






## SCIENTIFIC ARTICLE

# Germinative potential of 'Pata-de-Vaca' seeds at different maturation stages under various temperatures

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## Abstract

*Bauhinia monandra* Kurz has ornamental characteristics as the shape of its crown and the exuberance of its flowering, being and is widely cultivated in urban afforestation in Brazilian regions. The production of forest seedlings is essential not only to meet the demand of urban afforestation, but also to conserve forests. However, studies on *B. monandra* regarding the degree of fruit maturity on seed performance are scarce. Thus, the objective of the current work was to verify the germination potential of *B. monandra* seeds at different stages of maturation at five constant temperatures. The germination percentage, germination speed index (GSI), mean germination time (t), relative germination frequency, and mean germination speed (MGS) were evaluated. Initially, the mature seeds had a water content of 8.5% and the immature seeds of 68.3%. Mature seeds reached high germination percentages, above 96% for temperatures of 25, 30, and 35 °C. The immature seeds demonstrated low germination percentages, reaching the highest percentage of 33% for the temperature of 25 °C, followed by the temperatures of 30 °C and 35 °C, with respective percentages of 32% and 20%. Mature seeds demonstrated the highest GSI value (11.79) for the estimated temperature of 26.94 °C and immature seeds the highest value of t (7.85) for the temperature of 26.56 °C. It is concluded that mature seeds aggregate higher germination at an estimated temperature of 24.72 °C and that immature seeds present unsatisfactory germination performance.

**Keywords:** afforestation, *Bauhinia monandra* Kurz, germination percentage, physiological maturity.

## Resumo

### Potencial germinativo de sementes de pata-de-vaca de diferentes estádios de maturação sob temperaturas variadas

*Bauhinia monandra* Kurz possui características ornamentais como a forma de sua copa e a exuberância de sua floração, sendo amplamente cultivada na arborização urbana nas regiões brasileiras. A produção de mudas florestais é essencial não somente para atender à demanda da arborização urbana, mas também a conservação das florestas. No entanto, são escassos os estudos de *B. monandra* quanto ao grau de maturidade dos frutos no desempenho das sementes. Assim, o objetivo desse trabalho foi verificar o potencial germinativo de sementes de *B. monandra* de diferentes estádios de maturação em cinco temperaturas constantes. Foram avaliados o percentual de germinação, índice de velocidade de germinação (IVG), tempo médio de germinação (t), frequência relativa de germinação e velocidade média de germinação (VMG). Inicialmente as sementes maduras possuíam teor de água de 8,5% e as imaturas de 68,3%. As sementes maduras atingiram altos percentuais de germinação, superiores a 96% para as temperaturas de 25, 30 e 35°C. As sementes imaturas atingiram um percentual baixo de germinação, atingindo o maior percentual de 33% para a temperatura de 25 °C, seguido pelas temperaturas de 30 °C e 35 °C, com percentuais respectivos de 32% e 20%. As sementes maduras lograram o maior valor de IVG (11,79) para a temperatura estimada de 26,94 °C e as sementes imaturas o maior valor de t (7,85) para a temperatura de 26,56 °C. Conclui-se que sementes maduras agregam maior germinação sob a temperatura estimada de 24,72 °C e que as sementes imaturas tiveram desempenho insatisfatório na germinação.

**Palavras-chave:** arborização, *Bauhinia monandra* Kurz, maturidade fisiológica, porcentagem de germinação.

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## Introduction

Trees represent an extremely important element for adequate environmental management in cities, improving comfort requirements, as they influence microclimatic stability, promote a reduction in temperature and direct solar radiation, increase green coverage, and maintain biodiversity. Trees also represent a source of food and shelter for several species of fauna that coexist with the dynamics of the city, as well as having considerable potential to retain particles and polluting gases from the atmosphere and act as barriers, reducing wind speeds, and dampening the noises commonly found in the city (Silva and Oliveira, 2021).

