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Do scarification and seed soaking periods promote maximum vigor in seedlings of *Hymenaea courbaril*?

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ABSTRACT: The aim of this study was to establish a methodology to determine physiological vigor in small and large seeds of *Hymenaea courbaril* L. non-scarified seeds and scarified by the electrical conductivity test and period of seed soaking in water aiming at maximum capacity of emergence and seedling vigor. The design was completely randomized, in a 2 x 2 x 5 factorial scheme (two classes of seed mass, non-scarified seeds and scarified and five periods of soaking in water), with 10 replicates of 10 seeds each. The variables evaluated were electrical conductivity (µS $cm^{-1}.g^{-1}$), emergence speed (index), seedling emergence (%) and mean time of emergence (days), obtained by means of daily counts until stabilization in the values. Maximum vigor of *Hymenaea courbaril* seedlings is obtained with large seeds subjected to scarification and soaked in water for six hours. Water content of 18.7% in large seeds of *Hymenaea courbaril* scarified promotes the production of 100% of vigorous seedlings. Small and large seeds of *Hymenaea courbaril* non-scarified seeds and scarified, soaked for six hours, had shorter mean time for seedling emergence. Water content of 20.6% in small seeds of *Hymenaea courbaril* scarified promotes 100% seedling emergence. The electrical conductivity test applied to *Hymenaea courbaril* seeds can be adopted to determine seed vigor, showing speed in the obtaining of results and ease of execution.

Index terms: electrical conductivity, imbibition, jatobá, seedling emergence.

RESUMO: Objetivou-se estabelecer metodologia para determinar vigor fisiológico em sementes pequenas e grandes de *Hymenaea courbaril* L. não escarificadas e escarificadas pelo teste de condutividade elétrica e período de imersão em água das sementes visando máxima capacidade de emergência e vigor de plântulas. O delineamento foi inteiramente ao acaso, em esquema fatorial 2 x 2 x 5 (duas classes de massa de sementes, não escarificadas e escarificadas e cinco tempos de imersão em água), com 10 repetições de 10 sementes cada. As variáveis avaliadas foram: condutividade elétrica (µS $cm^{-1}.g^{-1}$), velocidade de emergência (índice), emergência de plântulas (%) e o tempo médio de emergência (dias), obtidas por meio de contagens diárias até a estabilização nos valores. O máximo vigor de plântulas de *Hymenaea courbaril* é obtido com sementes grandes escarificadas por seis horas de imersão em água. O teor de água de 18,7% nas sementes grandes de *Hymenaea courbaril* escarificadas promove a produção de 100% de plântulas vigorosas. Sementes pequenas e grandes de *Hymenaea courbaril* não escarificadas e escarificadas, imersas por seis horas exibem menor tempo médio para emergência de plântulas. O teor de água de 20,6% nas sementes pequenas de *Hymenaea courbaril* escarificadas promove 100% de emergência de plântulas. O teste de condutividade elétrica aplicado às sementes de *Hymenaea courbaril* pode ser adotado para determinação do vigor das sementes, apresentando rapidez na obtenção dos resultados e facilidade de execução.

Termos para indexação: condutividade elétrica, embebição, emergência de plântulas, jatobá.

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INTRODUCTION

Increased demand for native seeds for ecological restoration and rehabilitation has stood out in the large global industry of seed supply and sale (Smiderle et al., 2021). Therefore, research with native forest seeds is essential for obtaining seedlings of high quality and in quantities that meet the demands of the forestry sector segments (Leão et al., 2016), as seed propagation is the main form of dissemination of species and the study of seed technology and production becomes of great relevance in the process of management, conservation and genetic improvement (Menegatti et al., 2019).

Hymenaea courbaril L. seeds, when mature, have dormancy due to the impermeability of their coat to water, so it is necessary to apply a treatment to overcome dormancy, and mechanical and physiological scarifications are forms that enable the emergence of seedlings (Paixão et al., 2019).

