https://doi.org/10.1590/0034-737X2024710044
ISSN: 2177-3491

# New understanding of factors influencing the seed germination of *Jacaranda micrantha* (CHAM.), an aesthetically appealing native species[1]

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[1] This research is part of a dissertation prepared by the first author (one of the chapters), under the co-supervision of Professors Fabiana and Rogério.

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**Editors:**
Diego Ismael Rocha
Ricardo Silva Santos

**Submitted:** October 18th, 2023.
**Accepted:** August 7th, 2024.

## ABSTRACT

The factors and their levels influencing the seed germination process are well-known for most traditional forest species. But, for J*acaranda micrantha*, a native and aesthetically appealing species, some factors influencing the germination process are not well described. So, we evaluated the effect of different light conditions and soaking on the germination of seeds. Four light intensity treatments (5, 25, 50, and 100%) and four soaking times were tested (0, 12, 24 and 48 hours). In both procedures, a completely randomized design was applied, with four treatments of four replicates containing 25 seeds each. Daily evaluations were performed to determine the germination percentage and the germination speed index (IVG). Seeds of *J. micrantha* presented a better germinative potential in lower light intensities, but more abnormal seedlings were observed at very low-intensity light (5%). The imbibition of seeds was efficient in accelerating the germination process, and the 12-hour soaking time in the water was the most efficient, contrary to what is described in the literature.

**Keywords:** Caroba, germination process, native species, seedling production.

## INTRODUCTION

Seed germination involves a sequence of physiological and biochemical events (Schmidt, 2007) influenced by internal and external factors, mainly light, temperature and humidity conditions (Leão *et al*., 2015). These factors influence percentage, speed, and uniformity of germination, which affects seedlings quality and production costs (Dutra *et al*., 2016). Thus, information on seed germination patterns under different environmental conditions is crucial to understanding the ecological dynamics, as they determine seedling growth potential at each ecosystem (Figueroa & González, 2018; Gonçalves *et al*., 2020).

Regarding external factors, seed imbibition is the germination starting process (Schmidt, 2007) inducing tissues rehydration, which intensifies the metabolic activities, and increases energy and nutrients supply for embryo growth (Marcos Filho, 2015). Imbibition is an important procedure adopted to enhance speed and standardization of the germination process since water availability contribute to germination, as observed for different forest species (Maffra *et al*., 2011; Ataíde *et al*., 2016; Valdovinos *et al*., 2021), helping seeds to complete, partially or completely, the first phase of the germination process (Bewley & Black, 1994).

Another external factor influencing germination is the light environment, which can stimulate or inhibit seed development depending on the quality, intensity, and duration of light irradiation (Aud & Ferraz, 2012). Moreover, response to light change among species, as some have seeds that germinate equally well, both in light and dark, while others germinate better only in light or dark (Chanyenga *et al*., 2012; Taiz *et al*., 2017).

The effect of seed imbibition and their responses to different light intensities have been the subject of studies for different forest species to characterize the germination process. Regarding the effect of light on germination, *Jacaranda mimosifolia* (Bignoniaceae) seeds proved to be adapted to different light conditions (Socolowski & Takaki, 2004). Similarly, *Anadenanthera peregrina* (Fabaceae) presents a broad environmental spectrum for germination, going from full sun to 70% shaded (Fernandes *et al*. 2018). On the other hand, species such as *Platymiscium floribundum* (Fabaceae) show a greater germination vigor only in the presence of light (Oliveira *et al*., 2005; Alves *et al*., 2016). In this context, considering the large number of species and the recent advances in seed germination tests in Brazil (Pereira *et al*., 2022), understanding the effect of light and water resources on germination is necessary since these factors vary for different species and play a role on tree seedling establishment (Stork *et al*., 2009; Bhadouria *et al*., 2016).