Therefore, the production of forest seedlings, with quality and quantity, is essential not only to meet the demand of urban afforestation, but also to preserve forests and maintain the environment, for use in the recovery of degraded areas, formation of legal reserves, permanent conservation areas, and ecological corridors (Guariz and Guariz, 2020), in this sense, research and investigations of forest species are necessary.

Studies on forest species that involve methods related to the appropriate period for harvesting, processing, seed storage, vigor, and germination are becoming more frequent, in order to achieve greater success in seedling production projects and consequently in reforestation projects (Barros et al., 2021). Hereupon, the characterization of the appropriate stage of maturity for the collection of seeds at the right time is extremely important.

In this regard, the effects of abiotic factors that affect germination, such as temperature, must be considered. Temperature influences germination in a very expressive way and its variations affect the speed, percentage, and uniformity of germination, as well as the speed of water absorption and biochemical reactions that are inherent to the whole process. Thus, considering the relationship with these parameters, it is necessary to determine temperatures at which the process is most efficient, as well as the extremes (maximum and minimum) that can be tolerated by the seeds (Santos et al., 2022).

One of the species frequently used for afforestation of streets is *Bauhinia monandra*. Commonly found in Brazil, and popularly known as 'pata-de-vaca' or 'unha-de-vaca', the tree has remarkable ornamental characteristics, mainly the shape of its crown and the exuberance of its flowering. The species *B. monandra* has spread around the world due to its good landscape characteristics, with medium size, reaching up to 15 meters in height, an evergreen tree, with large rounded leaves, with two leaflets, moderate width canopy, flowers with good visual appearance, excellent for use in urban afforestation (Dapar, 2020). Although it is most commonly utilized in afforestation, *B. monandra* is also used as a medicinal plant, being a nutritional source of vitamin A, especially the seeds (Dapar, 2020).

Regarding the performance of *Bauhinia monandra* 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 information, it is necessary to define the appropriate time of collection during the maturation of the seeds, in order to facilitate and accelerate the production of seedlings in nurseries. Thus, the objective of the current work was to verify the germination potential of *B. monandra* seeds at different maturation stages under different temperatures.

## Material and Methods

The fruits were collected in September and October 2020 from 20 matrices that make up the urban afforestation in Londrina, northern Paraná State. The region's climate is warm and temperate with significant rainfall throughout the year, being classified as Cfa according to the Köppen classification, with an average temperature of 21 °C and an average annual rainfall of 1723 mm.

The visual indicators of fruits were used to determine the physiological maturity, based mainly on the changes in the epicarp color (Figure 1), as well as the water content of the seeds, which was determined as recommended by the Rules of Seed Analysis (Brasil, 2009). Fruit maturation stages were based on epicarp color, according to the MUNSELL color chart (Munsell, 1952), resulting in classifications 10Y 7/4 (immature fruit) and 5Y 6/6 (mature fruit).



**Figure 1.** Fruits (A) and seeds (B) immature and ripe of *Bauhinia monandra*.

The fruits were collected manually with the aid of a ladder and aerial pruning shears and all the seeds were removed from the fruits manually. The seeds were placed to germinate between two sheets of paper wrapped in the form of rolls and then placed in the germinator in a horizontal position and a volume of water 2.5 times the

weight of the paper was added to ensure the moisture maintenance (Brasil, 2009). Seed separation and sowing occurred successively and immediately, without intervals. Badly formed, discolored, necrotic, stained, or irregularly shaped seeds were sorted and properly discarded (Figure 2).



**Figure 2.** Seeds discarded or not used in the quantification of the germination process of *Bauhinia monandra*.

The design used was completely randomized in a 2×5 factorial arrangements, with two physiological maturity conditions (mature and immature) and five constant temperatures (15, 20, 25, 30, and 35 °C), distributed in four repetitions with 25 seeds each. The germination tests were carried out in germinators regulated (type B.O.D.) for constant temperature regimes, in the absence of light.