Although the species *Hymenaea courbaril* L. is considered endangered due to overexploitation and a rare tree, with only one tree per hectare according to the Institute for Forestry Research and Studies (*Institute de Pesquisa e Estudos Florestais* - IPEF), was classified as of little concern in the Red List of the International Union for the Conservation of Nature and Natural Resources, as it has a wide geographical distribution, for being considered common, for occurring in protected areas, and because its population is not threatened or declining.

Hymenaea courbaril L. is present in the Cerrado (lato sensu), Riparian Forest or Gallery forest, Dryland forest, Ombrophilous forest (Rain Forest) and Restinga (Flora Brasil, 2021), with natural preference for dry soils, of low fertility, with terrains of good drainage. It is tolerant to water deficit and, depending on the region, with an average annual temperature from 18 °C to 27 °C (Costa et al., 2018), it can reach 20 m in height and 50 cm in diameter in adulthood. Its flowering occurs from the end of the dry season to the beginning of the rainy season, with fruiting for a period of 3 to 4 months and alternated ripening (Oliveira et al., 2011). Thus, the determination of the physiological potential of seeds is performed by emergence and vigor tests. The emergence test is used to evaluate the physiological quality of seeds, thus making it possible to know the emergence potential of seedlings under favorable conditions (Nascimento et al., 2019). Moreover, it is important to evaluate the vigor of the seeds to complement and elucidate the information provided by the physiological tests based on vigor.

Among the physiological tests based on vigor, it is possible to mention the electrical conductivity test, which provides an evaluation of the vigor of seeds of forest species with satisfactory efficiency for being fast, easy to conduct and for offering the possibility of standardization (Oliveira et al., 2016).

It is known that vigor is responsible for promoting high performance of plants throughout their development cycle (vegetative and reproductive), such as in the formation of a well-developed root system, which will reach greater depths in the soil and thus provide conditions for the plant to have higher production (França-Neto et al., 2016).

In addition, physiological tests based on seedling development, which seek to determine the physiological activity of seeds, comprise: first germination count, germination speed, seedling growth, seedling dry mass, earliness in primary root growth and seedling emergence (Nakagawa, 1999).

In view of the above, the objective was to establish a methodology to determine the physiological vigor of small and large seeds of *H. courbaril* non-scarified seeds and scarified through the electrical conductivity test and period of seed soaking in water, aiming at maximum capacity of emergence and vigor of seedlings.

MATERIAL AND METHODS

The study was conducted at the Seed Analysis Laboratory (LAS) and in the seedling nursery of Embrapa Roraima's forestry sector from August to November 2020. The species used in the present study was *Hymenaea courbaril* L. Fruits were collected manually from eight parent trees, with distance of 100 m between the selected trees, located in an area of Dense Ombrophilous Submontane Forest with an emerging canopy, located at the geographic coordinates 1°38'29"

North latitude and 60°58’11” West longitude, in the municipality of Boa Vista (RR), in April 2020.

The climate of the municipality of Boa Vista - RR is Am type (tropical monsoon climate), with an average temperature of 27.2 °C in the hottest month and 23.3 °C in the coldest month, with an annual average of 25.4 °C. The average annual rainfall is 1808 mm with mean values of 365 mm and 26 mm in the months of highest (June) and lowest (February) precipitation, respectively (Smiderle et al., 2021).

The collected fruits of *H. courbaril* were selected by removing from the lot those with mechanical damage or deteriorated, which made it possible to obtain a uniform lot. Subsequently, they were thoroughly cleaned, de-pulped and the seeds were separated and washed under running water. For the morphological characters of *H. courbaril* seeds, measurements of length (mm), width (mm) and thickness (mm) were taken, using a digital caliper with accuracy of 0.01 mm. In addition, fresh mass (g) of the seeds was determined on a precision balance (0.001 g). *H. courbaril* seeds were classified into two classes of seeds referred to as small and large.

This classification by class of seeds was performed because *H. courbaril* shows variation in the size of its seeds, which is an adaptive strategy against unpredictable factors after their dispersion. It is worth pointing out that the main form of propagation of native forest species is through the use of seeds; for this reason, it is pertinent to classify their size, seeking to investigate the factors of influence during seedling emergence as well as seedling production.

