REHMANI, M.S., XIAN, B., WEI, S., HE, J., FENG, Z., HUANG, H. y SHU, K., 2023. Seedling establishment: The neglected trait in the seed longevity field. *Plant Physiology and Biochemistry* [en línea], vol. 200, [consulta: 7 agosto 2024]. ISSN 0981-9428. DOI 10.1016/j.plaphy.2023.107765. Disponible en: https://www.sciencedirect.com/science/article/pii/S0981942823002760.

RIFNA, E.J., RATISH RAMANAN, K. y MAHENDRAN, R., 2019. Emerging technology applications for improving seed germination. *Trends in Food Science & Technology* [en línea], vol. 86, [consulta: 7 agosto 2024]. ISSN 0924-2244. DOI 10.1016/j.tifs.2019.02.029. Disponible en: https://www.sciencedirect.com/science/article/pii/S0924224417307975.

RINALDELLI, E., 2000. Effect of ultrasonic waves on seed germination of Capparis spinosa L. as related to exposure time, temperature, and gibberellic acid. *Advances in Horticultural Science* [en línea], vol. 14, no. 4, [consulta: 7 agosto 2024]. ISSN 0394-6169. Disponible en: https://www.jstor.org/stable/42883273.

SHARIFIFAR, A., NAZARI, M. y ASGHARI, H.R., 2015. Effect of ultrasonic waves on seed germination of *Atriplex lentiformis*, *Cuminum cyminum*, and *Zygophyllum eurypterum*. *Journal of Applied Research on Medicinal and Aromatic Plants* [en línea], vol. 2, no. 3, [consulta: 7 agosto 2024]. ISSN 2214-7861. DOI 10.1016/j.jarmap.2015.05.003. Disponible en: https://www.sciencedirect.com/science/article/pii/S2214786115300024.

SHEKARI, F., MUSTAFAVI, S.-H. y ABBASI, A., 2015. Sonication of seeds increase germination performance of sesame under low temperature stress. *Acta agriculturae Slovenica* [en línea], vol. 105, no. 2, [consulta: 7 agosto 2024]. ISSN 1854-1941. DOI 10.14720/aas.2015.105.2.03. Disponible en: http://ojs.aas.bf.uni-lj.si/index.php/AAS/article/view/40.

SINGUREANU, Valentin, UNGUR, R., ONAC, I., KOVACS, M.H., MOLDOVAN, G. y SINGUREANU, Victoria, 2015. Automatic Germination Evaluation and Qualitative Analysis of Essential Oil of Mentha × piperita L. under the Influence of High Frequency Pulsatile Electromagnetic and Ultrasound Pulsatile Fields. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* [en línea], vol. 43, no. 1, [consulta: 7 agosto 2024]. ISSN 1842-4309. DOI 10.15835/nbha4319973. Disponible en: https://www.notulaebotanicae.ro/index.php/nbha/article/view/9973.

TRAKSELYTE-RUPSIENE, K., JUODEIKIENE, G., CERNAUSKAS, D., BARTKIENE, E., KLUPSAITE, D., ZADEIKE, D., BENDORAITIENE, J., DAMASIUS, J., IGNATAVICIUS, J. y SIKORSKAITE-GUDZIUNIENE, S., 2021. Integration of Ultrasound into the Development of Plant-Based Protein Hydrolysate and Its Bio-Stimulatory Effect for Growth of Wheat Grain Seedlings In Vivo. *Plants (Basel, Switzerland)*, vol. 10, no. 7, ISSN 2223-7747. DOI 10.3390/plants10071319.

VALLADOLID ONTANEDA, J., LEÓN MEJÍA, Á., PAREDES TOMALÁ, D., VALLADOLID ONTANEDA, J., LEÓN MEJÍA, Á. y PAREDES TOMALÁ, D., 2017. Selección de Árboles Semilleros en Plantaciones Forestales de la Península de Santa Elena. Ecuador. *Revista Científica y Tecnológica UPSE (RCTU)* [en línea], vol. 4, no. 2, [consulta: 7 agosto 2024]. ISSN 1390-7697. DOI 10.26423/rctu.v4i2.261. Disponible en: http://scielo.senescyt.gob.ec/scielo.php?script=sci_abstract&pid=S1390 - 76972017000100105&lng=es&nrm=iso&tlng=es.

VENÂNCIO, R.S. da S. y MARTINS, A.C.G., 2019. Overcoming dormancy of *Senna multijuga* seeds with an ultrasonic probe the comparison with ultrasound and sulfuric acid baths. *Ciência Rural* [en línea], vol. 49, [consulta: 7 agosto 2024]. ISSN 0103-8478, 1678-4596. DOI 10.1590/0103-8478cr20180904. Disponible en: https://www.scielo.br/j/cr/a/W39pMsvmr8dgm3xvy8ZYjRg /.

WANG, J., MA, H. y WANG, S., 2019. Application of Ultrasound, Microwaves, and Magnetic Fields Techniques in the Germination of Cereals. *Food Science and Technology Research*, vol. 25, no. 4, DOI 10.3136/fstr.25.489.

XIA, Q., TAO, H., LI, Y., PAN, D., CAO, J., LIU, L., ZHOU, X. y BARBA, F.J., 2020. Characterizing physicochemical, nutritional and quality attributes of wholegrain *Oryza sativa* L. subjected to high intensity ultrasound-stimulated pre-germination. *Food Control* [en línea], vol. 108, [consulta: 7 agosto 2024]. ISSN 0956-7135. DOI 10.1016/j.foodcont.2019.106827. Disponible en: https://www.sciencedirect.com/science/article/pii/S0956713519304165.

YALDAGARD, M., MORTAZAVI, S.A. y TABATABAIE, F., 2008. Application of Ultrasonic Waves as a Priming Technique for Accelerating and Enhancing the Germination of Barley Seed: Optimization of Method by the Taguchi Approach. *Journal of the Institute of Brewing* [en línea], vol. 114, no. 1, [consulta: 7 agosto 2024]. ISSN 2050-0416. DOI 10.1002/j.2050-0416.2008.tb00300.x. Disponible en: https://onlinelibrary.wiley.com/doi/abs/10.1002/j.2050-0416.2008.tb00300.x.

YANG, H., GAO, J., YANG, A. y CHEN, H., 2015. The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food Research International* [en línea], vol. 77, [consulta: 7 agosto 2024]. ISSN 0963-9969. DOI 10.1016/j.foodres.2015.01.011. Disponible en: https://www.sciencedirect.com/science/article/pii/S0963996915000137.

ZHANG, H., MO, W., LIAO, S., JIA, Z., ZHANG, W., ZHANG, S. y LIU, Z., 2023. Ultrasound promotes germination of aging Pinus tabuliformis seeds is associated with altered lipid metabolism. *Ultrasonics Sonochemistry* [en línea], vol. 93, ISSN 1873-2828. DOI 10.1016/j.ultsonch.2023.106310. Disponible en: https://pubmed.ncbi.nlm.nih.gov/36708697/.

Conflicts of interest:

The authors declare not to have any interest conflicts.

Contribution of the authors:

The authors have participated in the writing of the work and analysis of the documents.