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Methods of overcoming dormancy in seeds and initial development of Black Wattle

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ABSTRACT

The objective of this work was to analyze the efficacy of different methods of dormancy break in seeds of Acacia mearnsii De Wild. The work was performed at the UFSM Botany Lab and Forest Nursery, in Frederico Westphalen, RS. The treatments were: (T1) control; (T2) immersion in hot water (90 °C) for 5 minutes, followed by rest; (T3) immersion in hot water (90 °C) for 10 minutes, followed by rest; (T4) chemical scarification with concentrated sulfuric acid for 3 minutes, followed by washing under running water; (T5) chiseling with concentrated sulfuric acid for 5 minutes, followed by washing under running water. The seeds were sown in plastic trays with Oxisol and vermiculite. The results were submitted to analysis of variance and data were differentiated by Test t with 5% of probability. The most efficient treatment to overcome dormancy was T2, presenting 81.29% of germinated seeds and IVG of 18.23 days for germination. This treatment was also the best for the variables of physical vigor and best performing root length and aerial part, and did not differ from improved treatments for diameter and green mass weight.

Keywords: Acacia mearnsii; Forest seeds; Germination; Seed dormancy; Scarification; Physical vigor.

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1 INTRODUCTION

Acacia mearnsii De Wild, popularly known as Black Wattle, belongs to the Fabaceae family and Mimosoideae subfamily, and originates from Australia's South-Eastern, being a tree of great economic and ornamental importance (SCHUMACHER et al., 2003). The species was introduced in Brazil in 1918 and in 2018 ranks first among the most planted forest species in the country (IBÁ, 2018).

The economic role that the Black Wattle plays is closely related to the production of tannin and wood, which is used as firewood and coal, also serving as a raw material product for paper, cellulose and offset industries. In addition, it is evident its important ecological role in soil conservation and recovery (EMBRAPA, 1992). The benefits offered by this species are emphasized by its rapid growth, especially in the southern region of Brazil, where the pant finds optimal soil and climatic conditions for development (OLIVEIRA, 2007).

However, like most leguminous, the seeds have hard teguments that are impermeable to water and gases, making uniform establishment in the field difficult (BERTALOT; NAKAGAWA, 1998). According to BEWLEY and BLACK (1994), dormancy can manifest itself in three ways: dormancy imposed by the integument, embryonic dormancy and dormancy due to imbalance between promotor substances and inhibitors of germination. For Yap and Wong (1983), in Black Wattle this germination impediment can occur due to impermeability of the tegument to the water.

In view of this, methods proposed by Sacco (1974) to overcome this type of dormancy are based on dissolving this waxy cuticular layer or promoting striations in the seed coat. Mayer and Poljakoff-Mayber (1989) further add that this impermeability can be overcome by any treatment that results in the tearing or weakening of the integument allowing the passage of water and gas Exchange, initiating the germination process.

Among the methods used successfully to overcome the dormancy of species of the Leguminosae family are the chemical scarification (BIANCHETTI and RAMOS, 1981a; NICOLOSO et al., 1997; BIANCHETTI and RAMOS, 1981c; SILVA and SILVA, 1993) and mechanical scarification (BIANCHETTI and RAMOS, 1981b), as well as immersion in hot

water (BIANCHETTI and RAMOS, 1981b; RECH et al., 1980; BIANCHETTI and RAMOS, 1982; SILVA and SILVA, 1993). The application and efficiency of these treatments depends on the dormancy intensity, which varies considerably between species, provenances and years of seed collection.

The immersion of the seeds in hot water has been used to promote the germination of *Acacia mangium* (SILVA and SILVA, 1993; LIMA and GARCIA, 1996), *Acacia senegal* (TORRES and SANTOS, 1994) and *Acacia mearnsii* (RECH et al., 1980; BIANCHETTI and RAMOS, 1982).

According to Bianchetti and Ramos (1982), immersion of the seeds in hot water at 90 ºC followed by rest in the same water, outside the heating for 24 hours, was the most recommended treatment for the production of Black Wattle seedlings.

Results obtained by Lima and Garcia (1996) for seeds of *A. mangium* submitted to immersion in water at 80 ºC until reaching room temperature (2 hours), was the treatment that provided greater length of the seedlings. Already, for Alves et al. (2000), seeking to overcome *Bauhinia mondranta* dormancy, with the immersion in water at 85 ºC the death of all the seeds occurred, and in *B. ungulata* seeds, a low percentage of germination (6%).

