Iheringia
Série Botânica
ISSN ON-LINE 2446-8231

Jardim Botânico de Porto Alegre

# Substrate and temperature in germination of *Jatropha mollissima* (Euphorbiaceae)

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Recebido em 17.VII.2020
Aceito em 15.I.2024

**ABSTRACT** – Due to the lack of information about the germination of *J. mollissima* in different conditions, the research aimed to determine the best combination between substrate and temperature for the germination of *J. mollissima* seeds. Were used four substrates (vermiculite, between paper, sand, and paper roll) and four temperatures (25, 30, 35 and 40 ºC). Germination percentage (G), mean germination time (MGT), germination speed index (GSI) and relative frequency were calculated. There was no germination in the test conducted in 40 ºC, as well as the paper roll substrate. 30 and 35 ºC temperatures, obtained the best values for G (85.6 and 86.4), MGT (4.88 and 4.25) and GSI (4.7 and 5.25) on the vermiculite substrate. The most homogeneous frequency was observed on the substrate's vermiculite and sand. Vermiculite were the best options of substrate to germination of the *J. mollissima*, with temperature by 30 or 35 ºC.

**Keywords:** Caatinga, pinhão-bravo, seeds viability, semiarid, thermal demand.

**RESUMO** – **Substrato e temperatura na germinação de sementes de *Jatropha mollissima* (Euphorbiaceae).** Devido ao déficit de informações acerca da germinação da espécie em diferentes temperaturas e substratos, sob condições controladas a pesquisa objetivou determinar a combinação mais adequada entre substrato e temperatura para a germinação de sementes de *J. mollissima*. Foram utilizados quatro substratos (vermiculita média, entre papel, areia e rolo de papel) e quatro temperaturas (25, 30, 35 e 40 ºC). Calculou-se: porcentagem de germinação (%G), tempo médio de germinação (TMG), índice de velocidade de germinação (IVG) e a frequência relativa. Observou-se que não houve germinação nos testes conduzidos a 40 ºC, assim como, o substrato rolo de papel não apresentou resultados satisfatórios para germinação. As temperaturas de 30 e 35 ºC obtiveram valores para G (85,6 % e 86,4 %), TMG (4,88 dias e 4,25 dias) e IVG (4,7 e 5,25) superiores no substrato vermiculita. Nos substratos vermiculita e areia verificou-se frequência mais homogênea. Portanto, a combinação mais adequada para germinação das sementes de *J. mollissima* foi o substrato vermiculita média e as temperaturas de 30 ou 35 ºC.

**Palavras-chave:** Caatinga, demanda térmica, pinhão-bravo, semiárido, viabilidade de sementes.

## INTRODUCTION

*Jatropha mollissima* (Pohl) Baill. (Euphorbiaceae) or pinhão-bravo, is a shrubby-arboreal species endemic of Brazilian Savanah or dry forest, called by Caatinga, ecosystem semiarid with occurrence in Northeast Brazil (Brasil 2010; Secco *et al.* 2012; BFG *et al. 2015;* Gomes *et al.* 2016). It is widely used by the population due to its medicinal properties and the oil contained in the seeds has potential for biodiesel production, as well as the *Jatropha curcas* L. (pinhão-manso), in addition to its use for ornamentation and as a hedge (Neves *et al.* 2010; Lima *et al.* 2015; Zegarra 2015; Souza & Cavalcante, 2019).

Due to the wide diversity of biomes found in the world, the species have a range of adaptations for the optimal temperature for seed germination according to their environment (Masin *et al.* 2017; Nonogaki 2019), besides that, the successional group that they are inserted also influences in the species optimal temperature for seed germination (Brancalion *et al.* 2010). According to the authors, the optimal temperature is a physiological adaptation, that affects biochemical reactions (Oliveira *et al.* 2014) and consequently influences the germination, combined with the necessary water availability for the seeds (Rajjou *et al.* 2012; Nonogaki 2019).

Seed germination is characterized by the embryo growth recovery, with intensification of the metabolism, after seed imbibition, when subjected to favorable environmental conditions (Nonogaki 2019; Silva *et al.* 2019). Such conditions involve water availability, temperature, light incidence, and oxygen. Water is indispensable for rehydrating tissues and increasing metabolic rates, in the other hand, temperature influences germination with the acceleration of metabolic processes, affecting the speed and uniformity of seed germination (Guedes & Alves 2011; Guedes *et al.* 2013; Oliveira *et al.* 2014; Gonçalves *et al.* 2015). The optimal temperature for seed germination is defined when the highest percentage of germination is observed in the shortest time (Melo *et al.* 2017).