An important species of the Mixed Ombrophilous Forest, an ecosystem inserted in the Atlantic Forest Biome, with great aesthetic appeal for planting in cities, due to its beautiful flowering, and for the recovery of degraded areas (Saueressig, 2014) is *Jacaranda micrantha* (Cham.) (Bignoniaceae), popularly known as Caroba. Despite its importance, germination studies for the species and other Bignoniaceae species are focused on temperature and substrate analysis, indicating temperatures between 25 °C and 30 °C combining different substrates, reaching a germination rate of 74% on vermiculite substrate (Ramos & Bianchetti, 1983; Ferraz & Varela, 2003; Scalon *et al*., 2006; Bovolini *et al*., 2015; Lunkes & Franco, 2015; Ribeiro *et al*., 2018).

Thus, the study of factors affecting seed germination is critical for native species of Atlantic Forest, which can potentialize their use in reforestation programs and contribute to develop gemination test methods (Pereira *et al*., 2022). Hence, this research aimed to evaluate the germination potential of *Jacaranda micrantha* seeds under different light intensities, as well as to analyze the effect of seed water imbibition on the germination process.

## MATERIAL AND METHODS

### *Plant material, seed collection and seed processing*

Five matrices of *J. micrantha* were selected through phenotypic evaluation considering tree height, stem diameter, plant health and vigor. The matrices were in different forest fragments representative of the Mixed Ombrophilous Forest ecosystem, in a rural area of the municipality of Palmeira, southeastern of Paraná State, Brazil (Figure 1).

Seed collection was performed as soon as fruits discoloration and spontaneous opening started. The fruits were collected in February with a pole pruner and then dried in the sun to release the seeds (Saueressig, 2014). We proceeded to the identification and separation of the seeds from each selected matrix. All the experimental analysis was carried out at the Universidade Estadual do Centro-Oeste, Campus Irati (UNICENTRO).

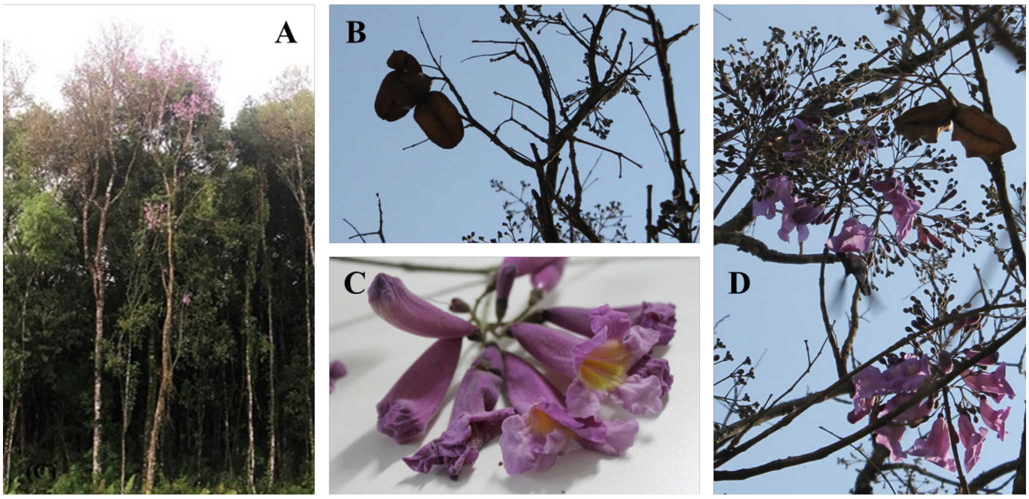

**Figure 1**: A tree from *Jacaranda micrantha* in the border of a forest fragment in Palmeira, Paraná, Brazil (A), with images from its fruits (B, D) and flowers (C, D).

### *Luminosity, imbibition, and germination evaluations*

For the evaluation of photoblastic responses, the germination experiment was carried out with 400 seeds divided into four light treatments. For each treatment, 100 seeds were incubated in four gerbox-type boxes (25 seeds in each box) of crystal polystyrene with 121 cm². The seeds were covered with a thin layer of commercial substrate based on bio-stabilized pine bark, previously sterilized in an autoclave at 1.0 Kgf.cm$^2$ for 30 minutes.

