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Overcoming seed dormancy of *Leucaena leucocephala* Lam. de Wit Seeds collected in Alagoas, Brazil

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ABSTRACT

Leucaena is a Fabaceae with environmental and economic importance, it can be used in the regeneration of degraded areas, for animal feed, for wood and handicrafts; its seeds have seed coat dormancy, which makes the propagation of this species difficult. Studies on overcoming seed dormancy are important to determine which efficient methods exist for breaking dormancy. Therefore, this study aimed to evaluate the best treatment for overcoming the dormancy of *Leucaena leucocephala* Lam. de Wit seeds from the state of Alagoas. The experimental design used was completely randomized, with four repetitions of 25 seeds for each treatment. Six treatments were tested, namely: (1) Witness (intact seeds); Chemical scarification, performed by immersing the seeds in H_2SO_4 (concentrated sulfuric acid) for (2) 5 and (3) 10 minutes, followed by washing in running water; (4) Notching and (5) Sanding, performed on the side opposite to the micropyle; and (6) Immersion in soda for 24 hours. It was concluded that the notching treatment was superior to the others, differing statistically, except for the variable dry mass in the sanding treatment. Thus, the notching treatment proved to be efficient and can be recommended for breaking dormancy in *L. leucocephala* seeds.

RESUMO

A leucena é uma Fabaceae com importância ambiental e econômica, pode ser usada na regeneração de áreas degradadas, na alimentação animal, para madeira e artesanato; as suas sementes possuem dormência tegumentar, o que dificulta a propagação dessa espécie. Estudos sobre superação de dormência de sementes são importantes para que se saiba qual ou quais formas eficientes para a quebra da dormência. Sendo assim, este trabalho teve como objetivo avaliar qual é o melhor tratamento para a superação da dormência de sementes de *Leucaena leucocephala* Lam. de Wit provenientes do estado de Alagoas. O delineamento utilizado para o experimento foi o inteiramente casualizado, com quatro repetições de 25 sementes, cada tratamento. Foram testados 6 tratamentos, sendo eles: (1) Testemunha (sementes intactas); Escarificação química, realizada imergindo-se as sementes em H_2SO_4 (ácido sulfúrico concentrado) por (2) 5 e (3) 10 minutos, seguido de lavagem em água corrente; (4) Desponte e (5) Lixa, realizada do lado oposto à micrópila; e (6) Imersão em refrigerante por 24 horas. Concluiu-se que o tratamento desponte foi superior aos demais, diferindo estatisticamente, exceto pela variável massa seca no tratamento com lixa. Assim, o tratamento desponte demonstrou-se eficiente e pode ser recomendado para a quebra de dormência em sementes de *L. leucocephala*.

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Introduction

Commonly known as leucaena, Leucaena leucocephala (Lam.) de Wit. belongs to the Fabaceae family, is native to Central America, and has multiple uses such as forage, wood, and green manure. It plays a role in nitrogen fixation thanks to its association with nitrifying bacteria (Felix *et al.*, 2018; Mendonça *et al.*, 2020). This species exhibits efficient characteristics that ensure its survival and allows it to establish in different environments, has rapid growth and reproduction, good adaptation, and acts as a restorer of degraded areas, even in adverse conditions as found in the Caatinga (Silva, 2022).

Despite being considered an invasive plant in the country (Brazil) due to the large amount of seeds produced annually by the plants and their natural regeneration capacity, this species is one of the most versatile tree-shrub legumes. According to Drumond and Ribaski (2010), *L. leucocephala* can be used in various applications such as nutritious forage for ruminants, erosion control on steep slopes, green manure, cover in agroforestry systems, recovery of degraded areas, source of wood, leaf biomass, charcoal, fuel, and others.

In addition to its practical uses, Leucaena plays a significant ecological role, as its nodulated roots have the ability to fix nitrogen from the atmosphere through a symbiosis with bacteria of the genus *Rhizobium*, and to solubilize phosphorus through interaction with vesicular-arbuscular mycorrhizal fungi (VAM) of the genera *Glomus* and *Gigaspora*, which results in soil enrichment (Moura *et al.*, 2020).

Although leucaena has several benefits, such as rapid growth and a high number of viable seeds, Souza and Santos (2018) observed the presence of physical dormancy in its seeds. Dormancy is a phenomenon that can occur in seeds, which can be observed when a seed that has it remains in the soil but does not germinate on its own. It is an evolutionary process aimed at preserving the long-term survival of species, which evolve under certain conditions (Oliveira *et al.*, 2023).