The experiment was ended on the 12<sup>th</sup> day, when the seeds showed stabilization of germination, ending the test on that day. At the end of the experiment, the following variables were calculated from the daily data on the number of germinated seeds, as suggested by Ferreira et al. (2022).

Germination was determined considering only normal seedlings, that is, those that showed the potential to continue their development and give rise to normal plants when developed under favorable conditions. Results were expressed as percentage of normal seedlings on the 12<sup>th</sup> day.

Daily counts were performed to determine the germination speed index (GSI), computing the number of protruded seeds, starting from the emission of 2 mm of radicle.

The average germination time (t) in days was performed simultaneously with the germination test, and the number of germinated seeds was counted daily.

Data were submitted to analysis of variance and, if significant, non-linear regression analyses were performed, considering the Brain-Cousens hormesis logistic model. The assumptions of normality of errors and homogeneity of variances were tested by Shapiro-Wilk and Bartlett, respectively. The analyses were performed with the aid of R software (R Core Team, 2021), using the software packages AgroR (Shimizu et al., 2022) and AgroReg (Shimizu and Gonçalves, 2021).

## Results and Discussion

The water content was 8.45% for mature seeds and 68.3% for immature seeds, with a considerable decrease in water content with advancing fruit maturation. Similar values were found by Antonelo et al. (2017), corresponding to 13% of the water content for mature seeds and 68% for green seeds of *Bauhinia forficata* Lindl. Lopes et al. (2007) found higher values of water content in mature seeds of *Bauhinia* spp., reporting up to 19% moisture.

According to Carvalho and Nakagawa (2012), the high moisture content, normally observed in the early stages of maturation, is due to the constant flow of water from the plant to the seed, as the seed requires abundant hydration to translocate, synthesize, or metabolize its reserves and, as maturation progresses, the seeds dehydrate until the stability of the percentage of moisture. As the maturation

process passes, this variable gradually decreases; while the opposite occurs with dry matter, in which the seeds increase their concentration with the course of maturation (Ristau et al., 2020).

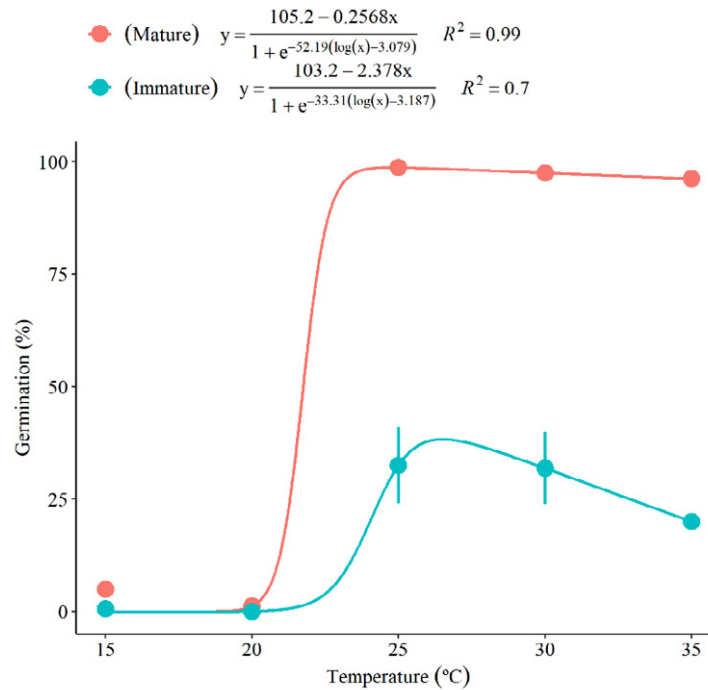
The color of the fruits and the water content were used as parameters to prescribe the stage of maturation of the seeds, since the fruits of pata-de-vaca change color during the development process, with this being an important index in the determination of the physiological maturity (Lorenzetti et al., 2018). Visual indicators of fruits and seeds can also be used to determine physiological maturity (Cruz et al., 2021; Duarte et al., 2020). These can be determined at the field level, using external changes in fruits and seeds, however, these indices must be combined with knowledge of the physical and physiological alterations.

