Ciências Agrárias
SEMINA

DOI: 10.5433/1679-0359.2021v42n6Supl2p3667

Germination potential of *Hymenaea courbaril* L. in different maturation stages

Potencial germinativo de sementes de *Hymenaea courbaril* L. em diferentes estádios de maturação

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Highlights

Jatobá seeds at different maturation stages are able to germinate satisfactorily.
Green seeds are recommended for planting and seedling production.
Mature scarified seeds are capable of generating vigorous seedlings.

Abstract

The production of quality forest seedlings in large quantities is essential for the restoration of environments that have been deforested and degraded. However, obtaining seeds with high vigor is a challenge for several tree species native to Brazil. The objective of this work was to verify the germination potential of jatobá-da-mata seeds at different stages of maturation, in order to favor the production of seedlings of this species in nurseries. The seeds were extracted from green and ripe fruits detached from the mother plant and ripe fruits collected from the ground. The germination percentage, average germination time, emergence speed index, average speed, relative frequency, leaf area of the seedling, and length of the aerial part and root were measured. The planting was carried out with mechanically scarified and intact seeds from each maturation group. The results indicated that non-scarified green seeds can be used for planting and seedling production, as they do not require pre-germination treatment and have a favorable germination percentage (79%). Fruit seeds harvested from the ground, on the other hand, needed a method to overcome integumentary dormancy, such as mechanical scarification, obtaining a germination rate of 85%. The seeds of ripe fruits harvested in the matrix showed greater vigor, with a higher percentage of germination (96 to 100%), a higher emergence speed index, shorter average germination time, and seedlings with greater leaf area and greater length of shoot.

Key words: Jatobá-da-mata. Germination. Vigor. Mechanical scarification.

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Received: May 24, 2021 - Approved: Aug. 24, 2021

Resumo

A produção de mudas florestais com qualidade e em quantidade é essencial para a restauração de ambientes outrora desmatados e degradados. Todavia, a obtenção de sementes com alto vigor é um desafio para diversas espécies arbóreas nativas do Brasil. O objetivo deste trabalho foi verificar o potencial germinativo de sementes de jatobá-da-mata em diferentes estádios de maturação, de modo a favorecer a produção de mudas dessa espécie em viveiros. As sementes foram extraídas de frutos verdes e maduros destacados da planta mãe e frutos maduros coletados no chão. Foram mensurados o percentual de germinação, o tempo médio de germinação, o índice de velocidade de emergência, a velocidade média, a frequência relativa, área foliar da plântula e comprimento de parte aérea e raiz. A semeadura foi realizada com sementes escarificadas mecanicamente e intactas de cada grupo de maturação. Os resultados indicaram que as sementes verdes não escarificadas podem ser utilizadas para semeadura e produção de mudas, por dispensarem tratamento pré-germinativo e possuírem percentual de germinação favorável (79%). Já as sementes de frutos colhidos no chão necessitaram de um método para superar a dormência tegumentar, como a escarificação mecânica, obtendo a germinação de 85%. As sementes de frutos maduros colhidos na matriz apresentaram maior vigor, com maior percentual de germinação (96 a 100%), maior índice de velocidade de emergência, menor tempo médio de germinação, plântulas com maior área foliar e maior comprimento de parte aérea.

Palavras-chave: Jatobá-da-mata. Germinação. Vigor. Escarificação mecânica.

Introduction

The production of quality forest seedlings, in large quantities, is essential in the preservation of forests and maintenance of the environment, for use in the recovery of degraded areas, formation of legal reserves and permanent preservation areas, urban afforestation, and formation of ecological corridors. Although it is pointed out that 66.3% of the area in Brazil is covered by forest (Gazzoni, Cattelan, & Nogueira, 2019), deforestation rates in all biomes are increasing. As detailed in a study by Global Forest Watch, considering the sum of all biomes, Brazil was the country that lost the most trees in 2018 worldwide, approximately 1.3 million hectares of primary forests (Weisse & Goldman, 2019). According to The Economist Newspaper (2019), forests still provide a livelihood for 1.5 billion people and a buffer against climate change. However, as reported by Loose and Balbé (2020), the fragile conception of development that has become hegemonic is mainly based on economic growth, in an unlimited perspective, leaving aside numerous aspects of human well-being and ignoring the fact that this path is unsustainable, as already known for some resources (such as tropical forests).

In this context, the creation of reforestation strategies, the choice of suitable species, and the production of seedlings that, in some way, not only contribute to the conservation and restoration of natural environments, but also serve as models that are replicable, become a priority in actions of this magnitude. In addition, knowledge of the fruit maturation process, as an indicator of the harvest point, is essential for obtaining seeds of high physiological quality, and should be considered in forest seed production programs.

Among the species that stand out in reforestation for projects of recovery or restoration of degraded areas, we can list the Jatobá or Jatobá-da-Mata (Hymenaea courbaril L.), a species found throughout the country, which is the most popular and important among the species of the genus, and is present as vulnerable on the list of endangered species, due to logging and forest fragmentation (Alonso et al., 2014). According to Lorenzi (2020), since it is a plant that is not very demanding in terms of fertility and soil moisture, it should not be lacking in the composition of heterogeneous reforestations and in the afforestation of parks and large gardens. Associated with these characteristics, Jatobá is a climax plant (reaches the forest canopy, being the last stage of ecological succession) (Regnier, 2020), heliophyte (needs exposure to light for its full development, when adult), or sciophyte (develops in a shaded environment in its young phase), and selective xerophyte (it develops in dry environments) (Lorenzi, 2020).

The formation of Jatobá seedlings is essentially seminal and it would be beneficial to investigate cultivation techniques used in the seedling production process, especially in the stages of collection and processing of fruits and seeds. For satisfactory and efficient germination, the handling of Jatobá fruits and seeds requires the fruit to be opened with the aid of a hatchet or hammer, followed by extraction, washing in running water, drying in the shade, and mechanical scarification of the seeds, steps which represent a lengthy and complicated process.