The gerboxes were placed on a suspended basis in a greenhouse at an average temperature of 25 ± 3 °C, humidity controlled by irrigation via intermittent fogging (RH ≥ 80%) and photoperiod according to environmental variation during the months of February and March, in Irati, South Brazil. Over the germination boxes we installed sun shading screens, composing four shading treatments:

- T1: Uncovered gerboxes in a 100% of light exposure;
- T2: Gerboxes covered with shading screen, in a 50% of light exposure;
- T3: Gerboxes covered with shading screen, in a 25% of light exposure;
- T4: Gerboxes covered with shading screen, in a 5% of light exposure.

The irradiance corresponding to each treatment was measured through the average of three light intensity measurements at different day times (8:00, 12:00 and 16:00 h) performed with a light intensity meter (luxmeter - LD-205A).

We evaluate the imbibition effect on germination by soaking seeds in deionized water for 0 (control), 12, 24 and 48 h and placing them in gerbox-type acrylic boxes containing the same commercial substrate described previously. Subsequently, they were allocated for germination in a BOD incubator, adjusted to 25 ± 1**ºC,** in a 12-hour photoperiod and 80% humidity.

For both experiments, evaluations were performed daily to describe the variables germination percentage and germination speed index (GSI). For this, we considered germinated seeds those that presented normal seedling formation (Figure 2), with the opening of the cotyledons and the appearance of hypocotyl (Bovolini *et al*., 2015). We considered as abnormal those seedlings that did not present the essential structures. The GSI was determined according to the equation proposed by Maguire (1962):

$$GSI = \left(\frac{G1}{N1}\right) + \left(\frac{G2}{N2}\right) + \left(\frac{G3}{N3}\right) + \ldots + \left(\frac{Gn}{Nn}\right) \quad (1)$$

where: *GSI*: germination speed index; *Gn*: number of seedlings computed in the first, second, third and last count; *Nn*: number of days from sowing to the first, second, third and last count.

### *Experimental design, evaluations, and statistical analysis*

The experiments were carried out in a completely randomized design with four replications of 25 seeds per treatment. The experiment evaluations were conducted until the germination process ceased. Thus, for the light intensities the experiment lasted 30 days, while the experiment for different imbibition times lasted 40 days. We applied the analysis of variance (ANOVA) to each experimental data after checking the homoscedasticity of the residues. Means were compared by Tukey's means test at a 95% confidence interval, using R statistical software (R Core Team, 2020).

## RESULTS

Germination under the different light environments started on the 8th day after sowing and the experiment was completed on the 30th day when germination process ceased. Among the treatments, the seeds that received 5, 25 and 50% light started their germination on the 8th day, whereas the seeds on 100% light treatment, started germination on the 9th day, with all the treatments reaching a plateau on the 24th day. At the end of the experiment, the number of germinated seeds was higher in treatments with light intensity reduced (5%, 25% and 50%) when compared to the 100% light intensity (Figure 3).

There was a significant effect (Table 1) of light intensities on the germination percentage (p-value = 0.0141) and GSI (p-value = 0.005) variables. The percentage and germination speed of *J. micrantha* seeds were higher at lower light intensities (Figure 4). The 5% light intensity treatment promoted a higher germination level of *J. micrantha*, reaching 79%, but with no difference (p-value > 0.05) from the treatments with 25% and 50% light (Figure 4A). However, despite the low light intensity (5%) treatment has enhanced the number of germinated seeds, we observed etiolated seedlings. Similarly, greater GSI indexes were observed in the shaded environments (Figure 4B).

Concerning the seed imbibition in water, we observed that treatments accelerated the starting point of the germination process. Seeds soaked for 12 and 48 hours started germinating on the 4th day after sowing, while unimbibed seeds started germinating on the 10th day (Figure 5). Treatments influenced the germination speed index (*p*-value = 0.0449), while seed germination percentage was not influenced by imbibition (*p*-value = 0.2407), although we observed the greater the time of imbibition the lowest was the germination percentage (Table 2).

