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## Effect of seed coat on the seed germination and seedling development of *Calophyllum brasiliense* Cambess. (Clusiaceae)

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**ABSTRACT.** This work aimed to study the effect of the *Calophyllum brasiliense* seed coat on the seed germination process. To this end, three experiments were conducted in laboratory, greenhouse and screenhouse. From a total of six treatments, five are related to the seed coat (mechanical scarification; mechanical scarification followed by 2 hours in water, chemical scarification, hot water immersion and complete seed coat removal) and one control. Laboratory and greenhouse experiments were conducted in a completely randomized design (CRD). Screenhouse experiment was conducted in a completely randomized block design (RBD). We evaluated the total percentage, the speed index and the average time of germination or emergence. Data were subjected to analysis of variance and means compared by LSD test, at 5%. Under the conditions of this work, it was possible to infer that, in laboratory, mechanical scarification followed by 2 hours in water increases the proportion and germination speed index (GSI), in the greenhouse, the complete seed coat removal increases the percentage and emergence speed index (ESI), and in the screenhouse, mechanical scarification followed by 2 hours in water and chemical scarification presented the best results. The average germination time was not significantly different in the three experiments evaluated.

**Keywords:** native species, diaspore, seed dormancy, *Calophyllum brasiliense*.

## Efeito do envoltório na germinação da semente e no desenvolvimento de plântulas de *Calophyllum brasiliense* Cambess. (Clusiaceae)

**RESUMO.** Objetivou-se com este trabalho estudar o efeito do envoltório da semente de *Calophyllum brasiliense* no processo de germinação. Foram montados três experimentos, sendo estes conduzidos em laboratório, casa de vegetação e telado. Avaliaram-se seis tratamentos, sendo cinco relacionados ao envoltório (escarificação mecânica; escarificação mecânica seguido por 2h em água; escarificação química; imersão em água quente; e retirada total do envoltório) e a testemunha. Os experimentos em laboratório e casa de vegetação foram conduzidos em delineamento inteiramente casualizado. No telado, o experimento foi conduzido em delineamento em blocos casualizados. Avaliaram-se a percentagem total; o índice de velocidade e o tempo médio de germinação ou emergência. Os dados foram submetidos à análise de variância e as médias comparadas pelo teste t (LSD) a 5% de probabilidade. Foi possível inferir que no laboratório, a escarificação mecânica, seguido por 2h em água, aumenta a percentagem e o índice de velocidade de germinação (IVG); na casa de vegetação, a retirada total do envoltório aumenta a percentagem e o índice de velocidade de emergência (IVE); e no telado, a escarificação mecânica, seguida por 2h em água, e a escarificação química apresentam os melhores resultados. O tempo de germinação não apresentou diferença significativa nos três experimentos avaliados.

**Palavras-chave:** espécie nativa, diásporo, dormência, *Calophyllum brasiliense*.

### Introduction

Family Clusiaceae includes trees, bushes, lianas and herbs of economic interest for timber production, edible fruit, chemical derivatives of pharmaceutical interest and others. Among the genera, there is *Calophyllum* L., which produces hardwoods (JÚNIOR et al., 2005).

*Calophyllum brasiliense* Cambess., commonly known as guanandi, a climax species (SANTOS et al., 2008), is a large arboreal plant, with height ranging from 20 to 30 m, diameter between 40 and 60 cm and wood density of 0.62 g cm<sup>-3</sup> (LORENZI, 2002). In Brazil, it occurs naturally from the Amazon region to the north of Santa Catarina State, and is mainly found in the Atlantic rainforest

(CARVALHO, 1994; LORENZI, 2002; NERY et al., 2007a; REIS et al., 2009).

In addition to presenting chemical and pharmacological importance, as reported by Júnior et al. (2005) and Noldin et al. (2006), it is recommended for riparian forest restoration, mostly in places subjected to periodic flooding from medium to long term (CARVALHO, 1994). This species has good quality wood and characteristics similar to the mahogany and cedar, providing a possible alternative to replace them.