Small seeds were those with mass between 2.9 and 4.7 g and large seeds were those with mass between 4.9 and 6.4 g (Table 1).

Soon after, the seeds of both classes were subjected to pre-germination treatment, on the opposite side of the hilum, with iron sandpaper N°. 100. Their water content was determined for each mass class, in an oven (105 ± 3 °C), where they were kept for 24 hours, according to the procedure described in Brasil (2009), with five replicates of 10 seeds.

Electrical conductivity test

The electrical conductivity test was performed in a completely randomized design, in a 2 x 2 x 5 factorial scheme (two classes of seed mass, with and without scarification and five soaking periods - EC readings), with 10 replicates of 10 seeds each.

For this test, the seeds were placed in 200 mL plastic cups containing 75 mL of distilled water according to Oliveira et al. (2016). Then, the material was taken to the Biochemical Oxygen Demand (BOD) chamber, regulated at 25 °C. Readings were performed at 0, 4, 6, 8 and 12 hours of soaking. The imbibition process was monitored by periodic weighing (0, 4, 6, 8 and 12 hours). After the last weighing, the water content of the seeds was determined (Brasil, 2009). For each weighing, the percentage of seed imbibition (%) was determined using the formula:

$$\%E = \frac{(Mf - Mi)}{Mi} x100$$

Where: M*f* = seed mass after imbibition; and M*i* = initial seed mass

At each period, the soaking solution was slightly stirred and the electrical conductivity of the seed soaking solution was determined with a conductivity meter (MCA 150 model), and the results were expressed in μS $cm^{-1}.g^{-1}$ seed.

Table 1. Mean values of length (mm), width (mm), thickness (mm), fresh mass (g) and average range of *Hymenaea courbaril* seeds classified as large and small.

Size	Length (mm)	Width (mm)	Thickness (mm)	Mass (g)	Range
Small	22.44	18.26	13.69	4.07	2.9 - 4.7
Large	26.38	20.57	12.37	5.17	4.8 - 6.4

Vigor test

In order to complement and elucidate the results obtained in the electrical conductivity test, the seeds of each soaking period were sown in sand with at 1.0 cm depth, in plastic trays with dimensions of 30 cm x 40 cm x 10 cm kept in a greenhouse with average temperature of 25 ± 5 °C and relative humidity of 60% to 70%.

The emergence test was conducted in a completely randomized design, in a 2 x 2 x 5 factorial scheme (two classes of seed mass, non-scarified seeds and scarified and five periods of seed soaking), with 10 replicates of 10 seeds each. The variables evaluated were: seedling emergence speed (ES, index) (Maguire, 1962), seedling emergence (%) and the mean time of emergence - MTE (Edmond and Drapala, 1958), obtained by means of daily counts until the stabilization of the counts of all treatments. The moisture of the sand substrate was maintained under manual irrigation, twice a day.

Seedling vigor were expressed as percentage of normal seedlings, obtained during the evaluations. Normal emerged seedling was the one that, after breaking the substrate surface, showed well-differentiated and well-developed plumula, cotyledons and hypocotyl.

To check the assumptions of the analysis of variance (ANOVA), the data were first analyzed for: a) normality by the Shapiro-Wilk test ($p > 0.05$), and b) homoscedasticity by the Bartlett test ($p > 0.05$). When there was normality of the residuals and homogeneity of the variances, the data were subjected to ANOVA, followed by Tukey test ($p < 0.05$) for means comparison. Quantitative variables were subjected to regression analysis to verify the response of seed vigor as a function of time. Data analysis was performed in the Statistical Program Sisvar (Ferreira, 2014).

RESULTS AND DISCUSSION

The mass of mechanically scarified small and large seeds of *H. courbaril* increased after 12 hours compared to non-scarified seeds (Figure 1).

In addition, small seeds that were scarified showed a mass gain of 8.13% in comparison to the initial mass. In turn, in small seeds non-scarified, the gain of the final mass was equal to 2.5% compared to the initial one.