In *Licania tomentosa* Benth. the seeds obtained from the yellow colored fruits resulted in a higher percentage and emergence speed index, when compared to the physiological quality of the seeds contained in the green, yellow-green, and dark-wrinkled fruits (Silva et al., 2018). In the study with seeds of *Allophylus edulis* (A. St.-Hil., A. Juss. & Cambess.) Hieron. ex Niederl. (vacum) at different stages of maturation, Kaiser et al. (2016) recommended that the fruits were harvested when they were red, regardless of the region, as they contained seeds with lower water content, coinciding with the maximum percentage and speed of seed germination, in addition to originating seedlings with greater size and biomass accumulation.

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 (Carvalho and Nakagawa, 2012; Silva et al., 2022). Thus, the pata-de-vaca seeds significantly reduced in size and changed color, becoming smaller, darker, and clearly harder and more rigid when compared to the green seeds.

Barros et al. (2021) report that the main criterion to be taken into account in the study of maturation should be the germination efficiency, since without this, the seeds have no value for sowing, making the quality of the seeds and the production of vigorous seedlings dependent on sowing.

Thereby, we observed that the immature seeds had an unsatisfactory performance in germination (Figure 3), demonstrating the highest germination percentage of 33% for the temperature of 25 °C, followed by temperatures of 30 °C and 35 °C, with respective percentages of 32 and 20%. Mature seeds demonstrated high germination percentages, above 96% for temperatures of 25, 30, and 35 °C. For both maturity conditions, temperatures of 15 and 20 °C were harmful to the seeds, providing very low percentages when compared to the other temperatures, being 5 and 1% for mature seeds and 1 and 0% for immature seeds, respectively.



**Figure 3.** Germination percentage of *Bauhinia monandra* seeds at different stages of maturation and temperatures.

This is different from the recommendation for *Hymenaea courbaril* Lindl., in which, according to Guariz et al. (2021), seeds capable of germination were obtained at all stages of fruit maturation (immature harvested from the matrix, mature harvested from the matrix, and mature harvested from the ground).

Regarding the alterations in the germination process that occurred as a result of the cold (temperatures of 15 and 20 °C), it could be seen that the stress caused by low temperatures led to damage to germination in all seeds evaluated, mature or immature, making germination unfeasible.

As reported by Cao et al. (2019), at the germination stage, the most commonly observed symptoms of cold damage are delay and decrease in germination percentage. Cold stress is largely related to the induction of oxidative stress, which leads to the generation of large amounts of reactive oxygen species, triggering lipid peroxidation reactions in membranes and compromising metabolic and cellular functions, causing significant damage to proteins, lipids, carbohydrates and DNA, and can even cause cell death. Temperature variation causes a decrease in germination speed and percentage and affects uniformity. This reduction is due to the slower imbibition of seeds, as well as the mobilization of reserves during the germination process (Marcos-Filho, 2015; Silva et al., 2021).

Marcos-Filho (2015) points out that although seeds in the early stages of maturation can germinate, as their structures are already formed, that is, the embryo is already morphologically formed, the highest germinations are achieved close to the maximum accumulation of dry matter. Furthermore, Ristau et al. (2020) working with seeds of *Albizia hasslerii* (Chod.) Burkart, as well as Silva et al. (2019) working with seeds of *Lophantera lactescens* Ducke, found higher percentages of germination in the

final stages of maturation and lower means when the fruits were green. The highest germination accumulation (99%) occurred for the estimated temperature of 24.72 °C for mature seeds, while for green seeds the best estimated temperature was 26.51 °C, with germination of 38%.

As observed in the current work, Nakagawa et al. (2010) report that seeds of *Peltophorum dubium* (Spreng.) Taub. with more accentuated decreases in water content, resulted in a decrease in dead seeds and the formation of abnormal seedlings, as well as improvement in the germination potential. According to the authors, dehydration, whether natural or permanently imposed, causes the seed to change the programmed expression of genes from maturation to the germination process, accompanied by redirection of metabolism towards germination and growth.