The Rules of Seeds Testing and the Instructions of Forest Seeds are Brazilian manuals for seed analysis laboratories, based on international rules of the International Seed Testing Association, with methods for quality verification of seeds lots, including the main cultivated species of the *Pinus* and *Eucalyptus* genera and the "most widespread" native species (Brasil 2009, 2013). However, a limited number of native species, given the diversity of Brazilian vegetation, is included in these manuals (Brasil 2009, 2013; Brancalion *et al.* 2010).

In the Rules of Seeds Testing (Brasil 2009) the use of paper, seed or soil as substrates for laboratory germination tests is indicated. Another commonly used material is vermiculite, since it is an inert material, just like the aforementioned, with standard granulometry and chemical composition (Martins *et al.* 2011; Silva *et al.* 2018).

The substrate used for seed germination, should promote structural aeration conditions, water retention capacity, absence of pathogens (Silva *et al.* 2008) and should remain with uniform humidity during all germination process to be efficient (Mello and Barbedo 2007; Guedes *et al.* 2010). The humidity of the substrates for germination are indicated in the RAS, however, each species has specificities in relation to its germination. According to Guedes *et al.* (2010), it is important to verify the responses of the species to the moistening of the substrates (Melo *et al.* 2017), since it could be an indication of the best area to insert the species.

From this information, it is necessary to know the germinative behavior of the species in different types of substrates and temperatures (Pereira *et al.* 2016), mainly because each material used with substrate has different characteristics of water retention and contact surface with seeds (Martins *et al.* 2011; Oliveira *et al.* 2016). The aim of present research was to determine the most suitable combination between substrate and temperature for the germination of *Jatropha mollissima* seeds.

## MATERIAL AND METHODS

*Jatropha mollissima* seeds were provided by the Rede de Sementes do Projeto de Integração do São Francisco, from the Núcleo de Ecologia e Monitoramento Ambiental (NEMA), based at the Universidade Federal do Vale do São Francisco, Brazil. The fruits were collected from 10 matrices, in the municipality of Floresta, state of Pernambuco, Brazil, with coordinates 08º36'04" S and 38º34'07" W at 316 m altitude, in May 2018, and remained in sieves covered by screens, due to the drupe type fruits and spontaneously exploding, thus preventing the loss of seeds. The analyzes were carried out at the Laboratório de Sementes Florestais of the Universidade Federal do Paraná, Brazil.

Preliminarily to the installation of the experiment, the water retention capacity (WRC) of the substrates, which were sterilized in an oven in accordance with the "Regra para Análise de Sementes" - RAS, were calculated according to the methodology applied in the RAS (Brasil 2009), with adaptations. For the substrate sand, two repetitions of 100 g were weighted and packed in funnels with filter paper and 300 mL of water were added, after 30 minutes, the volume of water drained was quantified, determining the WRC. The same procedure was performed with the vermiculite substrate, where two repetitions of 15 g were weighted.

It was defined that 65 mL of water would be used for 260 g of sand in each gerbox®, and 70 mL for the vermiculite substrate, containing 30 g of the substrate in each gerbox®, which represents 60 % of the WRC (Brasil 2009). For paper-based treatments, 100 % of WRC was used, through saturation in distilled water. During the experiment, distilled water was added to keep the substrates saturated, when observed that they were appearing not to be in saturation.

The gerboxes® were allocated in Mangelsdorf germinators, regulated at predetermined temperatures. The germinators were kept in a 20 ºC and constant lighting environment. The germination was monitored daily when root protrusion (radicle) ≥ 2 mm were considered germinated. The end of the experiment was determined when there was no germination for three consecutive days.

The experiment was conducted in a completely randomized experimental design in a 4x4 factorial arrangement, using four substrates (medium vermiculite, between paper, sand and paper roll) and four temperatures (25, 30, 35 and 40 ºC) with five replications with 25 seeds. As variable responses the germination percentage (G %), germination speed index (GSI) (Maguire 1962), mean germination time (MGT), measured in days (Labouriau 1983) and accumulated germination were counted. The germination percentage data were transformed by $\sqrt{G}$ and the mean germination time by $\frac{IVG^{0.5}}{0.5}$. The treatment data at 40 ºC were disregarded since it did not show germination and returned to the final analysis table.

Subsequently, the homogeneity and normality of the data were verified so that they could be submitted to analysis of variance (ANOVA), using the software R (R Core Team, 2020), followed by the Tukey at 5% probability of error.