In large seeds of *H. courbaril* scarified, that the final mass, after 12 hours of soaking, showed a gain in the water content of 6.27% compared to the initial mass. It is worth mentioning that large seeds non-scarified, after 12 hours of soaking, exhibited 2.6% lower mass compared to large seeds subjected to scarification after 12 hours of soaking (Figure 1).

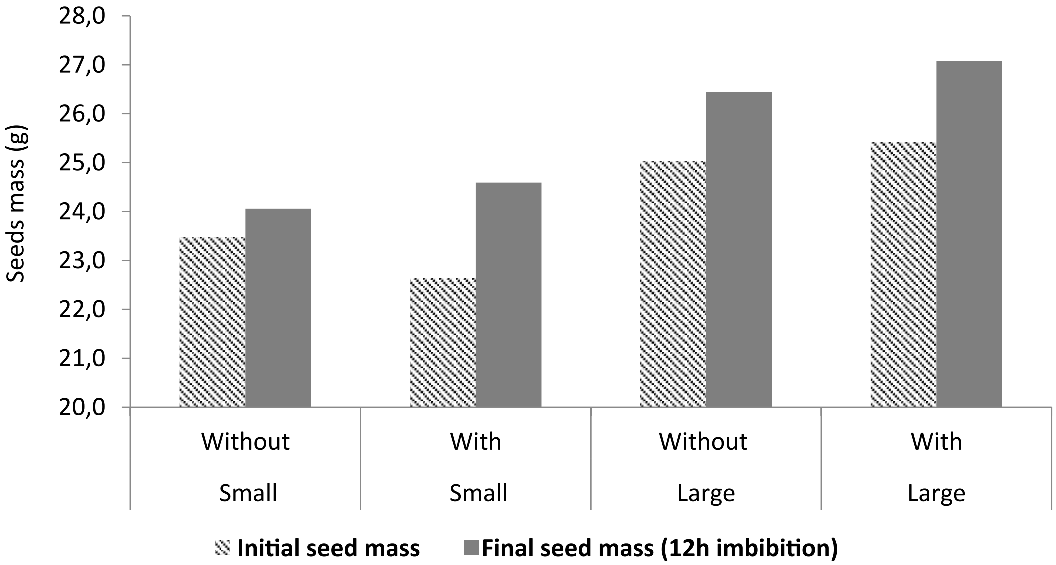

Figure 1. Initial mass and final mass after 12 hours of soaking in water of small and large seeds of *Hymenaea courbaril* scarified and non-scarified seeds.

In this context, the use of seeds with high physiological quality is important for the production of seedlings with higher quality to meet the forestry sector. Thus, the results of the present study showed that the six-hour period of soaking in water was sufficient to promote adequate changes in the physiological dormancy process, making seed metabolism more active and the embryo ready for resuming its development, consequently leading to greater reduction in the mean time of emergence (Table 2) of *H. courbaril* seedlings.

In turn, the average water content of *H. courbaril* seeds in both mass classes were between 9.0 and 10.5% in the period zero (Table 2). Similar result was obtained in a study conducted by Souza et al. (2016), where the average water content in the seeds of eight *Prunus persica* rootstock cultivars was 10.2%.

In addition, increments of 11.7% in the electrical conductivity were observed in large seeds scarified, between zero and six hours of soaking (Table 2), which was determinant in the result of 100% of vigorous seedlings (Table 3).

Oliveira et al. (2016), analyzing the release of exudates from *Acacia mangium* (Fabaceae) seeds, also observed the increase in the release of exudates between the initial water content of small seeds (9.0%) and large seeds (10.5%) and the water content obtained after eight hours of soaking, which reached between 16 and 20%, being determinant for obtaining more than 90% of vigorous seedlings of *A. mangium*. The similar result was observed in the present study (Table 2), since the seeds of *H. courbaril*, also of the Fabaceae family and recalcitrant, led to up to 100% of vigorous seedlings (Table 3).