## DISCUSSION

The light exposure influenced the germination percentage (G%) and the germination speed index (GSI) suggesting that luminosity can be a critical factor in the germination process of *J. micrantha* seeds. According to the results, the best conditions for germination of *J. micrantha* are at 25% and 50% light intensities, reaching a germination rate of 74%, This germination rate are similar to those observed by Lunkes & Franco (2015) and Bovolini *et al*. (2015) for *J. micrantha* seeds in different substrates, but with no light exposure variations. However, the conditions evaluated in our study promoted an earlier germination start. In a full sun environment, however, the germination was reduced, whereas, in limited light condition, the seedlings were visually etiolated.

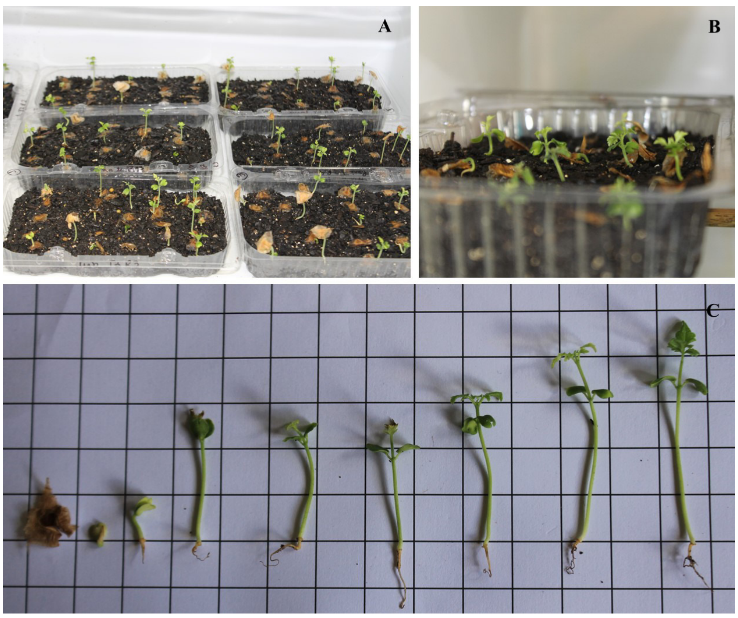


**Figure 2**: The germination process of *Jacaranda micrantha* seeds, with the seeds placed in the germination test after soaking treatments (A) showing the complete formation of seedlings (B) and the evolution of seedlings' development (C)

