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Ultrasound promoting vigor and germination in seeds of *Amburana cearensis*

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Abstract - Populations of *Amburana cearensis* (AC) present great diminution in Caatinga Biome due to extractive activities. Commercial plantations are emergent, demanding technologies as ultrasound (US) to improve AC seeds vigor and germination. This work has aimed to test seeds of AC with the US, for improving the vigor and quality of seedlings. Stored AC seeds were submitted to 0, 1, 2, 3, and 4 min in the US and tested for vigor and quality. The results were analyzed by ANOVA and Tukey's test. Stored seeds of *A. cearensis* after 3 min in the US (42 kHz) can be interesting for silvicultural management of this species.

Ultrassom para promover o vigor e germinação de sementes de *Amburana cearensis*

Resumo - As populações de *Amburana cearensis* (AC) têm diminuído no bioma Caatinga por conta da atividade extrativista. A implantação de campos comerciais é importante, demandando tecnologias sustentáveis, como o ultrassom (US), para melhorar o vigor e a germinação de sementes. Este trabalho teve por objetivo testar o tratamento de sementes de AC com o uso de US, para melhorar o vigor e a qualidade das plântulas. Para isso, sementes de AC foram submetidas à exposição de 0, 1, 2, 3 e 4 min em US, sendo testadas quanto ao vigor e qualidade. Os resultados foram analisados por ANOVA e teste de Tukey. O tratamento de sementes de *A. cearensis* em armazenamento com 3 min de exposição em US (42 kHz) pode ser interessante para o manejo silvicultural dessa espécie.

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Introduction

Amburana cearensis (Allemão) A.C. Smith (Fabaceae) is a tree from Caatinga Biome (Guedes et al., 2010b). The main characteristics of the seeds are the woody coat, of grayish color and hardened (Cunha & Ferreira, 2003; Guedes et al., 2010b, 2010c, 2010d), presenting elliptical shape (Guedes et al., 2010a); their seedlings emerge from the seeds around the fifth day after sowing (Cunha & Ferreira, 2003; Guedes et al., 2010a, 2010b, 2013), with total imbibition curve for around four days (Loureiro et al., 2013); during the seedling season it can form tuberous roots (Cunha & Ferreira, 2003; Guedes et al., 2015); its leaves are composed (Cunha & Ferreira, 2003; Guedes et al., 2010a, 2010c, 2010d) and its structure as an adult can reach a height of 10 m (Cunha & Ferreira, 2003).

This species is used for ecological and economic purposes. The main uses are the wood due to the excellent quality (Guedes et al., 2010a, 2010b, Pimentel & Guerra, 2011, 2015; Santiago et al., 2014); the peels and seeds, that present pharmaceutical, food and medicinal properties (Guedes et al., 2010d; Pimentel & Guerra, 2011, 2015; Loureiro et al., 2013); urban afforestation (Pimentel & Guerra, 2011, 2015); forest restoration (Cunha & Ferreira, 2003; Guedes et al., 2010a; Pimentel & Guerra, 2011) and forage (Pimentel & Guerra, 2015).

The production of *A. cearensis* seedlings are alternatives for forest management and commercial cultivation (Santiago et al., 2014; Pimentel & Guerra, 2015; Venâncio & Martins, 2019). They present potential to be used for the recovery of degraded areas (Guedes et al., 2010a; Pimentel & Guerra, 2011, 2015), and to decrease predatory extraction (Loureiro et al., 2013; Santiago et al., 2014; Pimentel & Guerra, 2015). It allows cultural management in favor of its commercial cultivation (Santiago et al., 2014; Pimentel & Guerra, 2015; Venâncio & Martins, 2019).