Regarding the performance of Jatobá-da-Mata seeds, there are no reports on the maturation stage of the fruits collected for the production of seedlings and germination analysis. In view of this, it is still necessary to define the appropriate time for collection during the maturation of Jatobá seeds, in order to facilitate and accelerate the production of seedlings in nurseries. Thus, the objective of this work was to verify the germination potential of Jatobá seeds at different maturation stages.

Material and Methods

The fruits were harvested from three matrix trees 10-12 m high, located in a mixed orchard of fruits and native trees, in the municipality of Primeiro de Maio/PR (22º53′14′′S, 50º56′42′′W). The region's climate is classified according to Köppen as humid subtropical Cfa, with an average annual temperature of 22.1 ºC and an average annual precipitation of 1290 mm. The fruit maturation stages were based on the epicarp coloration, according to the MUNSELL color chart (Munsell, 1952), resulting in the classifications 10R 3/6, 5GY 4/6, and 7.5YR 5/4.

The fruits were manually collected with the aid of a ladder and aerial pruning scissors, and classified into three stages of maturation, namely: ripe fruits collected from the matrix, unripe fruits collected from the matrix, and ripe fruits collected from the ground, as shown in Figure 1. The unripe fruits had completely green skin, with total visual growth achieved, and whitish-green arils.

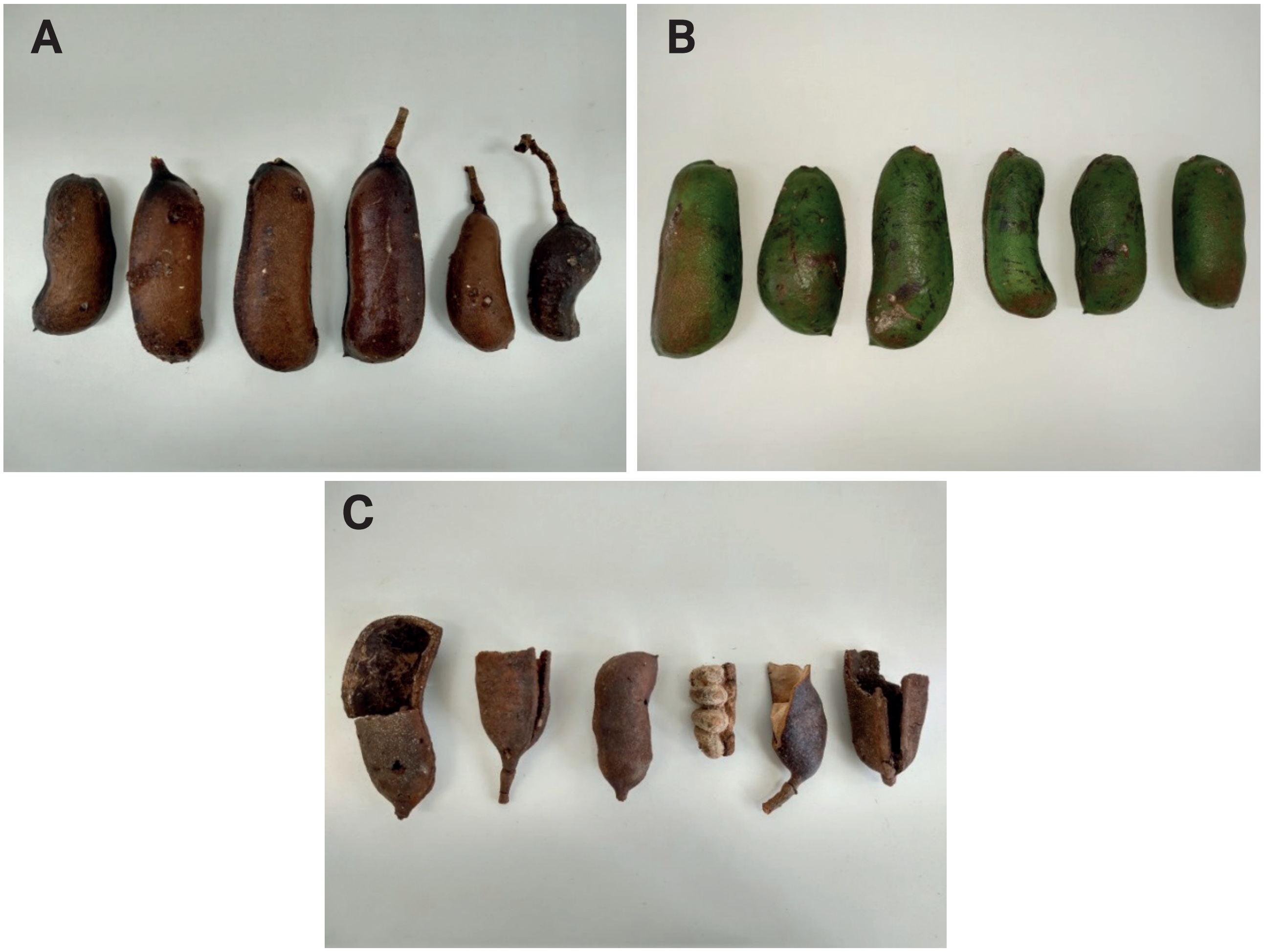

Figure 1. Morphological and maturation differentiation of Jatobá fruits. Ripe fruits collected from the matrix (A), unripe fruits collected from the matrix (B), and ripe fruits collected from the ground (C).

