



Effect of temperature and substrate on the germination of *Apuleia leiocarpa* (Vogel) J.F. Macbr (amarelão) seeds

Efeito da temperatura e substrato na germinação de sementes de Apuleia leiocarpa (Vogel) J.F. Macbr (amarelão)

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Abstract. This work aimed to evaluate the effect of different substrates and temperatures on the germination of amarelão (*Apuleia leiocarpa*) seeds. It was used four types of substrates (between paper, over paper, paper roll and sand) and five temperatures (constant: 20, 25, 30 and 35°C) and alternated (20/30°C). Germination was evaluated daily after imbibition counting seeds with radicle protrusion of at least 2mm. It was calculated the final percentage of germination and the indexes T50 and U7525 to estimate the speed and uniformity of germination, respectively. It was not observed differences in the percentage of final germination between the substrates in the temperatures of 25 and 30°C, However, when seeds were germinated at 30°C in paper roll an increase in speed of germination (reduction of T50) was observed. Germination was not affected in the tested temperatures, except for germination at 35°C, when a reduction in the percentage of final germination was observed. However, despite the lack of effect of temperatures on the percentage of germination for the majority of the tested temperatures, an effect on velocity and uniformity of germination was observed. Higher speed and uniformity were achieved when seeds were incubated at 30°C. In conclusion, paper roll and the temperature of 30°C (constant) are the best conditions for germination of *A. leiocarpa* seeds, which provide higher percentage of germination, germination velocity and uniformity.

Keywords. Forest species, germination uniformity, speed of germination garapiá, grápia,

Resumo. Este trabalho teve como objetivos avaliar o efeito de diferentes substratos e temperaturas na germinação de sementes de amarelão (*Apuleia leiocarpa*). Foram utilizados quatro substratos (entre papel, sobre papel, rolo de papel e sobre areia) e cinco temperaturas, sendo quatro constantes (20, 25, 30 e 35°C) e uma alternada (20/30°C). Foi avaliada diariamente após a embebição a germinação das sementes, verificada através da protrusão de radícula maior que 2 mm de comprimento para a determinação da porcentagem final de germinação, velocidade de germinação (T50) e uniformidade de germinação (U7525). Não foram observadas diferenças na porcentagem final de germinação entre os substratos utilizados nas temperaturas de 25 e 30°C, entretanto, quando as sementes foram germinadas a 30°C em rolo de papel houve aumento na velocidade de germinação (redução do T50). Nas diferentes temperaturas testadas, somente ocorreu redução na porcentagem final da germinação quando as sementes foram incubadas a 35°C. Apesar de não haver diferença na porcentagem final de germinação nas temperaturas de 20, 25, 30 e 20/30°C, foram observadas diferenças significativas nos índices T50 e U7525 quando as sementes foram incubadas a 30°C. Conclui-se que o melhor substrato para germinação de sementes de *A. leiocarpa* é o rolo de papel e que a temperatura de 30°C promove alta germinação, com maior velocidade e uniformidade de germinação.

Palavras-chave. Espécie florestal, uniformidade de germinação, velocidade de germinação, garapiá, grápia

Introduction

Apuleia leiocarpa (Fabaceae) is named locally as grápia, garapiá, garapa or amarelão. It is a forest species with ecological, ornamental and

timber production importance. The wood produced for this species is indicated for construction as beams, joists and rafters. It can be also used to obtain wood veneers used in



decoration and interior finish (Carvalho, 2003). The wood of this species has as a characteristic high level of lignin, considered very good for production of alcohol, coke and coal (Paula, 1981; Lorenzi, 2002). The bark can have up to 24% of tannin, which is used in tannery, especially for treatment of light leather. According to Mendonça Filho (1996), the leaves of this species are appreciated by the monkey bugio (*Alouatta fusca* É. G. Saint-Hilaire). Primates like miquiqui (*Brachyteles arachnoides* E. G. Saint-Hilaire) also feed from sprouts and flowers, what is considered an important resource at the end of the dry season. However, despite its economical and ecological importance, there are some limiting factors related to propagation that makes difficult the economic exploitation of this species (Nicoloso et al., 1997).