Sampaio et al. (2020) point out that the germination test conducted at 25°C with the batch of mature seeds of *Triplaris Americana* Lindl. presented significantly higher percentage means (42%) compared to the batch of immature seeds (0%), evidencing the importance of visual evaluation of seed maturation at the time of collection. As observed in the current work, Silva et al. (2019) found that the water content of seeds of *Lophantera lactescens* Ducke. reduced during the maturation process and the dry mass of the fruits increased during the process, as well as the germination and vigor of the seeds, concluding that the mature seeds maintained better development when compared to the green seeds.

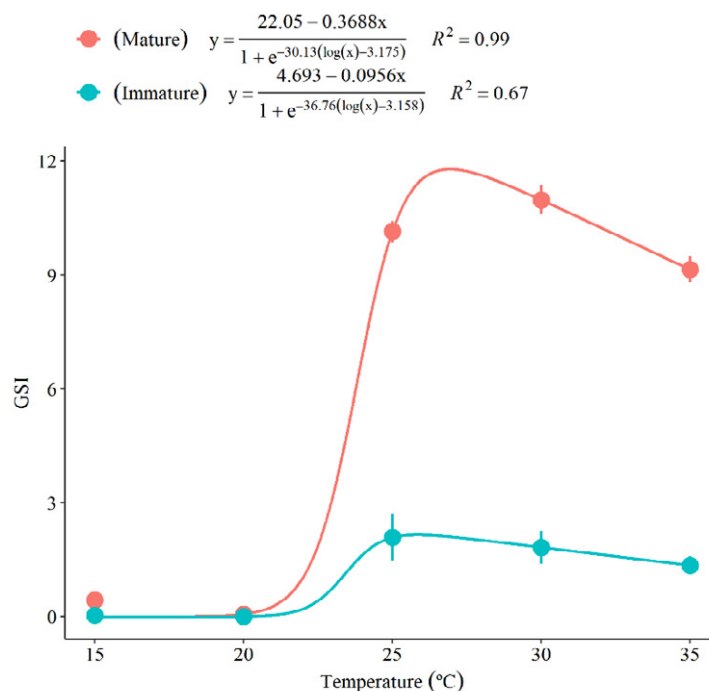
According to Brancalion et al. (2010) the temperature of 25 °C is optimal for seed germination of most Brazilian tree species, followed by 30 °C, in that these temperatures were considered as the most favorable in the germination process with 90.4% from a total of 272 forest species studied. This confirms the hypothesis that the optimal temperature for germination is related to the temperature



of the region of origin of the species at the favorable time for germination, constituting a physiological adaptation of the seeds to these environmental conditions.

As seen in Figure 3, the maximum germination occurred at a temperature of 24.72 °C, establishing this temperature as optimal for the process to occur with greater efficiency, thus, the optimal temperature is the one that allows the most efficient combination between the germination percentage and speed (Marcos-Filho, 2015). Therefore, as the temperature moved away from this optimal range, reducing in value, the germination percentage and speed drastically reduced.

In relation to GSI, treatments with seeds from mature fruits showed the highest values (Figure 4), with emphasis on the interval between 25-30 °C, according to the adjustment of the logistic model proposed by Brain-Cousens. Similarly, Costa et al. (2013) found that the temperatures with the highest values in relation to the germination percentage, germination speed index, and mean germination time for *Bauhinia forficata* Lindl. seeds are between 24.3 and 26.7 °C. The temperature that presented the maximum estimated response in the germination of *B. monandra* seeds (24.72 °C and 99% germination) was lower than the temperature that showed the highest GSI (26.94 °C and GSI of 11.79).



**Figure 4.** Germination speed index (GSI) of *Bauhinia monandra* seeds at different maturation stages and temperatures.