## RESULTS

In view of the results obtained for the germination of *J. mollissima* seeds, the influence of temperature on seed germination was observed, as well as the significant interaction of substrates with temperatures for all calculated variables (Tab. 1).

**Table 1.** Variance analysis of the germination percentage (%G), germination speed index (GSI) and mean germination time (MGT) of *Jatropha mollissima* seeds.

| Source of variation | DF | Sum of Squares (SS) | Mean Square (MS) | F Stat | Prob (> F) |
|---|---|---|---|---|---|
| **%G** | | | | | |
| Temperature | 2 | 149.8 | 74.90 | 39.03 | $8.62^{-11}$ * |
| Substrate | 3 | 349.8 | 116.60 | 60.76 | $2.27^{-16}$ * |
| Temperature x Substrate | 6 | 30.4 | 5.07 | 2.64 | 0.027 ** |
| Residuals | 48 | 92.1 | 1.92 | | |
| **GSI** | | | | | |
| Temperature | 2 | 61.81 | 30.90 | 55.27 | $3.51^{-13}$ * |
| Substrate | 3 | 90.93 | 30.30 | 54.21 | $1.91^{-15}$ * |
| Temperature x Substrate | 6 | 27.65 | 4.60 | 8.24 | $3.75^{-06}$ * |
| Residuals | 48 | 26.84 | 0.56 | | |
| **MGT (days)** | | | | | |
| Temperature | 2 | 46.39 | 23.19 | 49.22 | $2.36^{-12}$ * |
| Substrate | 3 | 68.27 | 22.75 | 48,29 | $1.56^{-14}$ * |
| Temperature x Substrate | 6 | 9.11 | 1.51 | 3.22 | 0.0097 ** |
| Residuals | 48 | | | | |

* Significant at $p < 0.01$.
** Significant at $p < 0.05$.

For the germination percentage, an increase in the values was observed when temperature was raised to 30 and 35 °C in all the substrates used (Tab. 2). The highest averages were seen in the treatments using medium vermiculite as a substrate, this elevation express the maximum germination potential of the species under the available conditions. The between paper treatment, at all temperatures, showed low germination compared to medium vermiculite and sand treatments, even being the most used substrate for laboratory analysis. The temperature of 30 °C provided greater germination in this treatment, yet, kept averages 49 % and 31 % lower than the substrates vermiculite and sand, respectively.

The biggest germination speed index (GSI) was obtained in the medium vermiculite substrate at temperatures of 30 °C (4.7) and 35 °C (5.25), which did not differ statistically from each other. The interactions between the substrates sand, between paper and paper roll with temperatures (25, 30 and 35 °C) showed that medium vermiculite as a substrate presents greater efficiency. At a temperature of 25 °C, the lowest GSI was observed in all substates used, with values close to zero (Tab. 2).

The mean germination time evaluates the average time to obtain the maximum germination of the seeds, with this, thereby, the lowest value must be obtained for it to be satisfactory. The treatments using medium vermiculite and between paper at 30 and 35 °C did not present significant differences, with the best results showing values between 4 and 5 (Tab. 2). The treatments submitted to the temperature of 40 °C did not show any germination during the experimental period. The paper roll as a substrate and the temperature at 40 °C, were not efficient for the germination of *J. mollissima* seeds. This substrate had the lowest results for the germination percentage at all the temperatures evaluated, with a maximum of 11% and lowest to GSI with an average of 0.25.

To analyze the germinative behavior of *J. mollissima* seeds, the accumulated germination was evaluated (Fig. 1). According to the results, greater homogeneity was observed in seed germination submitted to a temperature of 35 °C, in all tested substrates. Among the substrates, the medium vermiculite showed greater homogeneity in germination at 30 and 35 °C, with concentrated germination between the 3rd and 5th day of evaluation.

In the other substrates, variation in germination was observed depending on the temperature used. In the substrate between paper, it is possible to visualize behavior similar to that observed in the vermiculite substrate, with higher concentration of germination between 3 and 5 days at temperatures 30 and 35 °C. In the sand substrate, the temperatures 25 and 30 °C presented several germination peaks during the experimental period, presenting the most heterogeneous germination among the treatments used. Therefore, with the increase in temperature, germination becomes faster, homogeneous and in a higher percentage, corroborating with the average values of GSI, mean germination time and germination percentage (Tab. 2).