The seeds were removed from the fruits using hammers, manually separated from the farinaceous aril and sown in 1.00 x 1.00 x 0.60 m boxes, with sand, in the nursery belonging to LABRE - Laboratory of Biodiversity and Restoration of Ecosystems of the State University of Londrina. Prior to sowing, the seeds of the three maturation groups were submitted to the mechanical scarification procedure by means of a grinder coupled to an electric drill, breaking the integument on the opposite side of the hilum, while keeping the seed to be sown intact. Seed separation, scarification, and sowing occurred successively and immediately, without intervals. The moisture content of the seeds was determined as recommended in the Seed Analysis Rules (Ministério da Agricultura, Pecuária e Abastecimento [MAPA], 2009).

The experiment was conducted in a completely randomized design, in a 3×2 factorial scheme, in which the first factor is represented by the stage of fruit maturation (ripe fruits picked from the matrix, ripe fruits collected from the ground, and unripe fruits collected from the matrix) and the second factor is represented by scarification (with and without), totaling six treatments. Four replications were used, with 20 seeds each. Data were checked for normality by the Shapiro-Wilk test, and homoscedasticity by the Bartlett test, and submitted to analysis of variance (ANAVA) at the 5% probability level. Means were compared by the Tukey test at 5% significance. All analyses were performed using R software.

The experiment was carried out for 50 days, in January and February 2020, and the seeds were considered germinated when the plumular hook was fully immersed in sand, (which was the only substrate used). At the end of the test, from the daily data on the number of emerged seeds, the following variables were calculated:

1 - % of Emergence (E), calculated using the formula of Labouriau and Valadares (1976):

$$E = (N/A) \times 100$$

where: N = number of germinated seeds at the end of the test; A = total number of seeds placed to germinate;

2 - Emergence speed index (ESI), calculated by Maguire (1962) formula:

$$ESI = E1/N1 + E2/N2 + \cdots + En/Nn$$

Where: ESI = emergence speed index; E1, E2, En = number of seeds germinated in the first, second, and final count; N1, N2, Nn = number of sowing days in the first, second, and final count.

3 - Average emergence time (t) in days, calculated by the formula (Ferreira & Borghetti, 2013):

$$t = (\Sigma ni \times ti)/\Sigma ni$$

where: ni = number of germinated seeds per day; ti = incubation time;

4 - Average emergence speed (AES) in days, calculated by the formula (Ferreira & Borghetti, 2013):

$$AES = 1/t$$

where: t = mean germination time. The AES is another way to quantify the germination kinetics, being simply the inverse of the average germination time.

5 - Relative Germination Frequency, in percentage, according to the formula proposed by Labouriau and Valadares (1976):

$$Fr = \frac{ni}{(\sum ni)}$$

where: Fr = relative frequency of germination; ni = number of germinated seeds per day; Σni = total number of germinated seeds;

For the morphological determinations, 50 days after sowing, the following were measured: (1) the length of the root and the length of the aerial part, with the aid of a ruler graduated in millimeters. The measurement was made from the hypocotyl to the main root cap and from the hypocotyl to the base of the apical meristem, respectively, and (2) the leaf area, which was determined with the aid of a portable gauge (LI-COR Biosciences, Lincoln, USA), model LI-3000C, coupled to a mat where the detached leaves were inserted and the area was determined when passing through the measuring chamber. Ten plants were measured, randomly chosen per treatment, and the means were analyzed by the Tukey test at 5% significance.

Results and Discussion

A large variation in fruit size was recorded for the population of Jatobá-da-Mata, as was also seen by Pereira, Giraldelli, Laura and Souza (2011) and Santos et al. (2019) in H. stigonocarpa var. stigonocarpa showing great variability in relation to fruit size, number of seeds per fruit, and seed mass. Seed moisture content was 6.4% for mature seeds collected from the ground, 7% for mature seeds collected from the matrix, and 46.5% for green seeds collected from the matrix.

Fruit color and moisture content were used as parameters to prescribe the seed maturation stage, since most fruits, such as Jatobá, present color changes during the development process, especially in the bark, due to the degradation of chlorophyll and the synthesis of pigments such as carotenoids and anthocyanins (Barbosa, Rodrigues, Barbério, & Araujo, 2015; Duarte, Paula, Ferreira, & Nogueira, 2016). The size of the seeds also changed according to the evolution of the maturation process (Figure 2), due to the dehydration resulting from this process (N. M. Carvalho & Nakagawa, 2012). Thus, the size of the Jatobá seeds significantly reduced and their color changed, becoming smaller, darker, and clearly harder and stiffer when compared to green seeds (Figure 2).

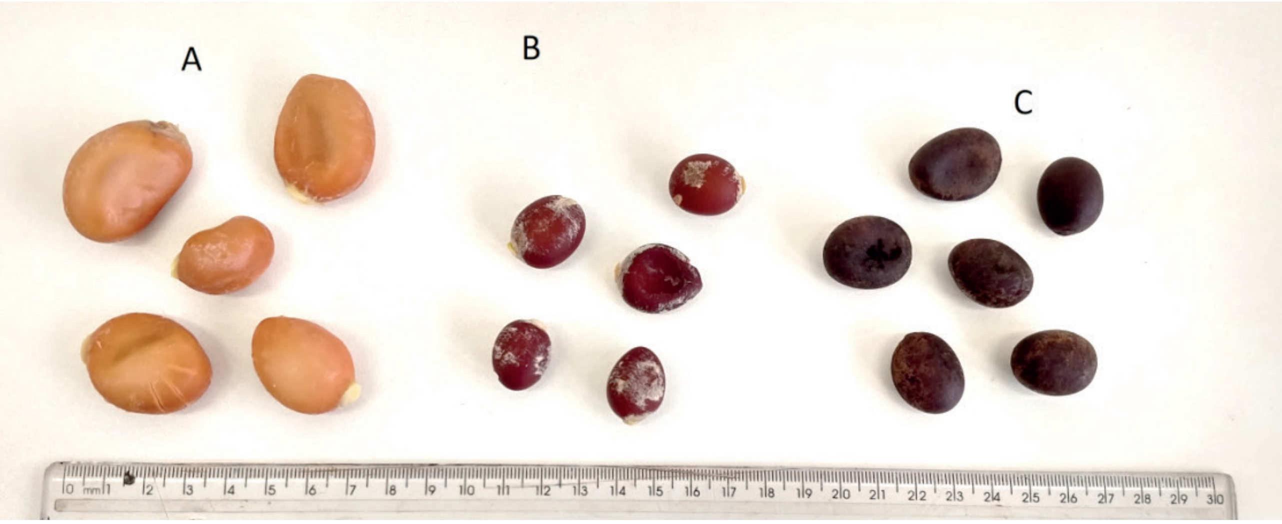

Figure 2. Variation in seed size according to the degree of maturity of the fruits., A - Unripe fruits; B - Ripe fruits collected from the matrix; C - Fruits and seeds harvested from the ground.