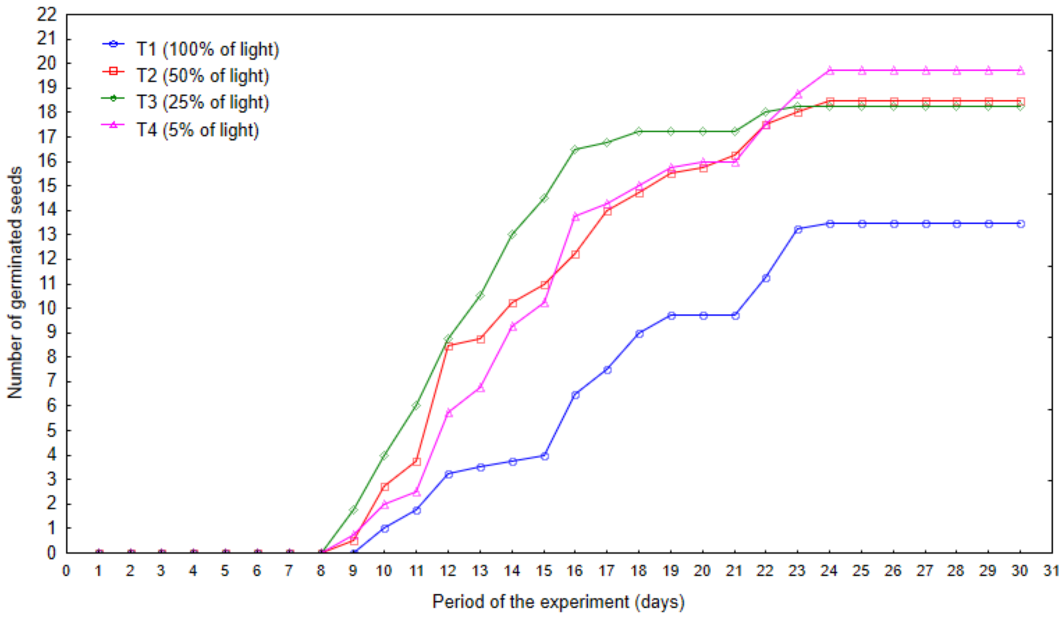


**Figure 3**: Cumulative germination of *Jacaranda micrantha* seeds in different light intensities exposure during the germination test (30 days) in greenhouse conditions.

**Table 1:** Analysis of variance for the germination percentage (G%) and germination speed index (IVG) for *Jacaranda micranta* seeds, due to different light intensities (5, 25, 50, 100%), and different soaking times (0, 12, 24 and 48 h)

| SV | Light intensities | | | Soaking times | | |
|---|---|---|---|---|---|---|
| | DF | Mean Square | | DF | Mean Square | |
| | | *G %* | *IVG* | | G% | IVG |
| Treatments | 3 | 482,666* | 0,206** | 3 | 246,666$^{ns}$ | 0,068* |
| Residues | 12 | 90,00 | 0,028 | 12 | 154,000 | 0,018 |
| Total | 15 | | | 15 | | |

*Significant at 5% probability. **significant at 1% probability. $^{ns}$ not significant

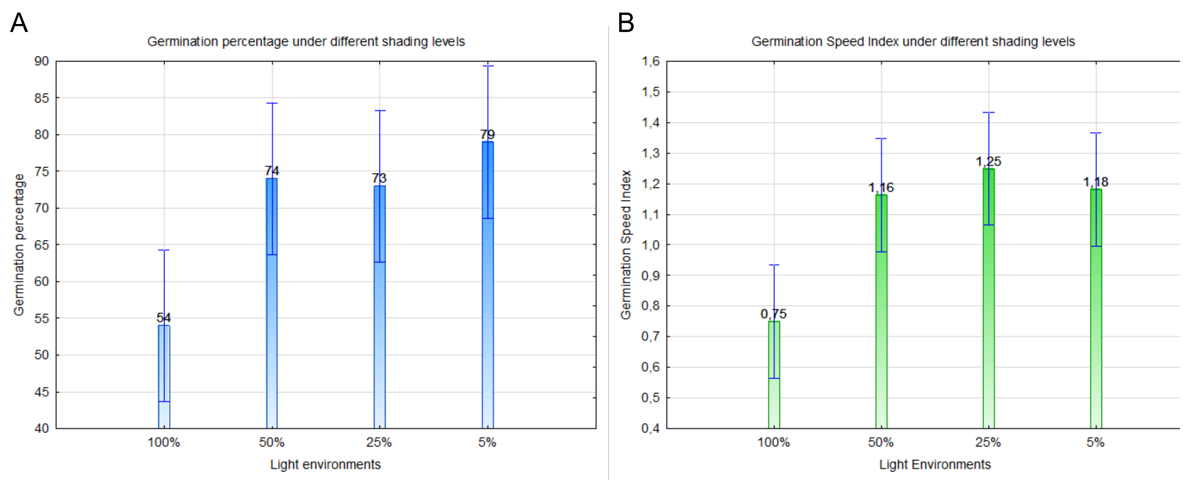


**Figure 4**: Average values for each light intensity treatment (100%, 50%, 25% and 5%) for the variables (A) germination percentage (G%) and (B) germination speed index (GSI) for seeds of *Jacaranda micrantha*. Means followed by the same letter do not differ significantly from each other at a 95% confidence interval.

