Tetrazolium and interaction of temperature and light under seed germination in *Ormosia arborea* (Fabaceae)

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**ABSTRACT**

The study aimed to define the best conditions to conduct germination tests for *Ormosia arborea* seeds and assess the viability of seeds using the tetrazolium test. The germination tests were conducted at temperatures of 10, 15, 20, 25, 30, and 35 °C and alternating temperatures of 20-30 °C, in the presence or absence of light. For the tetrazolium test, seeds were immersed in tetrazolium solution 0.075%, at 35 °C for three hours, and then the viability was evaluated. It was verified that the seeds did not present absolute photosensitivity. The tetrazolium test was effective in separating the seeds into two categories concerning seed viability, viable and nonviable. It was concluded that *O. arborea* seeds germinate in the presence or absence of light in a wide range of temperatures. Temperatures of 25 and 30 °C and alternating temperatures of 20-30 °C are indicated to conduct germination tests. The tetrazolium test was effective in assessing seed viability.

**Keywords**: Olho de cabra, Tetrazolium test, Native species.

**Tetrazólido e interação temperatura e luz na germinação de sementes de *Ormosia arborea* (Fabaceae)**

**RESUMO**

O estudo teve como objetivo definir as melhores condições para os testes de germinação de sementes de *Ormosia arborea* e avaliar a viabilidade das sementes utilizando o teste de tetrazólido. O teste de germinação foi realizado nas temperaturas de 10, 15, 20, 25, 30 e 35 °C e em temperaturas alternadas de 20-30 °C, na presença ou ausência de luz. Para o teste de tetrazólido, as sementes foram imersas em solução de tetrazólido 0,075%, a 35 °C por três horas, e a viabilidade foi avaliada. Verificou-se que as sementes não apresentaram fotosensibilidade absoluta. O teste do tetrazólido foi eficaz na classificação das sementes em duas categorias, quanto à viabilidade, viável e não viável. Concluiu-se que as sementes de *O. arborea* germinam na presença ou ausência de luz em uma ampla faixa de temperaturas. São indicadas as temperaturas de 25, 30 °C e alternada de 20-30 °C para o teste de germinação. O teste de tetrazólido foi eficaz na avaliação da viabilidade das sementes.

**Palavras-chave**: Olho de cabra, Teste de tetrazólido, Espécies nativas.
1. Introduction

*Ormosia arborea* (Vell.) Harms belongs to the Fabaceae family and is popularly known in Brazil as olho-de-cabra. Mainly found in the Atlantic rainforest and the broadleaf semideciduous forest, this species occurs in Bahia and Minas Gerais states and from Mato Grosso do Sul to Santa Catarina (Lorenzi, 2000). The trees are used for urban forestation in streets and avenues besides degraded lands (Lorenzi, 2000).

*O. arborea* is a native species propagated by seeds, and the success in seedling production depends on the knowledge regarding the germination process and seed quality. The germination process is influenced by external factors that include temperature, water, oxygen, and internal factors such as dormancy (Marcos-Filho, 2015). *O. arborea* seeds exhibit primary dormancy, which is characterized as the expression of dormancy as soon as its development is complete; that is, when harvested, the seeds present this type of dormancy (Lorenzi, 2000; Silva et al., 2014; Naves et al., 2018). According to Silva and Morais (2012), the most efficient method to overcome this dormancy is the chemical scarification in sulfuric acid for 20 minutes.

Temperature is a factor that influences the speed and uniformity of germination because it affects biochemical reactions that are involved in the process. The optimal germination temperature of a given species is when a higher percentage of germination occurs in the shortest time (Bewley and Black, 1982; Fenner, 2000; Marcos-Filho, 2015). In general, temperatures too high or too low inhibit this process. Hence, each species presents an optimal germination temperature related to climatic conditions from where the species naturally occur (Valadares and Paula, 2008).

In many species, seed germination is stimulated by light, whereas in other species, the light may inhibit germination. Seeds that require light for germination are positively photoblastic. Those that germinate better when there is a light limitation are negatively photoblastic, and those that are indifferent are lightly insensible (Lopes et al., 2005; Merai et al., 2019).

The ideal conditions for conducting the germination test, regarding temperature, light, and substrate, must be identified, aiming to identify the best conditions for the seeds of each species. Combinations of light and temperature must be assessed, and by doing so, one can gain insight into the species biology and its needs in the field.