Popinigis (1985) pointed out that the seed size increases gradually, from fertilization until reaching the maximum, when it still has a relatively high moisture content and, after reaching the maximum, the size of the seeds decreases, as it loses water. Marcos (2015) reported that, with the intensification of the transfer of reserves from the plant to the seeds, there is a progressive increase in size, in terms of thickness, so that the maximum size is reached approximately halfway through the period of dry matter accumulation, and then there is a reduction in size, with variable intensity, which is more evident in legumes, such as soybeans and Jatobá.

The results for percentage of emergence indicated that, in all maturation stages, seeds able to germinate were obtained. Figure 3 shows that for the percentage of emergence (E) the highest values were for ripe fruits harvested from the matrix, regardless of whether or not there was pre-germination treatment, although there were no significant differences for treatment 3 (ripe fruit picked from the ground under scarification) and for treatment 6 (non-scarified seeds of unripe fruits), whose treatments showed E averages above 79%. The lowest values (E<34%) were observed in treatments 4 and 5 (scarified seeds of green fruits and non-scarified seeds collected from the ground). In the latter case, the fruit ripening point had been exceeded, so that the seeds had begun to lose their vigor due to deterioration arising from environmental factors, confirming previous data that excessive fruit maturation can be harmful to seed quality (Castro, Bradford, & Hilhorst, 2004). In addition, fruits in contact with the soil may have increased water content, which, under unfavorable conditions, leads to the beginning of consumption of reserves without germination, affecting the entire germination potential (Nogueira & Medeiros, 2007; Mojena & Barreto, 2021).

The highest values of E for treatments 1 and 2 (96 and 100%) are justified by the fact that the seeds have greater germination potential and vigor, with minimal deterioration, maintaining active tissues and functional metabolism, which, according to Barbosa et al. (2015), marks the determination of the physiological maturity point of the seed. It is also noteworthy that the quality of the seeds is greatly affected when the harvest is performed before they reach physiological maturity or after this stage, as observed in the current work, for the percentage of emergence of seeds harvested from the ground without mechanical scarification intervention, which had the lowest value, as also reported by Pagliarini, Castilho, Nasser and Alves (2016).

For Costa, Bruno, Souza and Lima (2001), the highest percentages of emergence for treatments 1 and 2 are related to the endocarp, which, at this stage, is less lignified, a fact that does not occur in the same way with endocarps of fruits with advanced maturation, due to the difficulty of water penetration through its layers. This fact was also pointed out by Müller, Gibbert, Binotto, Kaiser and Bortolini (2016), with *Peltophorum dubium,* where percentage of germination was lower for seeds extracted from fully ripe fruits, with integument restriction to the entry of water inside the seed (18%), and higher for seeds in the maturation phase (43%).

Mechanical scarification resulted in a high percentage of emergence (treatments 1 and 3). This process allowed the entry of water and oxygen, in addition to favoring the action of hydrolytic enzymes that act with the activation of protein synthesis, enabling the mobilization of reserves from the germination process, as seen by Santos et al. (2019), who obtained 93% seed germination from *H. courbaril* when subjected to mechanical scarification. This was also seen by other authors, such as Coelho and Ribeiro (2018), above 93%, Cabral, Castilho and Pagliarini (2015), above 86%, Sampaio et al. (2015), with 84%, Souza et al. (2015), above 80%, and by Silva and Cesarino (2016) with 69.2%.