The first vegetative stage of the plant after seed germination is known as seedling, which has enormous value for the study of population dynamics, forestry, seed storage, works in nurseries and in forest conservation and restoration (SOUZA, 2003). According to Masetto et al. (2008), information regarding the positive characteristics about seed germination, through which it is possible to achieve a higher germination rate, although essential, is scarce in the literature. Procedures to conduct germination test for most native species are still limited in the Rules for Seed Analysis (BRASIL, 2009).

Therefore, given the importance and potential of *Calophyllum brasiliense* (Clusiaceae) and the lack of information, standardization, and analysis methods for the vast majority of forest species, especially for the species in question, this study evaluated the effect of *Calophyllum brasiliense* seed coat on the seed germination process, in order to achieve a greater percentage of germinated seeds in a faster and uniform process.

## Material and methods

Seeds of *Calophyllum brasiliense* Cambess. (Clusiaceae) were obtained from artificial populations containing trees between four and six years old, in Monte Alto City, São Paulo State. They were similar, originated from a population containing only trees of *C. brasiliense*. Seeds presented a 25% initial average humidity, which was determined by the oven method at  $105 \pm 3^\circ\text{C}$  for 24 hours, using a mechanical oven with forced air circulation, based on the water mass of seeds.

After selected, seeds were aseptically treated with bleach commercial solution containing sodium hypochlorite at 2.0%. Seeds were immersed in this solution for two min. and rinsed in distilled water five times.

To evaluate the seeds germination, three experiments were set up, which were developed one in the laboratory, one in the greenhouse and one in the screenhouse. To study the effect of seed coat in

each experiment, they were subjected to six treatments, five related to coat and one to the untreated control.

## Treatments

Control treatment: seeds with intact coat, without treatment after processing.

Mechanical scarification: the treatments using mechanical scarification submitted the seeds to a crack in their coat. Thus, a hammer was used to break the seed coat without any damage to the embryo.

Chemical scarification for 15 min.: seeds were immersed in concentrated sulfuric acid for 15 min. To perform this procedure, we used a beaker and a glass rod, under a lab fume hood.

Immersion in hot water: consisted of immersing seeds in hot water at boiling point for one min. Then, the seeds were immersed in water at ambient temperature for 48 hours.

Mechanical scarification and water for 2 hours: this treatment consisted of the mechanical scarification followed by immersing the seed in water at ambient temperature for two hours.

Total removal of seedcoat: the treatment consisted of completely removing the seed coat, adopting a procedure similar to mechanical scarification. However, after cracking, the whole seed coat was removed manually.

## Experiment 1

The germination test was conducted in the absence of light in a germination chamber (Tecnal<sup>®</sup>, TE-404), under constant temperature of  $30^\circ\text{C}$ . This temperature is considered optimal for *C. brasiliense* germination (FERREIRA et al., 2007; NERY et al., 2007b).

Paper towels were used as substrate, in a roll form, moistened with distilled water (2.5 times the dry paper mass), according to the Rules for Seed Analysis (BRASIL, 2009). Observations were made daily, during 90 days. Seeds were considered germinated when showed radicle protrusion and/or shoot equal to or exceeding  $\pm 2$  mm. The laboratory experiment was performed using a completely randomized design, which consisted of four replications with 25 seeds per each treatment.

## Experiment 2

At the greenhouse, not climatized, germination test was conducted on plastic trays with coarse sand as substrate. During the germination test, minimum temperature of  $7^\circ\text{C}$  and maximum of  $44^\circ\text{C}$  was observed. Treatments were watered twice a day, in the early morning and late afternoon. Observations

were made daily, during 90 days. Seeds were considered germinated when produced seedlings with all essential structures to the proper plant development in the nursery. To characterize the seedling, we used the concept proposed by Souza (2003), which establishes that a well-developed seedling must have the following structures: primary root, stem base, hypocotyl, epicotyl and eophylls. The greenhouse experiment was installed using a completely randomized design, consisted of four replications with 25 seeds per each treatment.