Studies about stored seeds of *A. cearensis* are necessary to enable the commercial production of this plant species (Cunha & Ferreira, 2003, Guedes et al., 2010b, 2010c, Loureiro et al., 2013; Santiago et al., 2014; Pimentel & Guerra, 2015; Lucio et al., 2016). One of the factors that may influence seedlings production is the uniformity of germination (Loureiro et al., 2013; Santiago et al., 2014). Therefore, technologies to improve plant growth of *A. cearensis* are very important to the conservation of this species (Cunha & Ferreira, 2003; Guedes et al., 2010a; Loureiro et al., 2013; Santiago et al., 2014; Pimentel & Guerra, 2015).

The seed coat and biochemical components from this species can compromise the seeds germination in a short period, as such natural processes aim to protect the seeds against adverse environmental conditions (Venâncio & Martins, 2019). The knowledge about the stored wood seeds from Caatinga is incipient (Guedes et al., 2010b; Loureiro et al., 2013; Santiago et al., 2014; Pimentel & Guerra, 2015). To overcome this problem, seed treatment processes using physical agents were recommended in the literature (Rifna et al., 2019; Venâncio & Martins, 2019; Xia et al., 2020). In addition, the use of sustainable technologies have been emphasized, aiming at promoting and improving the quality of wood seed germination (Rifna et al., 2019; Venâncio & Martins, 2019; Xia et al., 2020).

Among these clean technologies, the use of ultrasound frequencies (US) to break the tegument resistance and positive action regarding biochemical processes inherent in seeds culminating in the promotion of seed germination, have been reported in the current literature (Abramov et al., 2019; Venâncio & Martins, 2019). There are positive effects of using this technique for several plant species, including *Senna multijuga* (Venâncio & Martins, 2019), *Beta vulgaris* (Rifna et al., 2019), and *Oryza sativa* (Xia et al., 2020).

Thus, the aim of this work was to test the influence of ultrasound waves in improving the germination quality and vigor of stored *A. cearensis* seeds.

Material and methods

The assay was carried out at the Seed Laboratory of the National Institute of the Semiarid (Campina Grande, Paraiba State, Brazil).

Seeds of *Amburana cearensis* (AC) from the municipality of Teixeira, PB, were kindly provided by Dr. Maria do Carmo (UFCG). The seeds presented germination of 60% when received. They were stored in hermetically sealed containers in a refrigerated environment (10.0 ± 2.0 ºC) for 6 months. The tests were conducted in March of 2020.

For the ultrasound (US) test, the seeds were splited in lots of 200 and subjected to different incubation periods in the US at room temperature (25 ºC): 0, 1, 2, 3 and 4 min. We used an ultrasonic bath (STD model; frequency of 42 kHz; biowash), as suggested by Venâncio & Martins (2019).

The seeds were tested for pH, electrical conductivity, and leaching of K and Na, according to the methodology proposed by Guedes et al. (2010a, 2010b, 2010c, 2010d, 2013, 2015). Seeds were placed for germination in rolls of germitest paper previously soaked with 2.5 times the weight of the paper (Loureiro et al., 2013), being incubated at 25 ºC, for 14 days and12 h of photoperiod.

The following parameters were evaluated: pH; electrical conductivity (EC); leaching of K and Na; the percentage of germinated (G) and non-germinated (NG), and germinated/non-germinated ratio (G/NG). The results obtained were submitted to ANOVA analysis and Tukey's test (means) using the program R.

Results

The variables: K (*p-value* = 0.0167), Na (*p-value* = 0.0189), germinated (G) and non-germinated seeds (NG) (*p-value* = 0.0007), and the relation G/NG (*p-value* = 0.008) showed differences among treatments (Figure 1).

The pH and the electrical conductivity (EC) increased when increasing the time of exposure of seeds to the ultrasound (US) treatment (Figures 1A and 1B). However, there was no significant difference between treatments. It can be observed that K content (Figure 1D) in the treatment of 4 min of exposure was statistically different from 0, 1 and 2 min of exposure and the variable Na showed differences only between 1 min and 2 min of exposure (Figure 1C).

The best result of G was observed with 3 min of US exposure and the worst with 4 min and NG was exactly the opposite (Figure 1E). The G/NG ratio presented the best and statistically different for 3 min treatment of *Amburana cearensis* seeds exposure to US, when compared to the others (Figure 1F).