As previously mentioned, the paper roll substrate was not effective for the germination of the species. In the graph of accumulated germination, it is observed that only the temperature of 35 °C presented the same pattern as the other substrates, with a peak of germination in the first days after the installation of the experiment, even if with a lower germination percentage in relation to the vermiculite and sand substrates.

**Table 2.** Germination percentage (%G), germination speed index (GSI) and mean germination time (MGT) of *Jatropha mollissima* seeds under different temperatures and substrates.

| Temperature (ºC) | Substrate | | | |
|---|---|---|---|---|
| | Vermiculite | Between Paper | Sand | Paper Roll |
| | | %G | | |
| 25 | 31.20 bA | 4.80 bB | 7.20 bB | 0.80 bB |
| 30 | 85.60 aA | 36.00 aB | 67.20 aA | 4.00 abC |
| 35 | 86.40 aA | 20.00 aC | 47.20 aB | 11.20 aC |
| 40 | 0 | 0 | 0 | 0 |
| | | GSI | | |
| 25 | 0.97 bA | 0.16 bA | 0.24 bA | 0.02 aA |
| 30 | 4.7 aA | 2.00 aB | 3.14 aB | 0.16 aC |
| 35 | 5.25 aA | 1.17 abC | 2.77 aB | 0.82 aC |
| 40 | 0 | 0 | 0 | 0 |
| | | MGT (days) | | |
| 25 | 8.32 aA | 6.00 aA | 8.12 aA | 1.40 aB |
| 30 | 4.88 bA | 4.82 aA | 6.0 abA | 3.80 aA |
| 35 | 4.24 bA | 4.63 aA | 4.66 bA | 2.16 aA |
| 40 | 0 | 0 | 0 | 0 |

The averages followed by the same letter do not differ statistically from each other by the Tukey test at 5% probability. Lower case letters correspond to the column and upper-case letters correspond to the line.

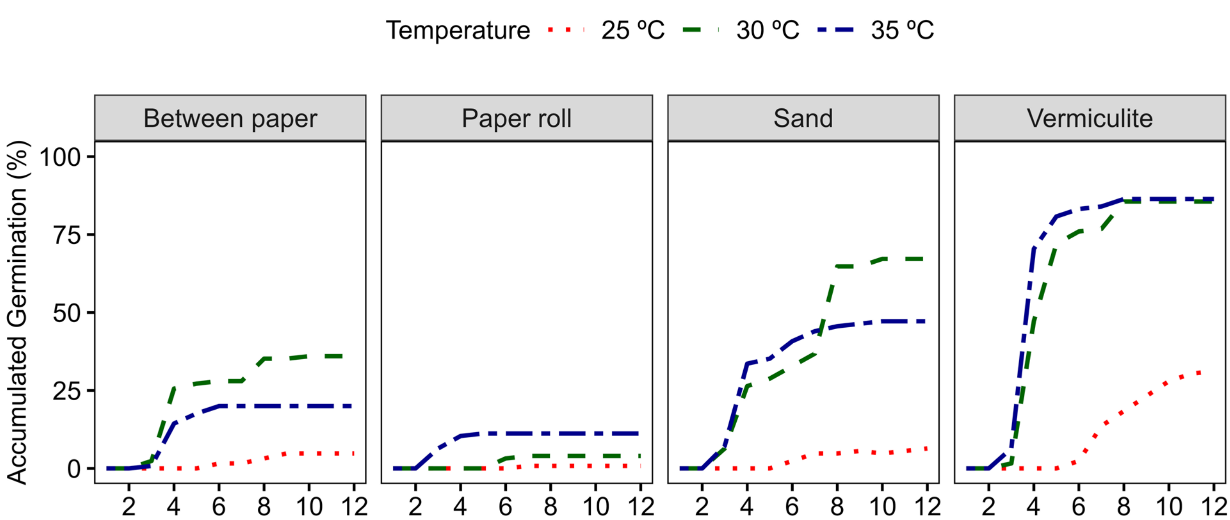


**Figure 1.** Accumulated germination of *Jatropha mollissima* seeds between paper, paper roll, sand, and vermiculite substrates at different temperatures.

## DISCUSSION

The temperature directly influences the water absorption speed and in germinators, one of the functions of the substrate is to retain moisture for soaking the seeds. Thus, these are factors that affect the germinative potential (Carvalho & Nakagawa 2012). The significant interaction between substrate and temperature was also observed by (Gomes *et al.* 2016; Oliveira *et al.* 2020) when they evaluated the germination of *Callisthene major* var. *Pilosa* (Vochysiaceae) Mart. & Zucc. (jacaré) and *Simiria gardneriana* M. R. Barbosa & Peixoto (pereiro vermelho), respectively.