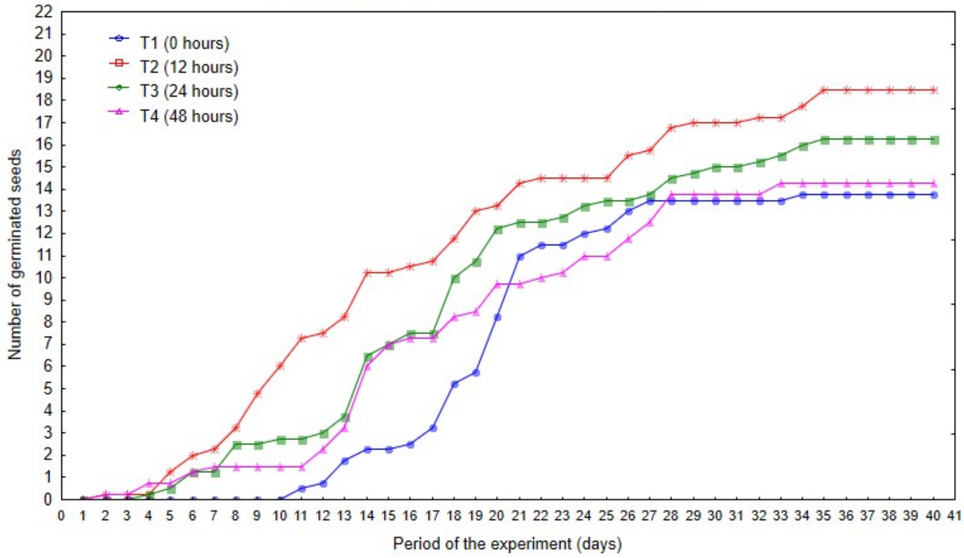


**Figure 5**: Cumulative germination of *Jacaranda micrantha* seeds in different imbibition times during the germination test (40 days) in BOD.

**Table 2:** Means test for germination percentage (G%) and germination speed index (GSI) for *Jacaranda micrantha* seeds, in relation to different seed imbibition treatments (0, 12, 24, 48 h)

| IMBIBITION TIMES (hours) | G% | IVG |
|---|---|---|
| 0 | 58,0 | 0,56 b |
| 12 | 74,0 | 0,86 a |
| 24 | 65,0 | 0,69 ab |
| 48 | 54,0 | 0,61 ab |

Note: means followed by the same letter do not differ from each other at a 95% confidence interval.

Based on the germination responses to the light intensities, the seeds of *J. micrantha* can be classified as neutral photoblastic since germination occurred equally in different light intensities. The lack of photosensitivity is characterized by no significative variation in total and speed of germination in different temperatures (Demuner *et al*., 2008). This suggests that the germination process is controlled by the phytochrome A, which characterizes the potential of seeds to germinate under light and darkness (Takaki, 2001; Holanda *et al*., 2015). However, although the light is not the limiting factor for germination of *J. micrantha* seeds, other factors may harm germination, such as temperature and water availability, that are deleterious when considered together (Locardi, 2011).

In the treatment that provided the highest level of shading, there was more abnormal seedlings (data not provided), because, in the absence of light, plants promote more stem growth, with etiolation, due to the high auxinic activity (Carvalho *et al*., 2009). This compromises the stability of the aboveground part, justifying the greater fragility of seedlings that germinate in the absence of light (Alves *et al*., 2016). Thus, even though seeds of *J. micrantha* germinate in any light environment (neutral photoblastic), seedlings demonstrated better development while growing in partially dark environments. Due to the light-sensitive, seeds change to the non-dormant stage after imbibition (Schmidt, 2007), and according to our results the *J. micrantha* seeds are, to some degree, dependent on light to enhance germination.