This species is widely distributed in the whole south America, with reported occurrence in the northeast of Argentina (Martinez-Crovetto, 1963), south of Bolívia (Killeen et al. 1993), east of Paraguai (Lopez et al., 1987), northeast of Peru (Encarnación, 1983) and northeast of Uruguai (Muñoz et al., 1993). In Brazil it is also widely distributed, occurring since the state of Para (North) until the state of Rio Grande do Sul (extreme south), however, its distribution is currently very discontinuous (Lorenzi, 2002).

Informations about seed germination are important to provide subsidies to reforestation projects such programs of reforestation of degraded areas or restoration of native forests and for standardization of germination tests. The knowledge of optimum conditions for seed germination, especially temperature and light are of fundamental importance, once seed germination is directly related to the ecological characteristics of the place of occurrence (Figliolia et al., 1993; Sousa et al., 2000).

Seed germination is a physiological process influenced by a number of intrinsic and extrinsic factors. Among the extrinsic factors light and temperature play a crucial role during seed germination, whereas among the extrinsic factors it can be highlighted the impermeability of the integument, embryo physiological immaturity and presence of inhibitory substances (Bewley and Black, 1994; Carvalho & Nakagawa, 2000).

During germination, the temperature influences the speed of water absorption and chemical kinetics (speed of reactions), which determine the whole process, thus affecting the

speed, uniformity and the percentage of germination in a population of seeds (Bewley & Black, 1994). Germination occurs only within certain limits of temperature, in which there is an optimum temperature or temperature range in which the process occurs with maximum efficiency (Carvalho & Nakagawa, 2000).

The substrate is the physical medium in which the seed is placed and has the function of maintaining conditions for germination and seedling development. It influences directly the germination, due to its structure, aeration, water holding capacity and as a source of possible pathogens, among others, and this way may promote or hinder the germination of seeds (Figliolia et al., 1993). In this context, it is necessary establish the appropriate substrate for each species, according to the characteristics of each seed, depending on their physical (size and shape) and physiological properties (dormancy, time needed for germination and demand for light). Thus, this study aimed to evaluate the effect of temperature and substrate on germination of *A. leiocarpa* seeds.

Material and Methods

Seed collection, processing and storage

The seeds of *A. leiocarpa* were collected in February 2010 in eight trees located in the Universidade Federal do Tocantins-UFT, campus Gurupi, Tocantins, Brazil. After collection the fruits were transported to Laboratório de Análise de Sementes da UFT-CAUG, processed manually for removal of the seeds from the pods and air dried in an acclimatized room (20 ± 5 °C, 50 % RH \pm 10) for 15 days. After cleaning and drying seeds were stored in paper bags at (17 ± 3 °C) for up to one month.

Effect of substrate on the germination

It was evaluated the effect of four types of substrates on the germination of the seeds: paper roll (PR), between paper (BP), over paper (OP) and in sand (OS). Seeds were germinated in B.O.D. incubators adjusted to the temperatures of 25 and 30 °C with 12 hours photoperiod.

Before germination seeds were chemically scarified with concentrated (98 %) sulfuric acid for 10 minutes, rinsed with distilled water and disinfected in sodium hypochlorite 1 % for 10 minutes and rinsed again in distilled water before germination.

Germination was carried on Petri dishes (9 cm of diameter) when the substrates BP and OP were used. Paper dishes were moistened in the proportion of 3:1 (paper:water), using distilled water.

For germination using the substrate PR seeds were put between two paper towel (Germitest®), rolled and placed inside plastic bags in order to avoid drying of the substrate during the incubation, inside incubators. Paper rolls were moistened in the proportion of 3:1 (paper:water), using distilled water.

Sand used in the germination tests had as characteristic the granulometry ≥ 0.05 e ≤ 0.08 mm. It was rinsed and sterilized in dry oven at 200 °C for 4 hours. Germination was carried on plastic box (Gerbox®). Sand was moistened at 60 % of its capacity of water retention (BRASIL, 2009).