### Experiment 3

The screenhouse is a structure fully covered with transparent plastic, and free sides, which softened the temperature. Germination test was conducted using seed beds, with four areas of 1 m<sup>2</sup> each, corresponding to the experiment blocks. Seeds were placed on the local soil and covered with manure, in order to maintain moisture. Each block was watered twice a day, in the early morning and late afternoon. Observations were made daily, also during 90 days. Seeds were considered germinated when seedlings presented all essential structures to the proper plant development. Screenhouse experiment was conducted in a randomized block design, consisted of four replications and 25 seeds per treatment in each block.

### Evaluation and data analysis

The treatments were evaluated through seed germination (G) or seed emergence (E), germination speed index (GSI) or emergence speed index (ESI) and average germination time (AGT) or average emergence time (AET) of *C. brasiliense* seeds. To calculate the GSI and average germination time, we used the data obtained in germination tests. GSI was calculated using the equation of Maguire (1962), suggested by Nakagawa (1999). The average germination time was calculated according to Santana and Ranal (2004).

Data for total germination, GSI or ESI, and average germination time or average emergence time, were subjected to analysis of variance. Means between treatments were compared by LSD test at 5% probability. All analyses were run using the statistical program Sisvar (FERREIRA, 2006).

## Results and discussion

### Experiment 1

The analyses of variance performed for germination and germination speed index in *C. brasiliense* seeds indicated significant differences at 5% among the treatments.

There was no significant difference ( $p > 0.05$ ) between control and treatment with immersion in hot water for 48 hours (Table 1), which presented the lowest germination percentage. This result is due to the immersion in hot water, which probably caused a seed coat rupture or damage to the embryo. Nery et al. (2007a) studying *C. brasiliense* seeds, found the necessity of removing the seed coat to occur imbibition, an essential fact for the germination process.

**Table 1.** Total average percentage of germination and germination speed index of *Calophyllum brasiliense* (Clusiaceae) seeds subjected to different treatments in laboratory.

Treatments	Germination (%)	GSI
Control	5 E	0.04 D
Mechanical scarification	62 C	0.45 C
Chemical scarification - 15 min.	77 B	0.58 B
Immersion in hot water - 48 hour	5 E	0.03 D
Mechanical scarification and water for 2 hours	92 A	0.72 A
Total seed coat removal	44 D	0.39 C

Means followed by the same letter, in the column, do not differ by LSD test, at 5% probability. GSI: germination speed index.

Seed coat rupture or even its complete removal promoted a significant increase in seed germination (Table 1). According to Marcos Filho (2005), seed coat regulates the seed imbibition speed, controls gas exchange, causes dormancy and may also prevent seed deterioration, acting as a barrier to microorganisms entry. This result differs from the results found by Nery et al. (2007b), in which the final germination percentage was not different between *C. brasiliense* seeds with and without seed coat.

Among the following treatments: mechanical scarification, chemical scarification treatment, mechanical scarification followed by immersion in water for 2 hours and total seed coat removal, there was no significant difference ( $p > 0.05$ ). In laboratory, the high humidity inside the germinator chamber besides the higher water amount favored the proliferation of fungi and microorganisms, directly affecting seeds without coat due to susceptibility (Table 1). Sulfuric acid acts on the seed coats removing the outer layers, such as cuticle and the cuticle layers, throughout the coat, allowing more homogeneous permeability degrees (SANTARÉM; ÁQUILA, 1995). In contrast, in mechanical scarification, seed coat is broken only in a small area, so it does not damage the embryo. In this way, the seeds mechanically scarified present only a small area allowing easy water entry, while chemically scarification may have an advantage in water absorption by presenting a more uniform scraping along the entire coat, facilitating and uniformizing water absorption. This may be the

reason for the higher germination percentage in chemically scarified seeds compared with those mechanically scarified (Table 1). On the other hand, seeds scarified mechanically and placed in immediate contact with water for 2 hours showed a higher germination percentage. *C. brasiliense* seeds are recalcitrant, as evidenced by Carvalho et al. (2006); so, seeds are sensitive to desiccation, demonstrating higher percentages of germination in immediate contact with water, after scarification, probably because such a procedure has maintained a high water content in the seeds and has contributed to a more uniform absorption. Water contact after scarification is an alternative to *C. brasiliense*, since after scarification, the permanence of seeds in water probably kept a high water content in seeds and caused a uniform absorption, and resulted in a higher germination percentage (Table 1).