Alves et al. (2000), suggest immersion in concentrated sulfuric acid (H_2SO_4), immersion in boiling water or scarification, as a treatment to overcome dormancy. The effectiveness of sulfuric acid was found by several authors: Frank and Baseggio (1998) in *Desmodium inacanum* DC and *Lathynus nervores* Lam; Bertalot and Nakagawa (1998) in *Leucaena diversifolia*.

According to Garcia and Cícero (1992), the use of H_2SO_4 in overcoming seed dormancy presents contradictory results, which can either favor or impair seed germination. This statement was verified by Goedert (1985) when studied the use of H_2PO_4 in overcoming seed dormancy of *Brachiaria humidicola* and *B. decumbens*. The author found that this treatment reduced the germination of *B. humidicola* seeds, however, the same treatment promoted a 50% increase in germination of *B. decumbens* seeds when compared to the control, although, apparently, has damaged part of the seeds.

According to Albrecht (1981), the exposure time to sulfuric acid is directly related to the hardness of the integument and, furthermore, after immersion the seeds must be washed in running water for 10 minutes to remove the residues. Barazetti and Sccoti (2010) stated that concentrated sulfuric acid for 4 minutes was the best treatment to evaluate the viability of *Mimosa scabrella* seeds, whereas, for Bianchetti and Ramos (1982), scarification with commercial sulfuric acid concentrated for 15 minutes was not efficient to overcome dormancy of Black Wattle.

The dendrometric variables evaluated as seedling length, stem diameter and green weight are important, because according to Silva et al. (2007) studies have reported that plant growth efficiency may be related to the ability of seedlings to adapt to ambient sunlight conditions.

The mechanism of seed dormancy, presented by most forest species, generates the necessity for studies that better explain this process. According to Roversi et al. (2002), it is necessary to test with practical methods of overcoming dormancy of Black Wattle, aiming at a significant improvement in germination and also in the performance of seedlings in the seedling nursery, accelerating and standardizing the establishment of plants in the field.

In view of the foregoing, the objective of this work was to evaluate the effect of different treatments on the dormancy overcoming of *Acacia mearnsii* De Wild. seeds, which may provide high percentages of germination and that may be applicable to laboratory tests and seedling nursery conditions.

2 MATERIALS AND METHODS

The seeds of *Acacia mearnsii* were purchased from the company MP Sementes, located in the county of Ijuí, Rio Grande do Sul. The work was carried out in the Botany Laboratory and in the Forest Nursery of the Federal University of Santa Maria (UFSM), campus Frederico Westphalen. In the laboratory, the first step was to separate the seeds according to their size, those smaller than 2.0 mm were eliminated. The selected seeds were submitted to these treatments to overcome dormancy: (T1) Witness (without intervention); (T2) Immersion in hot water (90 °C) for 5 minutes, followed by

standing in water at room temperature for 16 hours; (T3) Immersion in hot water (90 °C) for 10 minutes, followed by standing in water at room temperature for 16 hours; (T4) scarification with concentrated sulfuric acid for 3 minutes, followed by washing in running water for 10 minutes; and (T5) chemical scarification with concentrated sulfuric acid for 5 minutes, followed by washing in running water for 10 minutes. In the treatments with sulfuric acid the seeds were placed in Becker cups and immersed in enough acid to cover them.

The second stage was carried out in the Forest Nursery, with individual sowing in trays with polyethylene tubes containing 6 grooves with a capacity of 110 cm³, on 21th November, 2016. As substratum, three pieces of clayey soil of the type Red Latosol (EMBRAPA, 1999) sifted and one of vermiculite of fine texture were used. Each treatment consisted of 96 seeds, being made 2 replicates of 48, totaling 5 trays of 96 tubes each.

After sowing the trays were conditioned in an greenhouse in the Florest Nursery, equipped with a sprinkler irrigation system. The irrigation is automated four times a day, lasting ten minutes at a time. The greenhouse used has a measurement of 120 m² (10 m wide x 12 m long), covered with plastic film in the form of an arc.