Seed germination was evaluated daily from the third day after imbibition. It was considered germinated seeds with radicle protrusion ≥ 2.0 mm for up to 30 days.

Effect of temperature on the germination

After determining the best substrate for germination of *A. leiocarpa* seeds, germination tests were carried on B.O.D. incubators adjusted to different constant temperatures (20, 25, 30 and 35 °C) and alternating (20/30 °C) with a photoperiod of 12 hours, in paper roll.

The seeds were scarified before germination in concentrated sulfuric acid (98 %) for 10 minutes, rinsed with distilled water and then immediately disinfected in 1 % sodium hypochlorite for 10 minutes.

Germination was evaluated daily, starting at the third day after imbibition, using as a criterion for seed germination the radicle protrusion (≥ 2.0 mm), until it was no longer observed germination in any treatment.

The experiment was arranged in a completely randomized design with four replicates and five temperatures and 4 replicates of 20 seeds each.

Based on the daily counting of seed germination, data were analyzed using the software Germinator (Joosen et al., 2010), in order to perform curve fitting and determination of the indexes T50 (time (days) to reach 50% germination) and U7525 (time, in days, between 25 and 75 % of germination). The data were analyzed by using one-way ANOVA followed by Tukey's multiple-comparison test at 5 % probability using the software Sisvar (Ferreira, 2000).

Results and Discussion

Effect of substrate on the germination

Seeds that were germinated at the temperature of 30 °C showed higher percentage of final germination compared to those germinated at 25 °C, independently of the substrate used. At 25 °C it was verified that the germination in paper roll promoted higher germination compared to the other substrates, that did not showed significant differences. However, when the germination was carried out at 30 °C it was not observed significant differences between the substrates (Figure 1).

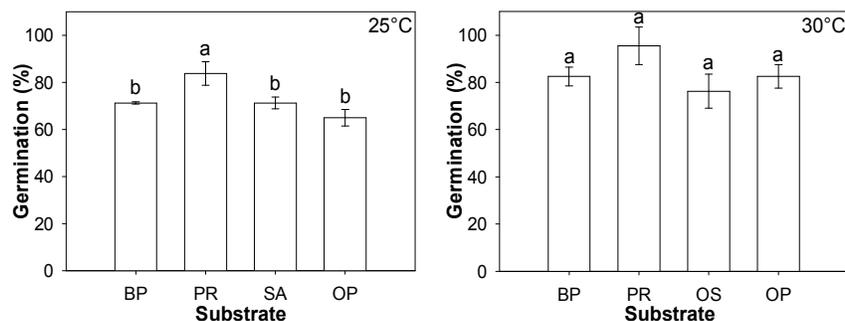


Figure 1. Effect of substrate on the germination of *Apuleia leiocarpa* seeds at 25 °C (left) and 30 °C (right). BP (Between Paper), PR (Paper Roll), OS (Over Sand) and OP (Over Paper). Error bars represent the standard error of the mean. Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$).

According to Albuquerque et al. (1998) the effect of substrate over the germination and speed of germination can be explained by its capacity of water retention and for the amount of light that reaches the seeds through the substrate, however, seeds of *A. leiocarpa* were classified by Henicka et al. (2006) as neutral photoblastic. In this context, Gomes et al. (1992) studying the germination of urucum (*Bixa orellana* L.) seeds in different substrates also verified that germination of this species was improved in paper roll. According to the authors the germination in paper roll presented as the characteristic the

maintenance of constant moisture throughout the period of germination, the absence of fungi contamination and easy to handling during the evaluation of the germination tests.

When the speed of germination was evaluated by the T50 index (Figure 2), it was not observed significant differences between the substrates when the germination was carried out at 25 °C, however, seeds germinated in paper roll at 30 °C present higher T50 (significant at 5 % probability, compared by Tukey test) compared to the other substrate.