On average, the results obtained for the GSI (Table 1) showed no significant difference between intact seeds (control) and seeds that received the immersion in hot water. The difficulty in absorbing water, due to the seed coat, was probably responsible for the lower values of GSI. Mechanical scarification and total seed coat removal were not significantly different to each other ( $p > 0.05$ ). The low GSI found in seeds subjected to mechanical scarification is due to an insufficient water absorption caused by a small rupture, thus affecting the germination rate. On the other hand, the complete removal of the seed coat may have caused an excessively rapid water imbibition. Seeds chemically treated were significantly different ( $p < 0.05$ ) from other treatments. Chemical scarification allowed water uptake throughout the seed coat, which positively influence the germination rate. The best germination speed index results in laboratory for *C. brasiliense* seeds was found in the mechanical scarification treatment, followed by seeds remaining in water for 2 hours, as presented in Table 1.

There were no significant differences ( $p > 0.05$ ) between treatments for average germination time. Seed coat rupture, although promoting a higher germination percentage and a higher germination speed index, showed no significant difference in average germination time between treatments with or without seed coat rupture. According to Ferreira and Borghetti (2004), the average time of germination is often directly related to the species, characterizing the strategies of each species to become established in the environment as soon as possible. Therefore, seeds of *C. brasiliense* showed no significant difference when comparing the different treatments related to seed coat.

## Experiment 2

In the greenhouse, no significant difference was detected between the control and hot water immersion treatment (Table 2). The no rupture of the seed coat probably acted hindering the absorption of water by the seed. Water absorption is essential for the germination; therefore the seed coat effect of such treatments resulted in the lowest accumulated percentage of emergence of *C. brasiliense* seedlings. Hot water immersion treatment may also have caused damage to the embryo, affecting seedling emergence.

**Table 2.** Total average percentage of emergence and emergence speed index of *Calophyllum brasiliense* (Clusiaceae) seeds subjected to different treatments in greenhouse.

Treatments	Emergence (%)	ESI
Control	6 D	0.04 D
Mechanical scarification	33 C	0.24 C
Chemical scarification - 15 min.	69 B	0.45 B
Immersion in hot water - 48 hour	8 D	0.06 D
Mechanical scarification and water for 2 hours	65 B	0.45 B
Total seed coat removal	83 A	0.58 A

Means followed by the same letter, in the column, do not differ by LSD test, at 5% probability. ESI: emergence speed index.

However, in the other treatments, there was a significant increase in the emergence percentage. The increase in the emergence percentage can be explained by the seed coat rupture, facilitating the water absorption by the seed, resulting in an increased germination percentage. The results indicated that the permanence of the mechanically scarified seeds in water significantly improved emergence percentage. Chemical and mechanical scarifications followed by 2 hours in water immersion were not significantly different (Table 2). The lack of difference between these treatments is likely to be related to the efficiency of chemical scarification in this study, allowing adequate water uptake by the seed, so that these results did not differ to each other. Nevertheless, the no rupture of the seed coat limited water uptake, thus limiting the emergence percentage.

The complete removal of seed coat resulted in a higher emergence percentage, due to the easier water absorption by the seed, evenly, facilitating the germination process. In the greenhouse experiment, it was observed that the water percolates through the sand, thus preventing its accumulation that harms the germination and favor microorganism attack. Additionally, unlike the laboratory experiment, with humidity approximately to 100%, favoring attack of seeds by fungi and other microorganisms, the greenhouse with sand substrate and total seed coat removal favorably affected the germination percentage. The sand facilitates water percolation,

favoring the water absorption by the seed and it is not conducive to fungi development and microorganisms, as occurs in environments with high humidity.

The analysis of variance with data of emergence speed index determined in the greenhouse evidenced a significant difference ( $p < 0.05$ ) among the treatments. Thus, the means for the emergence speed index were compared by LSD test at 5% probability and the results are presented in Table 2.