The results described in the present study as well as in the literature show that the dormancy of jatobá seeds is present (Pierezan et al., 2012; Soares et al., 2017; Costa et al., 2019; Dourado et al., 2020) and needs to be overcome

Table 2. Mean values of water content (%) in seeds, electrical conductivity (μS $cm^{-1}.g^{-1}$) and mean time of emergence (days) of *H. courbaril* seedlings as a function of the soaking period of scarified and non-scarified seeds.

	Soaking Periods (hours)				
	0	4	6	8	12
	Mean time of emergence (days)				
Small non-scarified	12.0 abA[1]	10.8 bA	10.0 aA	11.0 bA	11.5 bA
Small scarified	11.8 bA	10.8 bA	10.0 aA	10.5 bA	10.5 bA
Large non-scarified	13.5 aBC	13.3 aBC	11.8 aC	15.5 aA	14.8 aAB
Large scarified	12.5 abA	12.3 ab	10.3 aB	11.0 bAB	11.5 bAB
CV (%)	8.32	8.98	7.13	10.4	8.90
	Electrical conductivity (μS $cm^{-1}.g^{-1}$)				
Small non-scarified	1.14 bB	1.25 bB	1.45 cAB	1.61 cA	1.71 bA
Small scarified	1.24 bC	1.46 bC	1.83 bB	2.10 bB	2.79 aA
Large non-scarified	1.22 bB	1.28 bAB	1.54 bcAB	1.56 cAB	1.62 bA
Large scarified	1.72 aB	1.81 aB	2.23 aA	2.46 aA	2.48 aA
CV (%)	4.71	4.21	4.98	5.12	5.23
	Water content (%)				
Small non-scarified	10.5 aC	14.7 aB	16.0 bB	16.2 bB	19.1 bA
Small scarified	9.0 aC	15.5 aB	20.6 aA	21.9 aAB	22.9 aA
Large non-scarified	10.1 aC	14.2 aB	15.2 bAB	16.4 bAB	17.2 bA
Large scarified	9.8 aD	14.0 aC	18.7 aB	20.8 aAB	22.5 aA
CV (%)	5.21	4.25	7.89	8.90	8.93

[1] Lowercase letters (a,b) compare the means for the variables between the sizes of seeds without (WOS) and with (WIS) scarification. Uppercase letters (A, B) compare the means for the variables between the soaking periods, by Tukey test at 5% probability level. CV: Coefficient of variation.

scarified and adequate soaking period, which controls the process of physical and physiological dormancy, making seed metabolism more active and the embryo ready for resuming its development.

It is worth pointing out that the presence of lignified coat in *H. courbaril* seeds may limit the vigor of seedlings, resulting in uneven stand, which was confirmed in the results shown in Table 3, mainly in the six-hour soaking period. In general, for small and large *H. courbaril* seeds scarified and non-scarified seeds, as the soaking period increased to six hours and eight hours, the emergence percentages showed average values above 90% (Table 3).

In addition, non-scarified seeds soaked for six hours showed an increase of 21.0% compared to non-scarified seeds and without soaking. In turn, small and large seeds scarified seeds after six and eight hours of soaking reached maximum (100%) emergence of seedlings (Table 3). On the other hand, large seeds of *H. courbaril* non-scarified seeds in the 12-hour soaking period showed a decrease of 12.5% compared to seeds scarified seeds for the 12-hour soaking period.

On the other hand, soaking of seeds for four hours was similar to the control (zero hours) for both small and large seeds non-scarified seeds, with an emergence percentage below 80% (Table 3).

When comparing the emergence speed in small seeds of *H. courbaril* non-scarified seeds for the soaking periods of four, six, eight and 12 hours, the results were better than those of the control, as shown in Table 3. In turn, the maximum value for emergence speed was obtained with large seeds of *H. courbaril* scarified seeds (Table 3) in the soaking periods of six and eight hours, not differing from the small seeds with mechanical scarification in both soaking periods. The results of the present study showed that small and large scarified and non-scarified seeds, subjected to

Table 3. Mean values of seedling emergence (%), emergence speed (index) and vigor of seedlings (%) of *H. courbaril* as a function of the soaking period of scarified and non-scarified seeds.