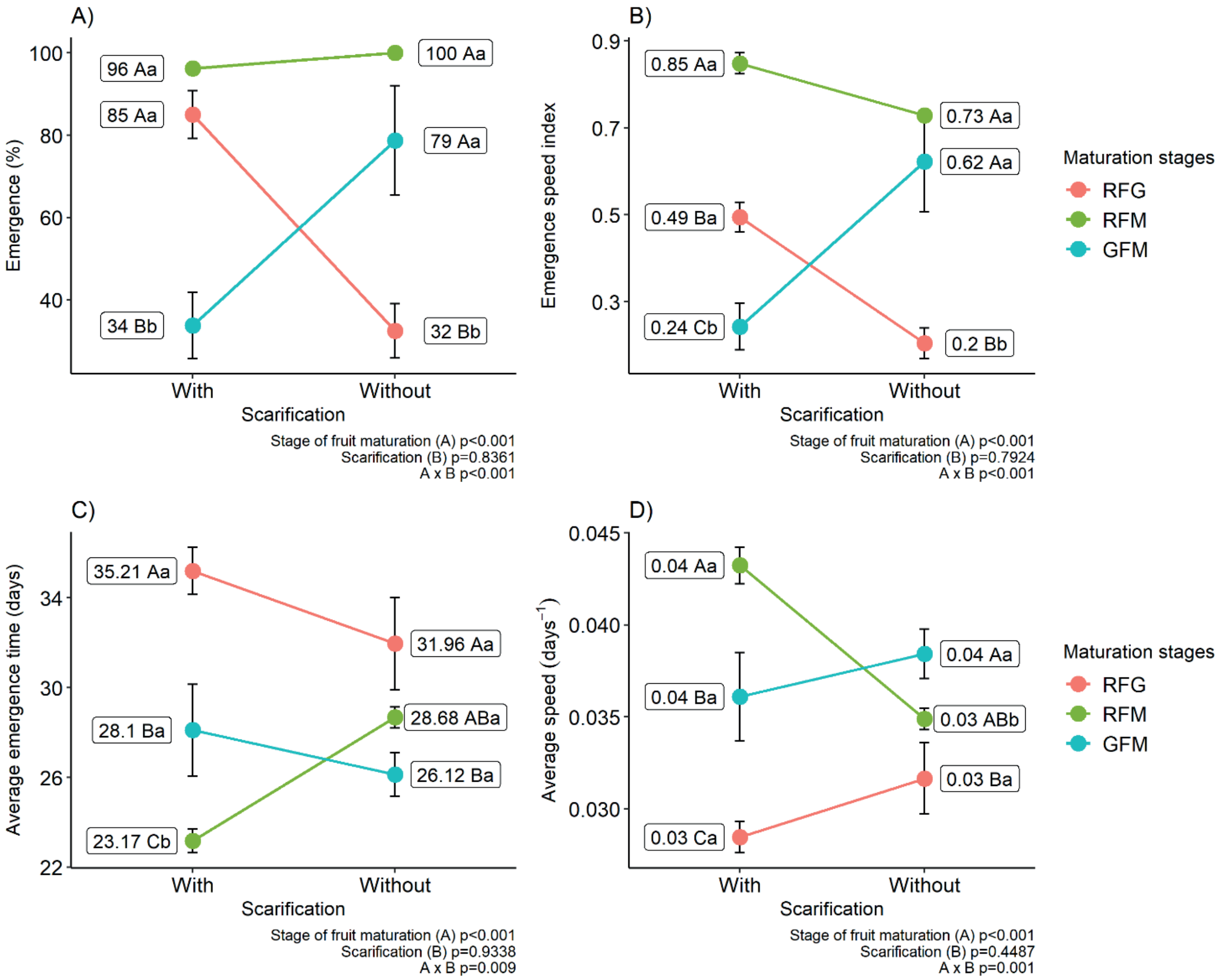

Figure 3. Emergence percentage (E), emergence speed index[1] (ESI), average emergence time (t), average speed (v) of *H. courbaril* seeds of different maturation stages.
* Means followed by letters of the same lowercase letter horizontally and uppercase vertically do not differ, according to the Tukey test at 5%. [1] data transformed. Where: RFM - Ripe fruits harvested from the matrix (treatments 1 and 2), RFG - Ripe fruits collected from the ground (treatments 3 and 4), GFM -Green fruits collected from the matrix (treatments 5 and 6). Germination CV=20.54%, ESI CV=12.7%, t CV=9.4%, and v CV=8.38%.

For Jatobá, it is necessary to open the fruit with the aid of a hatchet or hammer, followed by separation of the seeds, washing in running water and drying in the shade (Souza et al., 2015), with mechanical scarification of the seeds being more advantageous, as it is less expensive, safer and more convenient, however, this process must be performed with caution so as not to damage the embryo (Nascimento & Oliveira, 1999), showing efficiency in overcoming the tegumentary dormancy of Jatobá seeds.

Intact green seeds (treatment 6) demonstrated 79% emergence, not differing from the other treatments with a high percentage of emergence (treatments 2, 1, and 3). According to N. M. Carvalho and Nakagawa (2000), seeds that are not completely mature can germinate, however, they result in less vigorous seedlings than those resulting from the germination of seeds that have reached physiological maturity. Also according to Marcos (2015), seeds in early stages of maturation can germinate, as they already have their structures formed, that is, the embryo is already morphologically formed. The lower E value attributed to scarified green seeds (34%) is due to the mechanical damage caused to the seed, since at this stage the seeds do not have a completely formed integument, with the absence of rigidity and protection characteristics, as well as tissues in formation, so that mechanical intervention generates a decrease in the physiological potential of the seed, affecting the percentage and speed of seedling emergence.

Green seeds with germination capacity to form normal seedlings were also observed for the cultures of *Carica papaya* (A. W. P. Lopes, Seleguini, Boliani, & Correa, 2009), for lauraceous such as *Ocotea porosa, O. odorífera,* and *O. puberula* (Hirano & Possamai, 2008), and for *Physalis angulata* (Carvalho, Oliveira d'Angelo, Scariot, Saes, & Cuquel, 2014). In addition, some works pointed out species in which green seeds did not result in a satisfactory germination percentage when different stages of maturation were analyzed, as mentioned by (Costa et al., 2001) in seeds of *Spondias tuberosa,* in *Passiflora edulis f. flavicarpa* (Negreiros et al., 2006), in *Pseudobombax grandiflorum* (J. C. Lopes, Matheus, Correa, & Silva, 2008), in *Schinus terebinthifolius* (Vitória et al., 2018), in *Mimosa ophthalmocentra* (Leite, Nogueira, Freitas, Leite, & Guimarães, 2019), and in *Inga striata* (Mata et al., 2013). Thus, it is evident that the germination of green seeds can vary according to the species.

The emergence percentage (E) informs the total number of germinated seeds, however, it does not reflect how long it took for the seeds to reach this germination percentage. In this approach, the mean emergence time (t) gives a kinetic point of view for E, providing information on how long it took for a given seed lot to germinate, that is, it corresponds to the mean time needed for a set of seeds to germinate (Ferreira & Borghetti, 2013).

Regarding the ESI, the treatments with seeds from ripe fruits harvested from the matrix and unscarified green fruit seeds had the highest values, not differing statistically from each other (T1: 0.85, T2: 0.73, and T6: 0.62). The lowest values, as occurred in the percentage of emergence, were for treatments 4 and 5. Although this index is expressed without a unit, it relates the number of germinated seeds per unit of time (Ferreira & Borghetti, 2013), that is, the higher the ESI, the higher the emergence speed, which infers the vigor of the seed lot.