The germination test has been the most widely utilized method to evaluate seed viability. However, for native species, germination might require longer periods. For example, in *O. arborea*, the mean germination time, when evaluating different methods for overcoming seed dormancy, ranged from eight to 14 days (Silva et al., 2014). Therefore, it is important to develop and improve quick methods that evaluate seed viability and characterizes its physiological potential.

The tetrazolium test stands out from other tests as a method for evaluating seed viability and vigor based on the enzymatic activity of the dehydrogenase enzyme group in live tissues (Costa and Santos, 2010). This test is faster than the germination test and provides a diagnosis of seed quality (França-Neto and Krzyzanowski, 2019). In this sense, some researches had been carried out with forest species defining the methodology for conducting the tetrazolium test, such as in *Leucaena leucocephala* (Lam.) (Costa and Santos, 2010), *Helianthus annuus* (Silva et al., 2013), *Plinia cauliflora* (Hössel et al., 2013), *Acca sellowiana* (Sarmento et al., 2018), *Eugenia brasiliensis*, *Eugenia pyrifor mus* (Lamarca and Barbedo, 2014), *Tabebuia roseoa lba* (Abbad and Takaki, 2014), *Eugenia uniflora* (Kaiser et al., 2014), *Apuleia moral is* (Reis et al., 2016) and *Jatropha curcas* (Araújo et al., 2019).

Considering the lack of information regarding optimal temperature, photoblatism, and the efficiency of the tetrazolium test for *O. arborea* seeds, this study aimed to evaluate the germination of seeds under different temperature and light conditions and assess seed viability by the tetrazolium test.

2. Material and Methods

Seeds of *O. arborea* were collected from parent plants located in the left margin of the Ivinhema river (22°03′04.5″ S; 53°41′28.2″ W), in Nova Andradina, Mato Grosso do Sul state, in August of 2011 and stored for two years in a refrigerator at 10°C.

Initially, to overcome dormancy, seeds were chemically scarified with sulfuric acid for 20 minutes and subsequently washed in running water for 5 minutes (Silva and Morais, 2012). Non-scarified seeds were used as a control group in each temperature.

In sequence, seeds were distributed between three paper towels (two paper towels underneath and one over the seeds) and wetted with distilled water. The proportion of water was 2.5 times the dry weight of the paper. The paper towels sheets were then turned into rolls and were wrapped and kept in a germination chamber for up to 120 days. The sheets of paper towels were changed whenever necessary and were hydrated as needed to keep the substrate always moist.

The seeds were allowed to germinate under constant temperatures: 10, 15, 20, 25, 30 and 35°C in the presence of light (12 hours daily) and absence of light and, under alternating temperatures of 20-30°C (12 hours daily at 30°C in the presence of light, and at 20°C in the absence of light).

In the absence of light, the germination tests were conducted in a dark room, using a green light during the
evaluation of these treatments. For each temperature (10, 15, 20, 25, 30, 35 ºC and 20-30 ºC) and light conditions (presence and absence of light), four replicates with 50 seeds each were utilized.

The number of seeds showing primary root emissions (seeds that showed a primary root of at least 2 mm of length) and the number of germinated seeds (normal seedlings, showing all the essential structures well developed) were registered daily. The mean germination time, the mean germination speed, and the relative frequency of germination were calculated using the daily data. The mean germination time and the mean germination speed were calculated according to the following formulas (Labouriau and Valadares, 1976):

\[ t = \frac{\sum_{i=1}^{k} ni \cdot ti}{\sum_{i=1}^{k} ni} \]

Where \( t \) = mean germination time; \( n_i \) = number of germinated seeds per day; \( t_i \) = time (days).

\[ V = \frac{1}{t} \]

Where \( V \) = mean germination speed; \( t \) = mean germination time.

The relative frequency was calculated according to Labouriau and Pacheco (1978):

\[ F_r = \frac{n_i}{\sum_{i=1}^{k} n_i} \]

Where \( F_r \) = relative frequency of germination; \( n_i \) = number of germinated seeds per day; \( \Sigma n_i \) = total number of germinated seeds.

The criteria used to classify seedlings as having normal features are described in Brasil (2009). For the studied species, seedlings showing the absence of one of the eophylls were considered abnormal.