One of the main strategies for the perpetuation of *L. leucocephala* is seed coat dormancy, which ensures the species' persistence even in semi-arid areas. Planting this legume without treating the dormancy results in a germination rate of less than 50% (Silva, 2023). Due to this characteristic present in the species, it is necessary to seek pre-germination treatments to overcome its dormancy. Therefore, this study aimed to evaluate which treatment is the best for overcoming the dormancy of *Leucaena leucocephala* Lam. de Wit seeds originating from the state of Alagoas.

Methodology

The work was conducted in the Phytotechnology Laboratory belonging to the Campus of Engineering and Agricultural Sciences (CECA) at the Federal University of Alagoas (UFAL). The design used was completely randomized (DIC), with four repetitions of 25 seeds.

The ripe fruits of *L. leucocephala* were collected in June 2023 with the help of a pole pruner from eight trees located in the rural area of the municipality of Anadia - AL.

The treatments adopted for overcoming dormancy were: (1) Control (intact seeds); Chemical scarification, performed by immersing the seeds in H_2SO_4 (concentrated sulfuric acid) for (2) 5 and (3) 10 minutes, followed by washing in running water; (4) Trimming and (5) Sanding, done on the side opposite to the micropile; and (6) Immersion in refrigerant for 24 hours.

Immediately after the pre-germination treatments, the seeds were subjected to asepsis, performed by immersing them in 70% alcohol for one minute and washing in distilled water. Afterwards, the sowing was carried out on two sheets of germitest paper, already sterilized and moistened with distilled water in an amount equivalent to 2.5 times the weight of the dry paper, and placed in transparent plastic boxes of the Gerbox type (11.0 x 11.0 x 3.5 cm).

After the distribution of the seeds, all treatments were incubated in a Biochemical Oxygen Demand (B.O.D.) germination chamber regulated at a temperature of 30 °C for 15 days. Starting from the fourth day of the experiment, daily counts of germinated seeds were conducted at the same time.

Seeds that originated normal seedlings with all their essential structures were considered germinated, thus showing the potential to continue their development and produce normal plants when subjected to favorable conditions (Brasil, 2009).

Analyzed variables

First germination count: Conducted simultaneously with the germination test, being the accumulated percentage of germinated seeds on the fourth day after sowing.

Germination: Counts of germinated seeds were carried out daily over a period of fifteen days, with seeds that showed a primary root with length being considered germinated ≥ 2 mm (Giachini and Barneby, 2010).

Germination speed index: Carried out jointly with the germination test, counting the germinated seeds daily until germination stabilizes, and calculated using the formula proposed by Maguire (1962).

Average germination speed: V = 1/t where: V = average germination speed; t = average germination time (Labouriau and Valadares, 1976).

Average germination time: t = (Σniti)/Σni where: t = average incubation time; ni = number of seeds germinated per day; ti = incubation period (days) (Labouriau and Valadares, 1976).

Seedling length: At the end of the germination test, the seedlings from each repetition were used to evaluate the length, with the help of a ruler graduated in centimeters, with the results expressed in centimeters per seedling.

Dry mass of seedlings: After the completion of the germination test, the normal seedlings from each replicate were placed in paper bags, then placed in a forced ventilation oven at 80 ºC for a period of 24 hours. After this time, the samples were placed in desiccators with activated silica gel and weighed on an analytical scale with a precision of 0.0001g, and the result expressed in g/seedlings.

Statistical analysis

All statistical analyses were performed using the SISVAR 5.6 program from the Federal University of Lavras (*Universidade Federal de Lavras*) (Ferreira, 2014). The data were subjected to analysis of variance (ANOVA). When there was significance in the F test, the means were compared using Tukey's test at a 5% probability.

Results and discussion

Table 1 describes the results of each analyzed variable; in general, it is possible to observe that the treatments, cutting and sanding, stood out over the others, not differing statistically from each other. The first germination count (%) of leucena was carried out on the fourth day of the established experiment. The control and soda treatments had the lowest values of germinated seeds on the first reading day, which suggests that possibly part of the seeds from the population is released from the mother plant free of dormancy and that the soda treatment is not effective for breaking dormancy. Melo *et al.* (2018) and Melo Júnior *et al.* (2018), studying seeds of *Mimosa bimucronata* and *Colubrina glandulosa*, respectively, found similar results in relation to the control.