For t, it was observed that seeds with a mature and developed integument (treatments 3 and 4) had slower emergence than seeds from other maturation stages, with a mean time greater than 31 days. The lowest value of t was observed for treatment 1 (23.17 days), which had the highest value for ESI. The t is also related to seed vigor, as the high seed emergence rate occurred in a shorter time. Following this order, seeds from unripe fruits (treatments 5 and 6) and seeds from mature fruits harvested from the matrix (1 and 2) had the lowest *t* values, not differing from each other.

In the distribution of the relative daily frequencies of germination, differences were observed between seeds of different maturation stages. It can be seen in Figure 4, that in seeds from ripe fruits collected in the matrix, the germination peaks were distinct, occurring between the 20th and 22nd day for seeds under mechanical scarification (tract. 1) and for intact seeds (treat. 2) on the 24th day, with the presented values of ESI and t, being better than those evaluated for treatment 1. In figure 4, the germination peak was very accentuated on the 29th day for treatment 4, maintaining a characteristic of a leptokurtic distribution, that is, when seed germination is more concentrated in a certain time interval. Differently, treatment 3 did not present a sharp germination peak, maintaining a distribution spread over time, characterizing a platykurtic distribution (Figure 4C). Treatments 5 and 6 had a similar germination peak, which occurred between the 26th and 28th days.

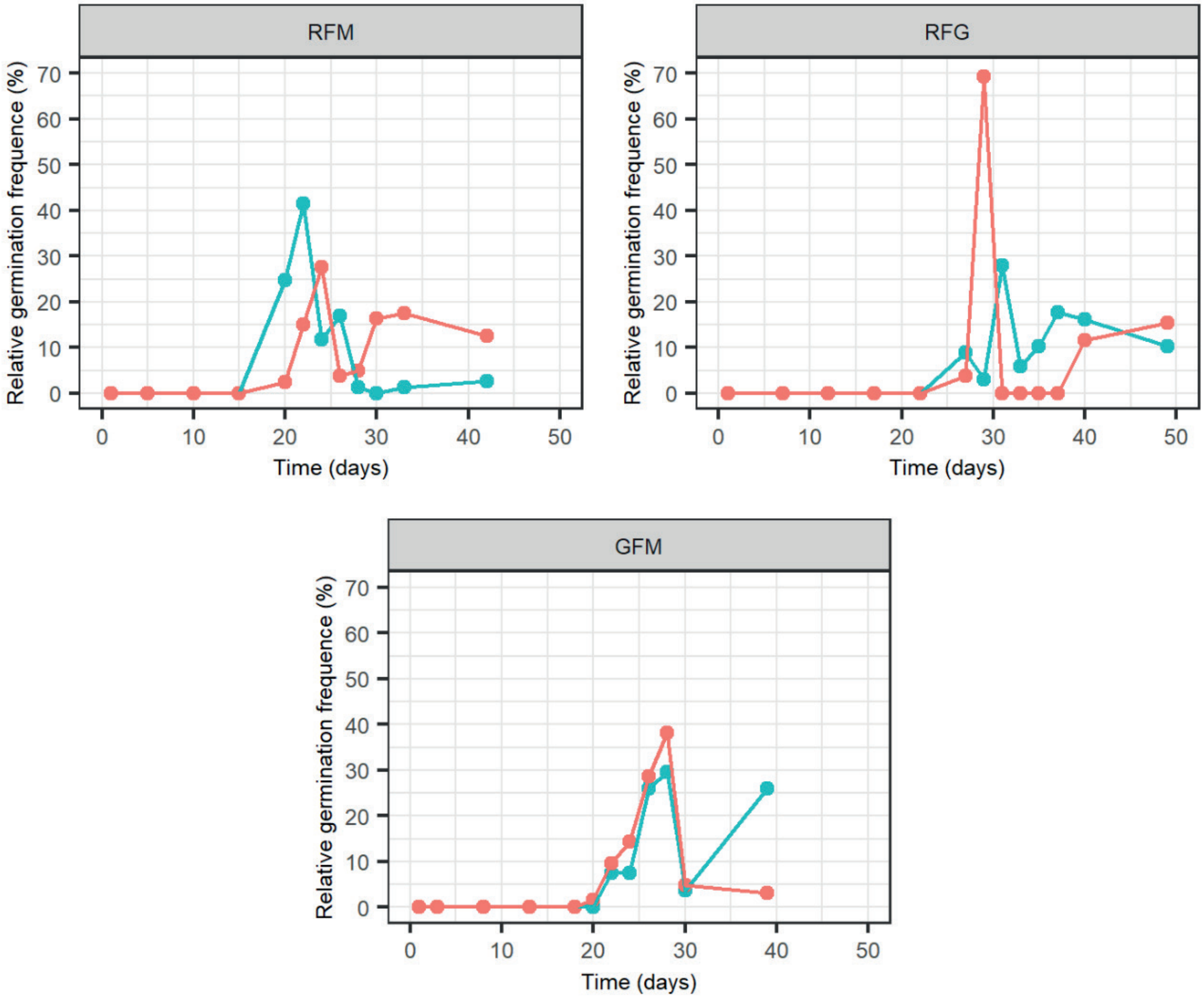

Figure 4. Relative Germination Frequency of scarified (blue) and intact (red) seeds of H. courbaril, clockwise, from ripe fruits collected from the matrix, from mature fruits collected from the ground, and from green fruits collected from the matrix.

Thus, we found that the same species can present different patterns of germination frequency distribution according to the treatment received. These distribution arrangements are widely used to evaluate the effects of different treatments on the temporal distribution of seed germination (Ferreira & Borghetti, 2013), enabling prediction or postulation of the germination behavior of a given species under certain conditions.