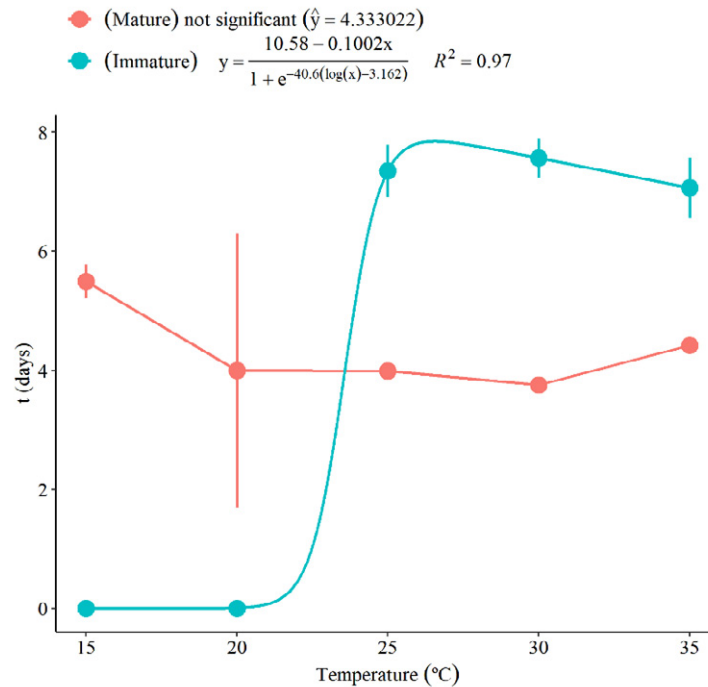
Regarding temperature, Otieno et al. (2020) describe that the temperature of 30-35 °C is suitable for conducting germination and vigor tests in seeds of *Tylosema fassoglense* (Kotschy ex Schweinf.) Torre & Hillc., and low (10 °C) and high ( $\geq 40$  °C) temperatures significantly reduced germination. In turn, Vitória et al. (2018) concluded that the seeds of *Schinus terebinthifolius* Raddi. extracted from ripe fruits showed superior physiological quality to the other stages evaluated, evidenced mainly at constant temperatures of 30 °C and alternating temperatures of 20-30 °C, which suggests that this is the ideal maturation stage for harvesting the fruits and extracting the seeds. The highest GSI (11.79) of mature seeds was reached at an estimated temperature of 26.94 °C, while for green seeds the GSI of 2.16 was reached at a temperature of 25.84 °C.

The speed at which the germination process occurs is fundamental for the survival and development of the species, as it reduces the time of exposure of the seed to adverse conditions and weather, being an unfavorable characteristic to the germination process, therefore, the shorter the mean

germination time, the higher the germination speed, which constitutes a good parameter for evaluating seed vigor (Rocha et al., 2018).

With different behavior to that observed in the current work, Santos et al. (2013) found that two araçá accessions (Y53 and Y52) when evaluated in the mature and immature condition did not present statistical differences for the evaluated characteristics of mean germination time, germination speed, and germination percentage, and the mature fruits presented the best GSI means. Still according to the authors, this demonstrates that seeds of fruits in their initial stage of maturation, have already reached physiological maturity and thus, this does not interfere with the germination process.

Regarding the temperature, we verified that it led to a reduction in the t of the mature seeds of *Bauhinia monandra* Kurz (Figure 5), as well as an increase in germination percentage and GSI. We observed that for green seeds, the highest t (7.85) was reached for the temperature of 26.56 °C, while for mature seeds there was no significant difference for the variable t.



**Figure 5.** Mean germination time (t) of *Bauhinia monandra* seeds at different maturation stages and temperatures.

#### Author Contribution

HRG, GDS, JCBP, HVS, and RYPM contributed equally to manuscript writing, data analysis and review.

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#### Conclusions

The mature seeds demonstrated high germination percentages, under the estimated temperature of 24.72 °C, this being around the ideal temperature for use for the production of seedlings. Immature seeds are not recommended for sowing.

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