In the germination variable (%), the debulking method enabled 100% germination; the debulking performed on the side opposite the micropyle likely allowed greater water entry into the seed, assisting in the germinative process. Araújo *et al.* (2013) found different results in their work that also studied ways of overcoming seed dormancy in leucaena, which had rates lower than 20% for the seeds treated with debulking. It is noteworthy that the sanding treatment had significant results for this variable and statistically did not differ from the debulking.

For the germination speed index (%) and average germination speed (%), it is interesting to have the highest value, as they are associated with the quickness of germination. The treatments of topping and sanding, as with other variables, had the highest values, which

means that the ease of water entry into the seed, as mentioned above, possibly contributed to the acceleration of the breakdown of the reserve tissues of the seeds and determined a quick and efficient germination. Santos *et al.* (2023) found similar results in their study on overcoming dormancy in seeds of *Albizia polycephala* (Benth.) Killip ex Record.

Table 1.

First germination count (PCG), germination (GER), germination speed index (IVG), and average germination speed (VMG) of *Leucaena leucocephala* Lam. de Wit seeds submitted to treatments for overcoming dormancy (CECA/UFAL, 2024).

Treatments	PCG (%)	GER (%)	IVG	VMG
1 Witness	15 c	20 c	3.502 c	0.076 c
2 H_2SO_4/5 minutes	62 b	70 b	4.111 b	0.161 b
3 H_2SO_4/10 minutes	57 b	70 b	4.010 b	0.160 b
4 Topping	90 a	100 a	5.102 a	0.255 a
5 Soda	12 c	20 c	4.110 b	0.125 b
6 Sandpaper	88 a	95 a	5.022 a	0.218 a
CV (%)	8.00	9.67	10.11	12.02

Means followed by the same lowercase letter in the column do not differ at 5% probability by the Tukey test.
Source: The authors (2024).

According to the results obtained (see Table 2) for the variables of mean time (TM), seedling length (CMP), and dry mass of seedlings (MS), it was observed that the treatment of topping stood out, differing statistically from the others, except for the MS variable of the sandpaper treatment (MS topping = 0.476 (g) and MS sandpaper = 0.458 (g)). The expressed results were different from those found by Mendonça *et al.* (2020), studying the overcoming of seed dormancy in *L. leucocephala* with physical and chemical methods, where the sandpaper scarification treatment showed better success compared to topping.

The treatments with sulfuric acid for 5 and 10 minutes showed negative results, with no statistical difference between them across all studied variables. However, Sousa and Santos (2018), when evaluating the germination rate of *L. leucocephala* subjected to different methods of breaking dormancy, in which the immersed treatments were H_2SO_4 for 5 to 10 minutes they obtained satisfactory results when compared to those subjected to physical methods.

In the present work, the soda and the witness obtained the highest average time and the lowest dry mass weight. According to Padilha *et al.* (2021), the parameters associated with germination speed, such as average time, can indicate a faster and more uniform germination process, displaying greater seedling length development, consequently leading to greater dry

mass weight. Melo *et al.* (2023) explained that for the TM variable, the best result is related to the lowest values found for it.

Table 2.

Average time (TM), length (COMP), and dry mass (MS) of seedlings from seeds of *Leucaena leucocephala* Lam. de Wit, subjected to treatments for overcoming dormancy (CECA/UFAL, 2024).

Treatments	TM (days)	COMP (cm)	MS (g)
1 Witness	9.0 d	3.60 e	0.150 c
2 H_2SO_4/5 minutes	4.8 c	6.70 c	0.309 b
3 H_2SO_4/10 minutes	5.0 c	7.00 c	0.330 b
4 Topping	3.0 a	12.00 a	0.476 a
5 Soda	9.2 d	5.50 d	0.175 c
6 Sandpaper	4.0 b	10.50 b	0.458 a
CV (%)	12.00	13.57	15.48

Means followed by the same lowercase letter in the column do not differ at 5% probability by the Tukey test.

Source: The authors (2024).

Conclusion

The despont treatment proved effective for breaking dormancy in seeds of *L. leucocephala* and can be recommended as a pre-germination treatment for this species.

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