	Soaking period (hours)				
	0	4	6	8	12
	Seedling emergence (%)				
Small non-scarified	75 aB	80 bB	95 aA	93 aA	93 aA
Small scarified	80 aB	90 aAB	100 aA	100 aA	90 aA
Large non-scarified	74 aB	75 bB	93 aA	91 aA	80 bB
Large scarified	80 aB	95 aA	100 aA	100 aA	90 aAB
CV (%)	11.0	10.9	10.1	10.3	12.0
	Emergence speed (index)				
Small non-scarified	5.32 bB	6.33 bA	6.64 bA	7.01 aA	6.42 abA
Small scarified	6.21 aB	7.57 aA	7.73 abA	8.32 aA	6.52 abB
Large non-scarified	5.40 bB	5.72 bAB	6.27 bA	5.86 bAB	5.31 bB
Large scarified	6.81 aB	7.16 aB	8.40 aA	8.12 aA	7.14 aB
CV (%)	8.78	9.1	10.2	9.6	9.1
	Vigor of seedlings (%)				
Small non-scarified	74 abB	74 bB	85 bA	80 bA	74 bB
Small scarified	75 abB	95 aA	95 aA	95 aA	80 aB
Large non-scarified	60 bBC	50 cC	85 bA	75 bB	70 bB
Large scarified	75 aC	90 aB	100 aA	100 aA	90 aB
CV (%)	10.9	9.9	12.6	15.7	14.2

[1] Lowercase letters (a,b) compare the means for the variables between the sizes of seeds without (WOS) and with (WIS) scarification. Uppercase letters (A, B) compare the means for the variables between the soaking periods, by Tukey test at 5% probability level. CV: Coefficient of variation.

adequate soaking periods, proved to be a viable and promising technique in the seedling production sector due to the longer time to obtain the emergence of *H. courbaril* seedlings.

According to Smiderle et al. (2021), native seeds with higher ES (index) are less subject to adverse conditions found in the soil, such as temperature variation, water stress and attacks of pests and pathogens.

The results obtained in this study reiterate those obtained by Oliveira et al. (2016) who found that the six-hour soaking period for large and small seeds of *Acacia mangium* is ideal in the optimization of ES (index). According to Singh et al. (2019), inadequate soaking periods can lead to deterioration duo to physiological, biochemical, and cytological changes in the seed, culminating in low vigor of seedlings.

Furthermore, the lowest percentage of seedling vigor occurred for both small and large scarified and non-scarified seeds. in the soaking period of 12 hours and in the control. On the other hand, the highest percentage of seedling vigor was obtained with small and large seeds of *H. courbaril* non-scarified seeds and scarified (Table 3), suggesting once again the need to adapt the soaking period for the continuity of the germination process by the embryo.

However, the adequate period of soaking in water was probably important to promote significant changes in the hormonal balance that controls the physiological dormancy process, making seed metabolism more active and the embryo ready for resuming its development, consequently leading to greater gain in vigorous seedlings obtained.

It is worth mentioning that the results described in the present study, as well as in the national and international literature, show that the dormancy of *H. courbaril* seeds is present (Soares et al., 2017) and needs to be overcome scarified. However, mechanical scarification in *H. courbaril* seeds requires labor and extra costs for the nursery manager.

As an alternative, adequate periods of immersion in water can be used. According to the results obtained in the present study, this management has the advantage of homogenization and shorter mean time for seedling emergence and better stand of seedlings in the nursery, resulting in better efficiency in the use of propagative material and in the quality of the seedlings produced.

CONCLUSIONS

Maximum vigor of *H. courbaril* seedlings is obtained with large seeds subjected to scarification and soaked in water for six hours.

Water content of 18.7% in large seeds of *H. courbaril* scarified promotes the production of 100% of vigorous seedlings.

Small and large seeds of *H. courbaril* non-scarified seeds and scarified, soaked for six hours, led to shorter mean time for seedling emergence.

Water content of 20.6% in small, scarified seeds of *H. courbaril* promotes 100% seedling emergence.

The electrical conductivity test applied to *H. courbaril* seeds can be adopted to determine their vigor, showing speed in the obtaining of results and ease of execution.

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