This difference in germination between fruits harvested from the matrix and from the ground may be related to the fact that mature seeds are not able to preserve their viability indefinitely, as their maturation involves processes that culminate in morphological, physiological, and biochemical alterations (Marcos, 2015). Wagner, Maciel, Radaelli and Guollo (2020) reported that when the storage environment is not suitable, the deterioration process is accelerated. When collected from the ground, the Jatobá seeds had probably already surpassed the physiological maturation point (when the seeds present maximum germination, vigor, and dry mass), which precedes the detachment of the fruit from the plant. Also with regard to seeds from green fruits, Marcos (2015) pointed out that most species have the ability to germinate well before reaching physiological maturity; however, the highest germinations are reached close to the maximum accumulation of dry matter.

Figure 5 shows the values obtained for the morphological determinations of the Jatobá seedlings. Regarding the root length, we found that treatments 4 (ripe fruit collected from the ground with non-scarified seeds) and 6 (green fruit collected from the matrix with seeds without scarification) obtained the highest values (10.89 and 10.18 cm, respectively). Lorenzetti et al. (2018) found similar values of root development with seeds of *Caesalpinia peltophoroides* at different maturation stages.

Seed maturation influenced the leaf area of the seedlings, with a higher value for treatment 1, with 83.173 cm² (Figure 6). Green seeds, on the other hand, had zero or very low values (Figure 6 E-F), presenting slower leaf development until the end of the experiment, although no unevenness in germination was noted. According to Marcos (2015), the reduction in vigor implies an increase in the period necessary for the emergence and a reduction in seedling growth, as observed in the current study.

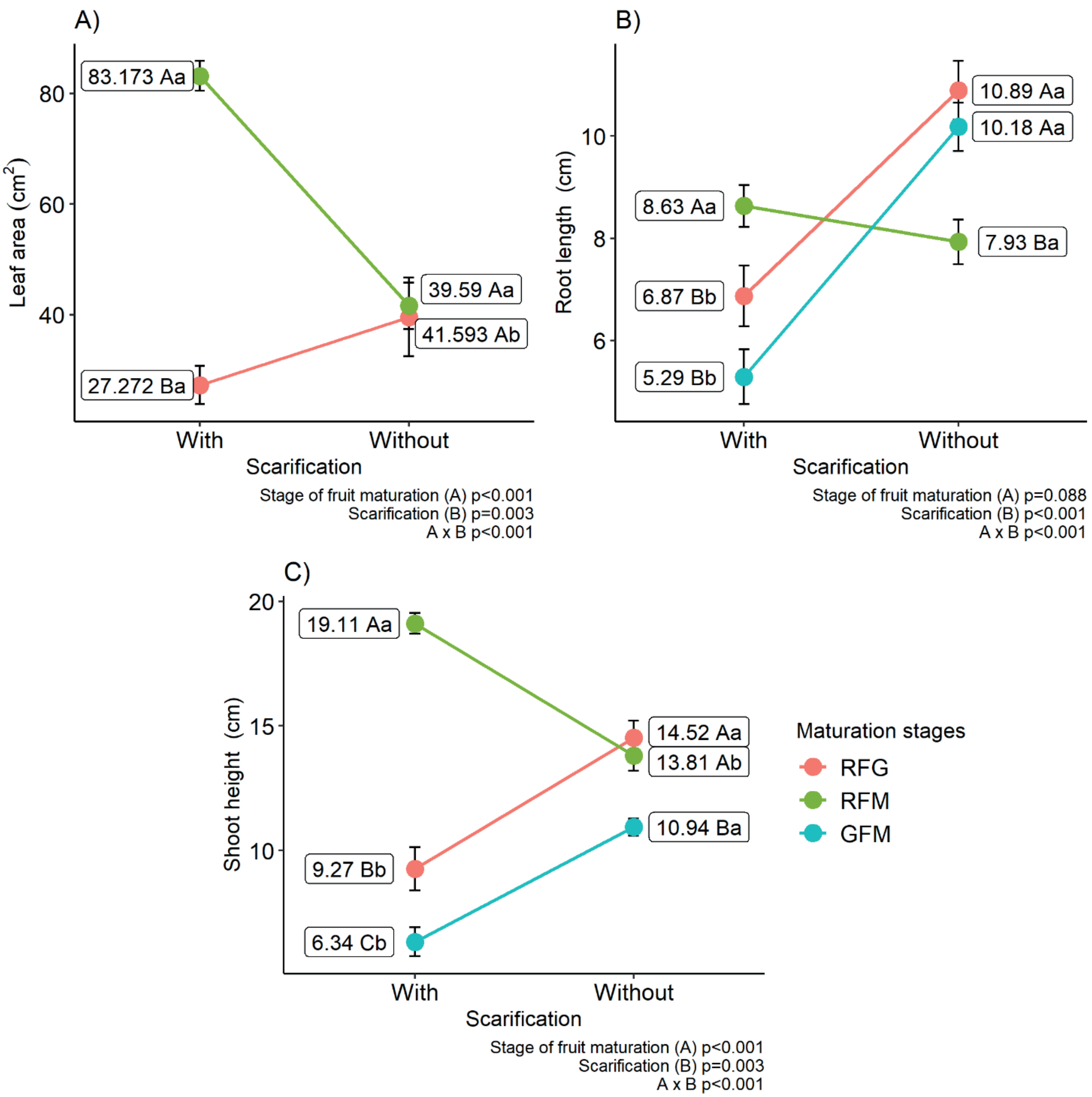

Figure 5. Shoot height (SH), root length (RL), and leaf area (LA) of *H. courbaril* seedlings from seeds at different maturation stages.
*Means followed by letters of the same lowercase letter horizontally and uppercase vertically do not differ, according to the Tukey test at 5%. LA CV=30.74%, Root Length CV=19.38% and Height CV=15.67% Where: RFM - Ripe fruits harvested from the matrix (treatments 1 and 2), RFG - Ripe fruits collected from the ground (treatments 3 and 4), GFM -Green fruits collected from the matrix (treatments 5 and 6).