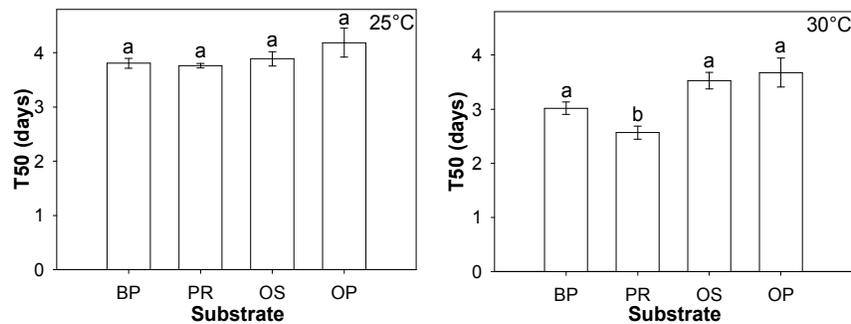


Figure 2. Effect of substrate on the speed of germination (T50) of *Apuleia leiocarpa* seeds at 25°C (left) and 30°C (right). BP (Between Paper), PR (Paper Roll), OS (Over Sand) and OP (Over Paper). Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$). Error bars represent the standard error of the mean.

It was not observed significant differences in uniformity of germination measured by the index U7525 comparing seeds germinated at different temperatures and substrates in the same

temperature (Figure 3), except for seeds germinated at 25 °C over paper, that present the highest U7525 (lower uniformity).

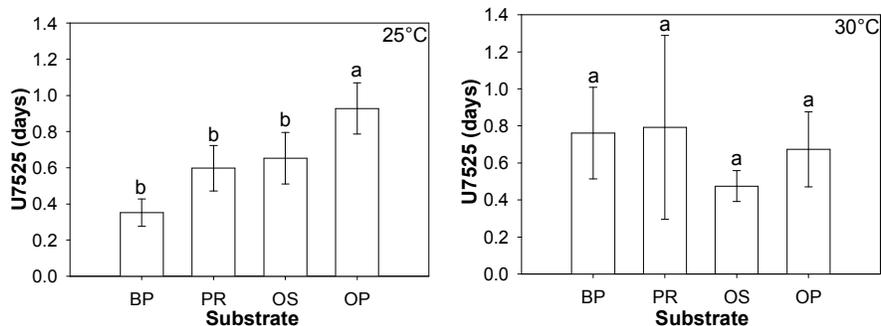


Figure 3. Effect of substrate on the uniformity of germination (U7525) of *Apuleia leiocarpa* seeds at 25 °C (left) and 30 °C (right). BP (Between Paper), PR (Paper Roll), OS (Over Sand) and OP (Over Paper). Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$). Error bars represent the standard error of the mean.



Testing different types of substrates, Varela et al. (2005) found the worse response when germination was carried out using paper (over paper) as substrate for germination of *Acosmium nitens* (Vogel) Yakovlev) seeds, which was attributed to its fast dehydration during the germination test, making necessary the re-wetting during the tests and also because of the development of fungi in the substrate, contributing to the reduction of the percentage of germination.

Effect of temperature on the germination of A. leiocarpa seeds

After the determination of the best substrate, paper roll (PR) was used as a substrate in further experiments, to test the optimal temperature for germination.

It was not observed significant differences between the temperatures of 20, 25, 30 and

20/30 °C. However, when the germination was carried out at 35 °C it was observed a decrease in final percentage of germination (Figure 4).

The optimal temperature for germination for the majority of the species is in the range of 20 to 30 °C (Marcos-Filho, 2005; Borges and Rena, 1993; Barbosa et al., 1985). For seeds of some forest species like sumaúma (*Ceiba pentandra* (L.) Gaertn) the temperature that promotes high level of germination is 30 °C, and variation in 5 °C (above or below), reduce the germination capacity (Varela et al., 1999). On the other hand, despite the observed reduction in germination at 35 °C, seeds of *A. leiocarpa* germinated in a wide range of constant and alternate temperatures, what can be explained by its environmental plasticity, once that its occurrence is reported in areas ranging from the tropical to subtropical locations.