The tetrazolium test was conducted to analyze its applicability in evaluating the seed viability of \( O. \ arborea \). Currently, there is no information about the tetrazolium test for this species in the literature. A total of 300 seeds was mechanically scarified with 100-grit sandpaper and subsequently immersed in distilled water for 120 hours in B.O.D., at 25 ºC, to assure that all seeds were fully imbibed.

Seeds of three replicates of 100 seeds had their coat removed and were longitudinally cut. Each replicate was immersed in a tetrazolium solution (2,3,5-triphenyltetrazolium chloride), at a concentration of 0.075%, and kept in an incubator at 35 ºC, for three hours. After that period, seeds were washed with running water and left submerged in water until the evaluation moment.

The seeds were classified as viable or unviable according to characteristics observed and, in the patterns indicated by Moore (1972), considering the integrity of the tissues, location, extension, and intensity of the color and the presence of milky-white areas concerning the essential areas for the development of the embryo (hypocotyl-radicle axis and cotyledons). In this way, the bright red or bright pink colors were related to the living and vigorous tissues, the crimson red to the deteriorating tissues, and white or yellowish to the dead tissues. Seeds were considered viable when the embryo presented bright pink and bright red coloring, milky-white seeds were considered unviable, as well as seeds showing embryo parts with milky white color. The results of the tetrazolium test were compared to the germination test.

**Statistical design.** The experiment was carried out with a completely randomized design in a 2x7 factorial scheme. Two light conditions (presence and absence of light) and seven temperatures (10, 15, 20, 25, 30, 35, and alternate 20-30 ºC) were evaluated. The percentage data (seeds showing primary root emission and germinated seeds) were transformed using the function arcsen \( \sqrt{x/100} \). When significant in ANOVA, the means obtained for the temperatures were compared by Tukey test at 5% probability. The F test compared the means obtained for the treatments with the presence and absence of light at a 5% probability.

### 3. Results and Discussion

In intact seeds of \( O. \ arborea \) (control), the maximum percentage of seeds showing primary root emission was only 2%, and for this reason, their data was not included in the analysis. These results agree with Silva et al. (2014), the species has tegumentary dormancy, and to germinate these seeds, this dormancy needs to be broken.

The chemical scarification method resulted in a higher percentage of seeds showing primary root emission (above 70%), proving the efficacy of the method to overcome seed dormancy (Table 1). Furthermore, these seeds were stored for two years before the germination test. This result shows the importance of dormancy for the survival of this species, which maintained high viability even after the storage period. Moreover, these seeds are orthodox, supporting storage at low temperatures (10 ºC, in the fridge) for two years.

It was verified that the seeds of \( O. \ arborea \) did not present absolute photosensitivity. A higher percentage of seeds showing primary root emission was observed in the absence of light from 15 to 30 ºC. However, at the alternate 20-30 ºC, the seeds showing the percentage of primary root emission was indifferent to the presence of light only in the extreme temperatures (sub and supra optimum) of 10 and 35 ºC (Table 1).
Table 1. Percentage of seeds showing primary root emission, percentage of germination, mean germination time, and mean germination speed of *Ormosia arborea* seeds, chemically scarified in sulfuric acid for 20 minutes, kept at different temperatures (10, 15, 20, 25, 30, 35 e 20-30 ºC), in the presence and absence of light.

| Temp (ºC) | Primary root emission (%) | Germination (%) | Mean germination time (days) | Mean germination speed |
|----------|---------------------------|-----------------|-----------------------------|------------------------|
|          | Light | Dark | Light | Dark | Light | Dark | Light | Dark |
| 10       | 79 Aa | 81 Ab | 0 Ac   | 0 Ad  | 0 Aa   | 0 Aa  | 0 Ac   | 0 Aa  |
| 15       | 80 Ba | 94 Aab | 67 Aa  | 74 Abc | 83.2 Bf | 74 Ad  | 0.82 Ad | 0.99 Ad|
| 20       | 84 Ba | 94 Aab | 78 Aa  | 84 Abc | 40.1 Ae | 46.2 Bc | 1.97 Abc | 1.62 Acd |
| 25       | 77 Ba | 96 Aab | 71 Ba  | 89 Aab | 26.1 Ac | 30.9 Bb | 2.74 Aab | 2.94 Aa |
| 30       | 70 Ba | 90 Aab | 62    | 78 Abc | 19.3 Ab | 28.6 Bb | 3.31 Aa | 2.82 Aab |
| 35       | 79 Ba | 85 Aab | 41 Bb  | 67 Aa  | 34.8 Ad | 32.3 Ab | 1.24 Bcd | 2.17 Abc |
| 20-30    | 85 Ba | 98 Aa  | 79 Ba  | 95 Aa  | 25.9 Ac | 29.9 Bb | 3.11 Aa | 3.31 Aa |
| CV (%)   | 7.1   | 11.1  | 5.9   | 18.2  |        |        |        |        |