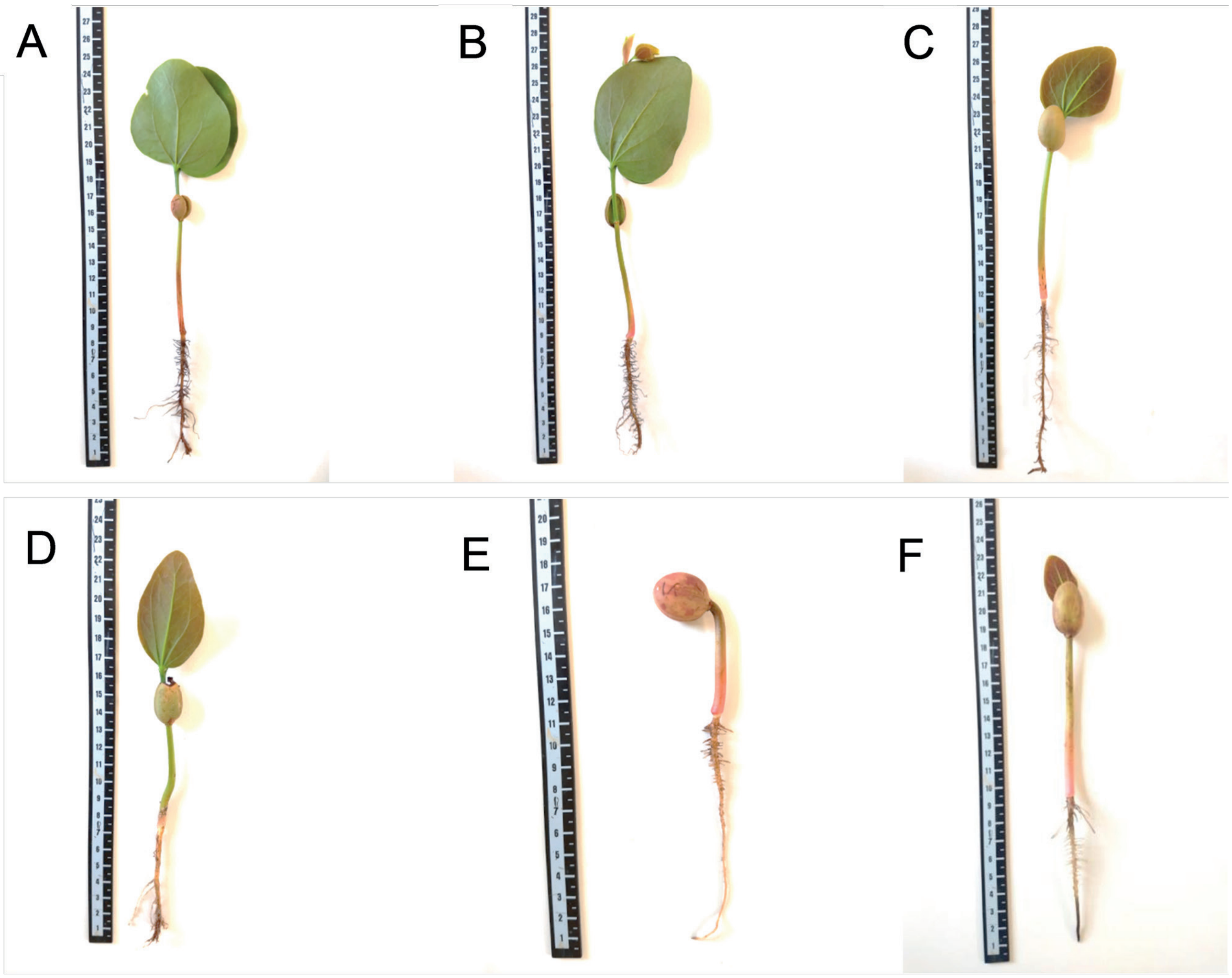

Figure 6. Development of *H. courbaril* seedlings from unscarified (A) and scarified (B) seeds from mature fruits harvested from the matrix; from unscarified (C) and scarified (D) seeds from ripe fruits collected from the ground; and from unscarified (E) and scarified (F) seeds from green fruits collected from the matrix.

With respect to treatment 4, the fact that the seeds had a period of rest in the soil after detachment from the matrix proved to be advantageous, as this period promoted a gradual reduction in the degree of moisture of the seeds, which resulted in seeds with the capacity to develop and the formation of seedlings with adequate root and aerial systems.

For treatment 6, it is possible that the high levels of gibberellin stimulated the alpha-amylase enzyme and other enzymes that promote hydrolysis of the reserve material, necessary for root growth (Antunes, Picolotto, Vignolo, & Gonçalves, 2012). The same was not evidenced for treatment 5, as scarification was harmful to the germination of green seeds and consequent seedling formation.

Treatment 1 presented an average shoot height value of 19.11 cm, being 27.73% higher than treatment 2, 51.5% higher than treatment 3, and 66.18% higher than treatment 5. This result demonstrates the high vigor of the seeds, as well as the fact that they reached higher values of % E and ESI, and lower t. As evidenced by Mata et al. (2013) green seeds of *Inga striata* presented lower shoot development values when compared to mature seeds.

Conclusions

Jatobá seeds from different maturation stages are able to germinate.

Seeds from ripe fruits harvested from the matrix present greater vigor, as they show a higher percentage of emergence, higher emergence speed index, shorter average emergence time, and seedlings with a larger leaf area and longer shoot length.

Intact green seeds are recommended for sowing and seedling production, as they do not require pre-germination treatment and have a favorable emergence percentage, however seedling development is slow, which implies longer nursery time.

Seeds from fruits harvested from the ground require the help of a method to overcome tegumentary dormancy.

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