Martins *et al.* (2011), as well as in the present research, identified an increase in the germination percentage when using vermiculite as a substrate. Such behavior can be explained due to the water retention capacity of the substrate used (Martins *et al.* 2011). Considering that the most used material for germination analysis in laboratory is the germitest® paper, it is possible to identify that the use of paper as a substrate for germination of this species is not effective and does not express the real germination potential.

The result observed in the paper substrate is due to its lower water retention capacity, when compared to medium vermiculite (70 mL) and sand (65 mL). Thus, a greater capacity of retention implicates in a greater availability of water or moisture for the seeds, and the availability of water is the most determining factor in germination, as it rehydrates the seed tissues, providing the necessary energy for the growth of the embryonic axis (Carvalho & Nakagawa 2012). In addition, because they were wrapped in plastic packaging, oxygenation was low, and if lower than the existing value in the atmosphere, germination tends to be delayed (Oliveira *et al.* 2019), and the high moisture content, which may have led to the rotting of the seeds.

According to Mello & Barbedo (2007), the temperature exerts influence in the speed and germination percentage (Guedes *et al.* 2013; Masin *et al.* 2017), with this, higher temperatures increase the speed of chemical reactions culminating in a quicker germination (Sorana *et al.* 2019). These behaviors explain the best results at temperatures of 30 and 35 °C on the vermiculite substrate.

These high temperatures are consistent with the conditions found in the semiarid region of Northeast Brazil and, according to (Oliveira *et al.* 2014) the temperature is related to the adaptation of the species to its natural environment, in order to de combinate germination with conditions that favor the development and establishment of seedlings. The lower GSI values, at the mildest temperature (25 °C), becomes one more indication of the adaptation of the species on its place of occurrence (Hernández-Nicolás *et al.* 2017; Melo *et al.* 2017).

The species behave in different ways in relation to the optimal temperature of germination (Oliveira *et al.* 2015), even though they are inserted in the same region, due to the adaptive differences and amplitude of establishment of the species. Species like *Schinopsis brasiliensis* Engl. (baraúna), *Sideroxylon obtusifolium* (quixabeira) and *Amburana cearenses* (Allemão) A. C. Sm. (umburana de cheiro) have better germinative performance at 30 °C, while *Simira gardneriana* (pereiro vermelho) and *Chorisia glazovii* O. Kuntze (barriguda) show better performance in germination and seedling formation in milder temperatures (Guedes & Alves 2011; Oliveira *et al.* 2014; Oliveira *et al.* 2016).

According to Oliveira *et al.* (2014) seeds of native Brazilian species, present satisfactory germination in the temperature range between 20 and 30 °C and also when there is variation between these temperatures, however there is no a standardization, due to the different behaviors of the species (Guedes *et al.* 2010; Oliveira *et al.* 2019). Brancalion *et al.* (2010) indicates that Brazilian tree species show higher germination at temperatures of 25 °C and/or 30 °C, however the authors still comment about the variation according to the species biome, and that it is related to the water availability.

The lack of germination in treatments at 40 °C may be an indication of the acceleration of the seed metabolism in order to cause the denaturation of proteins and, consequently, the deterioration of the seeds (Oliveira *et al.* 2014). Even with high temperatures occurring in the Caatinga, the seeds, because they present mostly thick integuments, are able to keep the internal structures unchanged until they have favorable conditions for germination. Associated with litter being a thermal insulation (Mateus *et al.* 2013).

The paper roll substrate did not present the structural characteristics of aeration and water retention capacity for germination (Silva *et al.* 2008), since the paper rolls remained inside plastic bags and kept inside the germinators, causing the creation of a microclimate inside the packaging. These conditions may have raised the temperatures and associated with high humidity, accelerated the degradation of the seeds, as well as in the treatment at 40 °C, in addition to low oxygenation due to the closed environment providing favorable conditions to the appearance of fungi (Mello & Barbedo 2007; Guedes & Alves 2011), a factor that adds to the deterioration of the seeds. Therefore, we concluded that the most suitable combination for germination of *Jatropha mollissima* seeds is the medium vermiculite substrate and temperatures of 30 or 35 °C.

## ACKNOWLEDGEMENTS

This study was carried out with the support of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Financing code 001. The authors also thank the Núcleo de Ecologia e Monitoramento Ambiental (NEMA/UNIVASF) and the Projeto Integração do São Francisco (PISF) for the collect and processing of the seeds used in this work.

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