From the emergence of the first seedling were counted, every two days, the germination. This procedure was carried out until the 30th day after sowing and on this date the final count of the germination test was done, being evaluated the percentage of germination (BRASIL, 1992) and the rate of germination (IVG) (MAGUIRE, 1962). IVG was determined by the sum of the number of normal seedlings germinated each day, divided by the number of days elapsed between sowing and emergence, according to the formula (Maguire, 1962):

$$IVG = \frac{G_1}{N_1} + \frac{G_2}{N_2} + ... + \frac{G_n}{N_n} \quad (1)$$

on what:

G_1, G_2, G_n = number of seedlings in the first, second and last counts.

N_1, N_2, N_n = number of days of sowing at the first, second and last count.

The final evaluation of the variables root length, aerial part length, stem diameter and green mass weight was made 60 days after sowing. The seedlings were carefully removed from the tubes, washed and placed in bags numbered with the treatment and the repetition to which they belonged. After this process, they were taken to the Botany Laboratory and deposited on filter paper to remove excess water. To measure the length of the primary root and aerial part was used a ruler millimeter and to measure the diameter of the stem was made use of a pachymeter. Data were expressed in millimeters. After that, the seedlings were weighed in a precision scale to obtain the green mass. These data were expressed in grams.

The results were analyzed according to the completely randomized design and the treatments were compared by the t test at the 5% probability level. The statistical analysis of the data was performed by the software Assistat (SILVA and AZEVEDO, 2002).

3 RESULTS AND DISCUSSION

From the seventh day after sowing the first germinated seeds were observed, and the first count was made. The treatments T2, T3, T4 and T5 presented respectively: 3, 5, 1 and 2 germinated seeds. The witness, however, had its first germination on the 9th day, with 2 emergencies. As shown in Figure 1, treatments T2 and T3 had higher germination expression. This happened during the second week of evaluation, with 36 and 37 seeds germinated, respectively. Already, for T1 and methods of immersion in H_2SO_4 for 3 and 5 minutes, less germination occurred and no week was highlighted, remaining almost constant.

Table 1 shows the results obtained at the end of the 30 days of evaluation, where it was found that the treatments that used water as a scarifier (T2 and T3) in both immersion times promoted greater final germination of the seeds when compared to other methods tested. For the treatments evaluated, there were significant statistical differences for the germination variable, so that in T1 the percentage of germination was 9.37% being characterized as the worst treatment observed. The T2, with 81.29%, promoted the highest percentage of germination and was characterized as the best treatment for the overcoming of dormancy in Black Wattle seeds. However, T3

presented 81.25% and did not differ statistically from T2, and could also be considered as the best treatment. The percentages of germination for T4 and T5 were lower. With 14.58% and 13.54%, respectively, these treatments differed statistically from the best, but outweighed the witness treatment. The seeds treated chemically took the same time to initiate the germination when compared to the treatments with immersion in hot water, but possibly the action time of the acid should have been longer, as Bianchetti and Ramos (1982) affirm, regarding the method immersion in concentrated sulfuric acid pa (96% purity). The authors state that it is possible to promote the immersion of the seeds for times of 5 to 20 minutes to break dormancy of the Black Wattle.

The low percentage of germination in seed without treatment proves the presence of seed dormancy. In spite of this, it is noticed that 4.5% of the seeds found adequate environmental conditions for their development, occurring thus the breakdown of dormancy by natural processes.

Figure 1 – Absolute frequency of seed germination in the different treatments (a)(b)(c)(d)(e), during the four weeks of evaluation