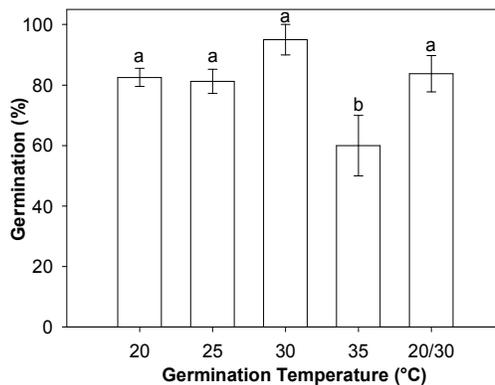


Figure 4. Effect of different temperatures (°C) on the germination of *Apuleia leiocarpa* seeds. Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$). Error bars represent the standard error of the mean.

Studying the effect of temperature and salt stress on the germination of *A. leiocarpa* (Henicka et al., 2006) concluded that the best temperature for germination was 25 °C, however, in this study, in all the tested conditions the germination was below 50 %, what can be justified by the type of substrate used (over paper).

However, when compared the speed of germination (T50) and uniformity of germination

(U7525), despite no significant differences in percentage of germination at 20, 25, 30 and 20/30 °C, seeds germinated at 25, 30 and 20/30 °C presented the lowest T50 and U7525 (higher speed and uniformity of germination). Germination at 30°C showed the higher velocity and uniformity of germination (Figure 5 e 6).

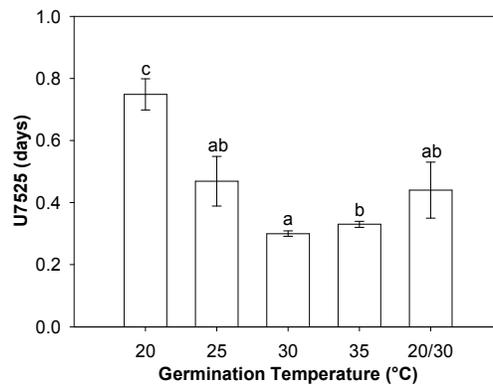


Figure 5. Effect of different temperatures (°C) on the uniformity of germination (U7525) of *Apuleia leiocarpa* seeds. Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$). Error bars represent the standard error of the mean.

According to (Mayer and Poljakoff-Mayber, 1989), temperature is one of the factors that affect germination and there is an optimal temperature, which is defined as the temperature where the maximal germination is obtained, with the higher speed. Above or below this temperature

the germination is hampered. However, in some cases, especially for some tropical forest species the optimal temperature for the maximum germination cannot coincide with the temperature that promotes the faster germination (Carvalho and Nakagawa, 2005).

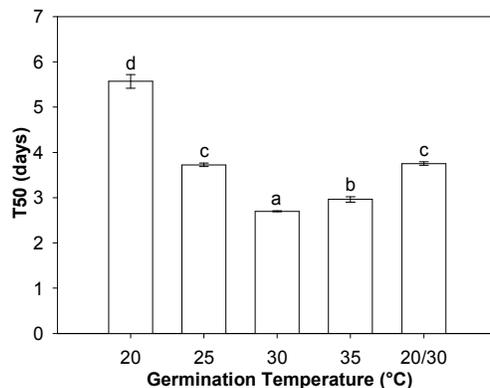


Figure 6. Effect of different temperatures (°C) on the speed of germination (T50) of *Apuleia leiocarpa* seeds. Different lower case letters indicate significance of difference based on Tukey test ($p \leq 0.05$). Error bars represent the standard error of the mean

It was verified in this work that despite no effect of the temperatures on seed germination (except for seeds germinated at 35°C), the higher speed and uniformity of germination was attained in seeds germinated at 30°C.

Conclusions

The best substrate to germinate *A. leiocarpa* seeds is the paper roll. The temperature of 30°C promotes higher germination, speed and uniformity of germination in *A. leiocarpa* seeds.

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