Considering that *J. micrantha* is a pioneer to early secondary species (Carvalho, 2012), the results obtained in this research suggest that the germination potential is influenced by the environmental conditions in which seeds are presented. As stated by Aud & Ferraz (2012), environmental conditions and seed size influence the germination of pioneer species, so that, under constant temperature, some seeds germinate under diffused light or in the dark. Furthermore, small seeds need light stimulation for germination to start and larger seeds do not depend so much on light exposure, but on the alternation of temperature as a stimulus for germination (Bovolini *et al*., 2015). In this sense, the results of this research suggest a divergent characteristic from the statements of Aud & Ferraz (2012), since *J. micrantha* presents small-sized seeds that germinate under light and dark conditions.

Similar to what was observed in the present research, seeds of other forest species are not sensible to light intensity, like *Jacaranda mimosifolia* (Socolowski & Takaki, 2004), *Jatropha curcas* (Silva *et al*., 2016) and *Mimosa bimucronata* (Melo *et al*., 2018). These species are considered neutral photoblastic, and this characteristic can promote a greater tendency to the survival and perpetuation of the species through germination in different light exposure conditions.

The light intensities of 25% and 50% promoted better results and environments with these light intensities represent the best condition for germination and seedling development, which is optimal to obtain the highest percentage of germination and normal seedlings of *J. micrantha*. Therefore, the species requires higher intensity of extreme red light for germination and good seedling development. This characteristic poses a challenge for the species' development in open-grown environments, primarily due to the abundant presence of red light throughout most of the day. In contrast, understory environments exhibit a higher proportion of extreme red light, partly due to its absorption by the tree canopy (Borghetti & Ferreira, 2004). This unique light environment can be further affected by the presence of litter, inhibiting germination in tropical forests (Vázquez-Yanes *et al*., 1990). In this context, the imbibition process becomes a limiting factor for light-sensitive seeds, as they exit their dormant stage only upon water uptake. Furthermore, seeds buried deep within the substrate receive an increased amount of far-red light, which also acts as a hindrance to germination (Schmidt, 2007).

On the other hand, following hydration, the seeds of many species are stimulated for germination by light. High-red or far-red light promotes the activated form of phytochromes (Allen *et al*., 2007), leading to an increase in bioactive gibberellin content (Hilhorst, 2007). In this regard, the light environments tested in this study may have promoted the germination of *J. micrantha* to some extent. For *Zeyheria tuberculosa* (Vell.) Bur., a forest species also belonging to the Bignoniaceae family, the presence or absence of light, as well as the imbibition process, had no effect on the germination of the species (Lima, 2003). In contrast, seeds of *J. micrantha* responded to the different conditions tested, allowing for the selection of optimal conditions for germination and seedling production. The positive effect of light is also observed in dry tropical forests, where shading has a greater impact on germination than watering (McLaren & McDonald, 2003).

In relation to pre-soaking treatment in water, imbibition during 12 h was suitable to promote *J. micrantha* germination, since it accelerated and triggered the germination process. The percentage of germination achieved with the 12-hour soaking surpasses the patterns observed by Wielewicki *et al*. (2006) and Bovolini *et al*. (2015) which obtained an average germination of 60% for the species. The GSI reduces with seed aging, being the index observed in the 12 h imbibition is in line with the GSI observed by Souza *et al*. (2016) for *J. micrantha* seeds stored for 11 months.

The optimal imbibition period observed in this study is similar to the pre-conditioning period of 16 hours reported for *Handroanthus spongiosus* (Bignoniaceae), which facilitated tegument removal and the entry of the tetrazolium solution into the tissues (Silva *et al*., 2023). Furthermore, it has been observed that germination in forest species of the Bignoniaceae family decreases under water stress (Valdovinos *et al*., 2021). This suggests that the absence of imbibition can hinder the germination of *J. micrantha*. Therefore, a 12-hour water imbibition period is recommended to enhance the seed germination of this species.

The positive effect of soaking *J. micrantha* seeds in water can be attributed to the requirement for a high-water content over a specific period to initiate the germination process. This process involves the mobilization of reserves, cell respiration, ATP production, protein and mRNA synthesis, as well as the readjustment of the cell membrane. These processes collectively culminate in root protrusion, directly impacting the time and speed of emergence (Bradford, 1986; Castro *et al*., 2004; Borghetti, 2004). This result is commonly observed in forest species, where water imbibition tends to accelerate the germination process, as seen in *Dalbergia nigra* (Ataíde *et al*., 2016), *Trichilia claussenii* (Maffra *et al*., 2011), *Parkia multijuga* (Calvi *et al*., 2008) and *Bowdichia virgilioides* (Gonçalves *et al*., 2008).