There was a similar behavior among treatments for the emergence percentage and emergence speed index in the greenhouse. In Table 2, treatments related to the seed coat rupture promoted a higher emergence speed index. Similar results to those obtained in this work were found by Nery et al. (2007b), who reported for *C. brasiliense*, higher GSI values in seeds without coat, and noted a possible coat interference with water uptake by the seed, which slows germination and causes dormancy. The analysis of variance performed for the average emergence of *C. brasiliense* seedlings in the greenhouse showed no significant differences at 5% probability among the treatments. The treatments applied to the seed coat of *C. brasiliense* did not affect the average emergence time probably because it is a survival mechanism of the species.

### Experiment 3

The results obtained in this experiment showed the species development subjected to conditions very close to the field, however with partial protection by the effect of the clear plastic cover, which softens the direct effects of weather.

The analysis of variance for treatments performed in the screenhouse indicated non-significant differences between blocks used in the experiment. This result is probably due to the uniformity of the area where the experiment was conducted. However, we observed significant differences between treatments, and the means for percentage emergence were compared by LSD test, at 5% probability.

Based on the results presented in Table 3, we found higher seedling emergence percentages in seeds subjected to treatments related to the seed coat rupture. As previously described, rupture favors the water uptake by the seed, favoring germination process. Meanwhile, seeds with complete seed coat removal, despite showing significantly higher emergence rates when compared to intact seeds or seeds with hot water immersion had a lower emergence percentage in the screenhouse. This low emergence percentage can be explained by the ability of soil water retention when compared with sand

substrate. Probably, this retention resulted in a rapid imbibition, affecting the germination process and the high water content favors the proliferation of fungi and microorganisms, which mainly affect the seeds without coat because of their susceptibility.

**Table 3.** Total average percentage of emergence and emergence speed index of *Calophyllum brasiliense* (Clusiaceae) seeds subjected to different treatments in screenhouse.

Treatments	Emergence (%)	ESI
Control	4 D	0.03 D
Mechanical scarification	63 B	0.42 B
Chemical scarification - 15 min.	74 A	0.51 A
Immersion in hot water - 48 hour	7 D	0.05 D
Mechanical scarification and water for 2 hours	78 A	0.50 A
Total seed coat removal	31 C	0.23 C

Means followed by the same letter, in the column, do not differ by LSD test, at 5% probability. ESI: emergence speed index.

Mechanical scarification showed significantly better results when compared with seeds without seed coat. This result is due to the function of seed coat as a protection to the embryo. According to Santos et al. (2009), the coat acts as seed protection, so that its absence becomes a limiting factor.

According to the results in Table 3, there was no difference between seeds that were mechanically scarified followed by water immersion for 2 hours and chemically scarified seeds. Both treatments resulted in greater seedling emergence in the screenhouse, which is due to enough water absorption, due to seed coat rupture, thus favoring the germination process, compared with other treatments. At the same time, the seed coat permanence worked as protection for the embryo, water output control by the seed and possibly against attack by fungi and/or predation.

The analysis of variance for the emergence speed index determined in screenhouse is presented in Table 3. No significant differences were observed between blocks used in the experiment. This fact is due to the uniformity of the area where the experiment was set up. However, there were significant differences between treatments analyzed, and the means for the emergence speed index were compared by LSD test, at 5% level of probability.

The results for the emergence speed index in the screenhouse were similar to the results of emergence in the same conditions (Table 3). Similar results were found by Cardoso and Pereira (2008) in a study on the effect of water potential on seed germination of *Drymaria cordata*. These authors observed that the treatments that affected the germinability also affected the germination speed, showing similar behavior. The similarity in behavior between the total emergence percentage and ESI is probably related to the same limiting factor; in this study, it is believed that water was the limiting factor.

The results obtained for the average time of *C. brasiliense* seedling emergence in screenhouse showed no significant differences at 5% between the blocks used in the experiment, even among the treatments.

## Conclusion

The partial or complete removal of seed coat of *Calophyllum brasiliense* seeds increases germination and promotes a higher germination speed. In more conducive environments to fungi and/or in the presence of insects, seed coat is needed for a better result in the emergence percentage. When sowing the seeds in sand, the complete seed coat removal leads to an increased seedling emergence and emergence speed. For seeds directly sown in soil, the chemical or mechanical scarification of seeds followed by water immersion for 2 hours provides increased emergence and higher emergence speed.

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