Means followed by the same lowercase letters in the column do not differ by the Tukey test at 5% probability. Means followed by the same uppercase letter comparing light and dark conditions do not differ by the F test at 5% probability.

Similar results were found by Teixeira et al. (2011) concerning the absolute requirement of light. In this case, seeds of *O. arborea* did not present an absolute requirement for light; conversely, they presented a tendency to a higher percentage of primary root emission in the absence of light. Other species are also indifferent to the light requirement for seed germination, as it was verified for *Magonia pubescens* St. Hil. (Souza Filho et al., 2012) and *Kielmeyera coriacea* Mart. & Zucc. (Pimenta et al., 2011).

In light conditions, the temperature did not affect the primary root emission percentage of *O. arborea* seeds. In the absence of light, the highest primary root emission percentage was obtained by alternating temperatures of 20-30 ºC (98%); however, this value was superior only to the germination percentage obtained at 10 ºC (Table 1).

During the period of evaluation (120 days), seed germination was not observed at the temperature of 10 ºC (Table 1), a result also found by Rosseto et al. (2009) in *Parkia pendula* seeds. For *P. pendula*, the temperatures of 10 and 15 ºC inhibited the formation of the normal seedling.

The germination percentage was higher in the absence than the presence of light for the temperatures of 25, 30, 35 ºC and the at alternating temperature (20-30 ºC) (Table 1). In the presence of light, there was a higher percentage of seed germination at the alternating temperature of 20-30 ºC (79%) (Table 1). However, this value only differed from those obtained at 10 ºC (0%) and 35 ºC (41%) (Table 1). In the absence of light, the higher germination percentage was also obtained in alternating temperatures of 20-30 ºC, not differing at 20 and 25 ºC (Table 1).

In a study by Brancalion et al. (2010), which evaluated the optimal germination temperature of tree native species in Brazil, it was found that the species in which alternating temperatures are exclusively indicated as optimal are typically early species in the forest succession. However, according to the same authors, many species that are last in the forest succession also have germination favored by alternating temperatures.

Thus, alternating temperatures may be essential to overcome the physical and physiological dormancy of pioneer species, even though later species in the successional stage may benefit from it.

Several studies have shown that with alternating temperature is possible to overcome the physical dormancy (Souza et al., 2012; Daibes et al., 2017; Jaganathan et al., 2019). Temperature is probably the factor involved in overcoming physical dormancy in *Schizolobium parahyba* var. *parahyba*, a forest gap species from the Brazilian Atlantic Forest (Souza et al., 2012). According to these authors, alternating temperatures 20-30 ºC overcame dormancy faster than the constant high temperature at 30 ºC. *Phytelephas macrocarpa* presents morpho-physiological dormancy, and the stratification at alternating temperatures (26 to 40 ºC) helped to overcome seed dormancy (Ferreira and Gentil, 2017).

Due to the wide range of temperatures in which relatively high germination percentages were obtained, this species presents great ecological plasticity capable of germinating in many environmental conditions. This ecological plasticity increases the chances of success in recruiting seeds in their natural environment, where they may be subject to high irradiances and a great temperature variation. However, it is not recommended to initiate the production of seedlings when temperatures are low.

Larcher (2006) highlighted that for species with wide distribution, as well as for those adapted to large temperature fluctuations in their habitat, the temperature range for germination is extensive. According to the author, the optimal temperature for tropical species in natural conditions lies between 20 ºC and 35 ºC. On the other hand, and to a certain limit, the increase in temperature may promote changes in the performance of certain enzymes, which play important roles in the biochemical processes of germination and may favor the contamination by microorganisms.