In contrast, prolonged imbibition tends to decrease seed viability in *J. micrantha*. These seeds are considered intermediate in relation to desiccation and storage, and they may be damaged by extended imbibition (Wielewicki *et al*., 2006). Similar observations have been made for related species like *Jacaranda cuspidifolia*, where excessive humidity can interfere with seed germination (Martins *et al*., 2008), and for *Handroanthus spongiosus*, where imbibition for more than 18 hours impairs seed quality, leading to observable dark spots on the seeds (Silva *et al*., 2023). Additionally, imbibition has been associated with seed deterioration after periods longer than 8 hours, particularly in the case of *Handroanthus spongiosus* (Rizzini) S. Grose.

Another technical aspect regarding *J. micrantha* germination and seedlings production, is the substrate humidity condition, that according to the normative instruction number 44 of the Agriculture Ministry (Brasil, 2010), the substrate must be dryer than usual for germination tests. As a result, a dryer substrate may reduce the imbibition time, which meets the results observed herein for an imbibition period of 12 hours in comparison to longer periods. Thus, defining an imbibition limit on pre-conditioning treatment is a significant aspect regarding seed germination success, influencing seed staining with tetrazolium for other tests as well (Silva *et al*., 2023).

Moreover, as noted by Wuebker *et al*. (2001), seed imbibition can lead to deterioration due to the disruption of various physiological mechanisms. These include issues like ethanol toxicity, reduced oxygen availability, and the accumulation of carbon dioxide, which can collectively cause damage and ultimately result in seed death. Such damage typically occurs when seeds undergo rapid water absorption, hindering the complete restructuring of the cell membrane system and leading to increased leaching of cell contents. These effects, as observed by Castro *et al*. (2004), can reduce seed performance during subsequent stages of the germination process. Additionally, it's worth noting that while water availability is vital for processes like tegument rupture, embryo growth, and the preparation for radicle emergence, it alone cannot account for gene and metabolism activation in seeds (Nonogaki *et al*., 2007).

Prolonged imbibition and the absence of imbibition both resulted in reduced germination percentages. However, when seeds were soaked for 12 hours, germination reached 74%, which was 16% higher than seeds that were not soaked. *J. micrantha* exhibits better Germination Speed Index (GSI) rates at temperatures between 25°C and 30°C (Bovolini *et al*., 2015). These conditions can be achieved under intermediary shading levels, which appear to provide the most balanced conditions for the germination of the seed bank (Cordeiro *et al*., 2021). Therefore, environmental conditions, especially the level of shading, may indeed play a crucial role in seed bank germination.

According to Fonseca *et al*. (2006), the success of reforestation and planting activities in natural forests is closely linked to understanding the light requirements of different species. This environmental factor plays a crucial role in promoting seed germination and facilitating seedling development, depending on the species. Additionally, the availability of water is another key factor influencing the success of forest species in various environments, affecting their growth and biomass allocation (Ferreira *et al*., 2015). In this context, the seeds of *J. micrantha* performed improved germination under moderately shaded environments. This suggests their ability to germinate in areas at different successional stages, which is a crucial factor for the management, conservation, and long-term survival of the species.

## CONCLUSIONS

*Jacaranda micrantha* seeds can be classified as neutral photoblastic, as they exhibit the ability to germinate in various light environments. Specifically, germination is enhanced in shaded environments with solar incidence levels between 25% and 50%, coupled with a 12-hour seed imbibition. This suggests the species' adaptation to shaded environments under the forest canopy or in production conditions that mimic shading.

## ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

The authors are grateful for the financial support of the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) for the master's scholarship provided to the first author.

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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