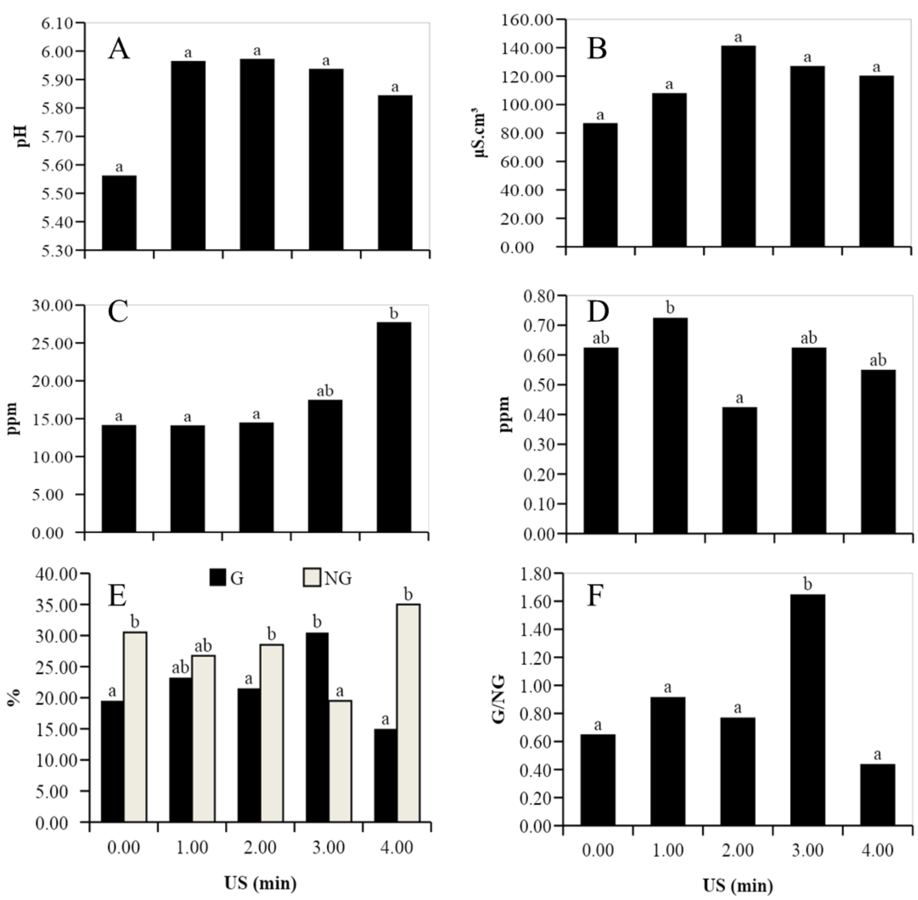

Figure 1. Averages of pH (A); electrical conductivity (B); Na content (C); K content (D), percentage of germination and non-germinated Seeds (E) and G/NG index analyses (F), for ultrasound exposition periods from 0 to 4 min, of *Amburana cearensis* seeds. The different letters indicate statistic differences by Tukey test ($p < 0.05$).

Discusion

Stored seeds of *Amburana cearensis* may present a moderate inhibition of germination, a controversial factor according to the literature, possibly linked to genetic issues (Santiago et al., 2014, Pimentel & Guerra 2015) and aggravated when stored (Guedes et al., 2010a; Pimentel & Guerra, 2011). Likely, the seeds used in this work showed similar characteristics observed by differences of vigor between treatments (Figure 1). Guedes et al. (2010b) also describe that stored *A. cearensis* seed lots have differences in resistance to environmental factors, linked to their genetic structure.

It is also important to highlight that, as described by Loureiro et al. (2013), there is an action of ceride compounds not present in the seeds coat, but it is not known if they act at *A. cearensis* s in storage. Probably, this impermeability was reduced while the periods of exposure to ultrasound (US) treatment were increased. We observed in this research that there were numerical differences in relation to the control (0 min) to the other treatments (1-4 min) for all variables (Figure 1), concerning the leachate and vigor of the worked seeds. These factors are directly related to the US treatment (Rifna et al., 2019).