As for the effect of alternating temperature, its response is difficult to quantify. According to Cardoso
(2004), it may vary according to exposure time, the variation range of high and low temperatures, and the number of exposure cycles, among other aspects.

It is noteworthy that the reasons regarding what determines the effect of alternating temperatures are not well known. Still, it is supposed that temperature variation may affect the promoter/inhibitor balance of the germination. Promoter/inhibitor concentration is lower at lower temperatures, while in higher temperatures, promoter concentration tends to increase (Huate and Benech-Arnold, 2010; Footitt et al., 2011; Marcos-Filho, 2015).

The optimal temperature is the one that efficiently combines the percentage and speed of germination (Bewley and Black, 1982; Marcos-Filho, 2015; Fenner, 2000). The author Marcos-Filho (2015) highlights that the gradual reduction of temperature causes a sharp decrease in germination speed due to the effects on imbibition speed and reserve mobilization. This process contributes to a greater seed sensibility to adverse environmental factors.

When the mean germination speed data is compared between treatments (Table 1), a higher germination speed was observed in the presence and absence of light, at the temperatures of 25 and 30 °C, and in the alternating temperatures of 20-30 °C. (Table 1). O. arborea seeds can germinate both in the presence and absence of light and germinate from 15 to 35 °C and under alternating temperature (20-30 °C). When the data of mean germination speed was compared in presence or absence of light in the same temperature, it was possible to see that just at 35 °C in the absence was better than in the presence of light (Table 1). In general, alternating temperatures in the absence of light provided a higher percentage of seeds showing primary root emission and seed germination.

The lowest Mean germination time for seed germination occurred at 30°C in the presence of light, while for the absence of light, the lowest mean germination time occurred at 25, 30, 35, and 20-30 °C. The temperatures of 25, 30, and 20-30 °C promoted faster germination speeds both in the absence and presence of light (Figure 1). In another study with the same species, the authors indicated the temperature of 30 °C as the best for seed germination on the substrate containing sand (Oliveira et al., 2016).
O. arborea seeds require a long period to germinate, as do many native species. Since there are no records in the literature regarding the tetrazolium test for this species, the improvement of tests that assess seed viability and characterize its physiological potential is greatly important.

Using the tetrazolium test, it was possible to separate O. arborea seeds into two categories concerning seed viability (Figure 2): Viable seeds, which presented reddish colored embryos, and unviable seeds, the ones that presented injuries in the embryonic axis. Most (80%) of the seeds presented reddish colored embryos in the present work, classified as viable (Figure 2). However, further experiments with seed lots showing different physiological potential, salt concentrations, and exposure time are necessary to validate the tetrazolium methodology.

The viability results are following the mean germination percentage (Table 1) observed in this study. For Leucaena seeds (Costa and Santos, 2010), Amburana cearensis (Guedes et al., 2010), Guibourtia hymenaefolia (Oliveira and Pereira, 2014), Tabebuia rosealba (Abbare and Takaki, 2014), Enterolobium contortisiliquum (Nogueira et al., 2014) and Apuleia moralis (Reis et al., 2016), the tetrazolium test also was efficient to assess the viability of these seeds.
4. Conclusions

The germination of *Ormosia arborea* seeds occurs in a wide range of temperatures, from 15 to 35 °C and regardless of the presence or absence of light. The temperatures of 25 and 30 °C, and alternating temperatures of 20-30 °C are indicated to conduct germination tests. The tetrazolium test corroborates the obtained data in the germination test. It is efficient to analyze the viability of seeds with coat removed, longitudinally cut, immersed in a 0.075% tetrazolium solution at 35 °C, for three hours. However, further experiments with seed lots of different qualities can establish a single temperature and light condition and establish a method of exposing seed tissue for staining. Also, different salt concentrations and exposure times are necessary to validate the tetrazolium test.

Authors’ Contribution

Aparecida Leonir da Silva was responsible for the planning, material collection, development, and evaluation of the experiments. Halisson César Vinci Carlos and Rodrigo Cyrino Rivaben were responsible for the development and evaluation of the experiments. Prof. Laércio Junio Silva responsible for statistical analysis, Prof. Denise Cunha Fernandes dos Santos Dias responsible for correcting and interpreting the data, and Prof. Glauzia Almeida de Morais and Liana Baptista de Lima guided the planning, compiled the data, and coordinated the writing of the manuscript.

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