Regarding the variable's pH and electrical conductivity (EC), represented in Figure 1, the changes in the external and internal constitution inseeds of *A. cearensis* were not sufficient to promote significant differences between treatments. Rifna et al. (2019) and Xia et al. (2020) observed that the levels of loss of solids in seeds are different due to the phenotypic action of each batch. If stored *A. cearensis* seeds had lost a greater number of solids, they could lose their vigor, already worn out by the referred storage (Pimentel & Guerra, 2011).

About specific leaching of compounds (K and Na), Pereira et al. (2014) mentioned that these elements are constituents of organic substances important for seeds in general. For stored *A. cearensis* seeds, it would be not different, as described by Guedes et al. (2010a, 2010b, 2010c, 2010d). It is clear, therefore, that there was an increase in the leaching of these elements when increasing the exposure to US waves (Figures 1C and 1D). The exception was the exposure for 4 min of US. Probably, the incidence of these waves would compromise the quality of the seeds, due to the increase in the number of solids released from the seeds.

It is possible to observe that the increase in germination (G) and decrease in the number of non-germinated (NG) seeds with exposure of 3 and 4 min of US shows the positive threshold for the use of this technology with the conditions described in this work. These are linked to the results showed in Figure 1, as previously described. It is worth noting that the germination obtained in this research was similar to that described by Guedes et al. (2010a) when testing similar conditions.

According to Rifna et al. (2014), the use of US treatment for seeds may allow the transmission of substances harmful to germination, a fact that may have occurred with the stored seeds of *A. cearensis* used in this research, promoting greater G (Figure 1E). Regarding the G/NG ratio, we observed positive results of the US exposure for 3 min (Figure 1F). Probably, the seeds that did not germinate were in physiological pause, a factor easily found and described in seeds of that species (Cunha & Ferreira, 2003; Guedes et al.,2010a, 2010b, 2013). Loureiro et al. (2013) mention also that improving the condition of water absorption in seeds would optimize the production of *A. cearensis*.

Oliveira et al. (2011) tested the thermotherapy treatment (60 ºC for 20 min) and the fungicide Captan, obtaining similar results as showed by our method. However, we consider our protocol more environmentally friendly since only one technology promoted results similar to the use of two technologies with a higher and less sustainable energy expenditure than using US treatment.

The results obtained in this research (Figure 1) are similar to other studies. Abramov et al. (2019) studied the exposure of barley seeds up to 3 min of US, and they considered 2 min the positive limit. Song et al. (2019) noted that US (2 MHz) in barley seeds for 2 min was good to improve germination, Venâncio & Martins (2019) stated that 2 to 4 min (2 MHz) was sufficient to improve the germination of S*enna multijuga* and Xia et al. (2020) noted that the use of US (28 kHz, for 5 min) in rice seeds stimulated germination and vigor, plant growth and the production of the species.

In addition to all the factors previously discussed, on the effectiveness of the US exposure for 3 min of stored *A. cearensis* seeds, the following points can be added. The production of technologies to standardize the germination of *A. cearensis* could promote the reduction of extractives by facilitating the production of seedlings for commercial plantations (Guedes et al., 2013, 2015) and could also improve seed health (Paredes-Villanueva et al., 2015; Rifna et al., 2019).

Conclusions

The treatment of stored *Amburana cearensis* seeds with 3 min of ultrasound exposure (42 kHz) can be interesting for the silvicultural management of this species.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

Thiago Costa Ferreira:Conceptualization, formal analysis, investigation, methodology, writing – original draft and writing – review & editing.
Fábia Shirley R. Silva: Formal analysis and methodology.
Mayara Ferreira Barbosa: Methodology.
Manoel Rivelino Gomes de Oliveira: Methodology.
Aldrin Martin Pérez-Marin: Supervision.

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