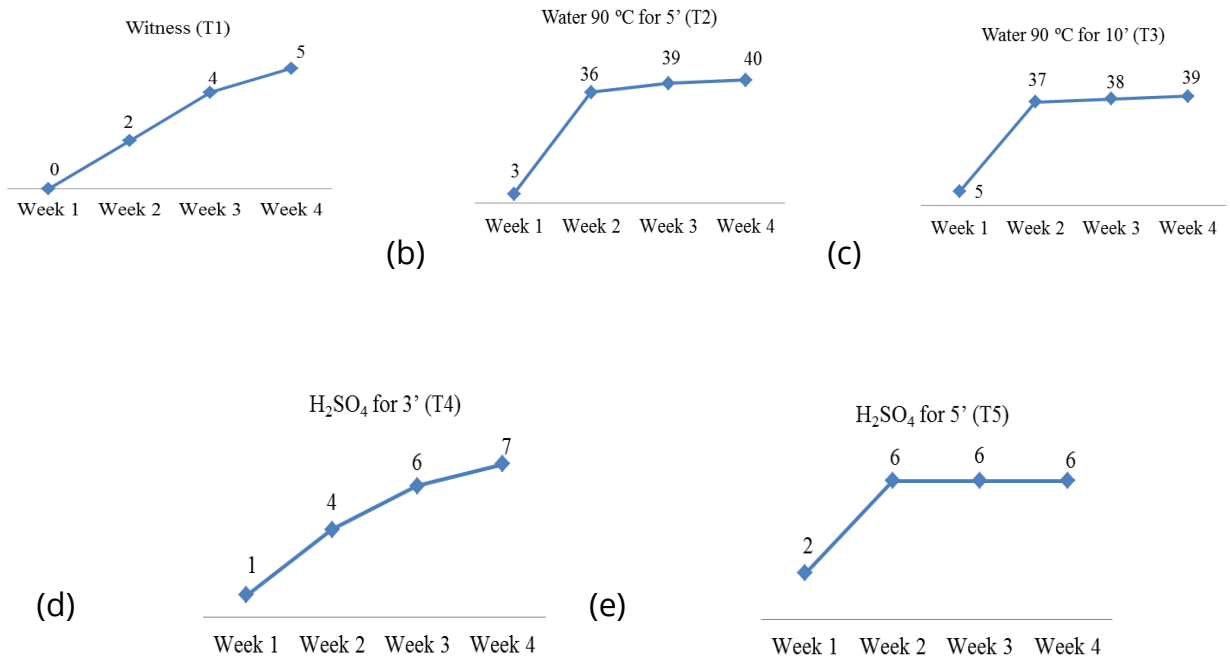


Table 1 – Averages of germination percentage in Acacia mearnsii De Wild seeds. after applying the methods of overcoming dormancy

Treatments	% germination[1]
T1 - Witness without intervention	9,37 c*
T2 - Immersion in water at 90 ° C for 5 ', followed by standing in water at T (° C) ambient for 16 h	81,29 a*
T3 - Immersion in water at 90 ° C for 10 ', followed by standing in water at T (° C) ambient for 16 h	81,25 a*
T4 - Immersion in concentrated H2 SO4 for 3 ', followed by washing in running water for 10'	14,58 b*
T5 - Immersion in concentrated H2 SO4 for 5 ', followed by washing in running water for 10'	13,54 b*
CV(%)[2] = 6,33	

*Means followed by different lowercase letters differ from each other by the t test at the 5% probability level; [1] Percentage of germination (%); [2] Coefficient of variation (%).

It is known that an important criterion for the cultivation of forest species is that they have a rapid and homogeneous development (KREFTA et al., 2012), so that the IVG was calculated, an important parameter to evaluate the homogeneity and speed of germination. Was observed that the best result was obtained by T3, with an average germination time of 19.66 days, although T2 also reached a high index, which differed statistically from the best, according to Figure 2.

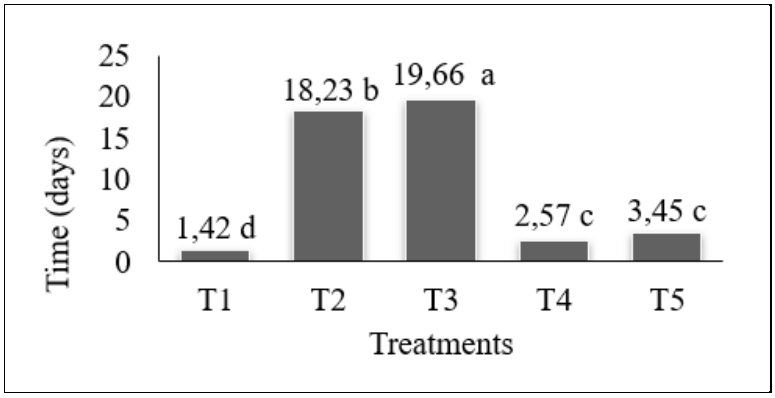
For the seeds treated with immersion in concentrated sulfuric acid (T4 and T5), there was no significant difference between them for the germination percentage and IVG, but these differed from the best treatments, in both cases, only the results of the control. This showed the lowest index: 1.42 days of average time for germination.

The data presented above make it clear that it was the immersion in water at 90 °C for 10 minutes, followed by rest for 16 hours in water at room temperature which led to the greater speed of dormancy breaking of the seeds of this species. The association of immersion in hot water at 90 ºC with water at room temperature was intended to verify if there was an increase in the germinative performance of this species. It was observed that the germination speed was higher and resulted in a significant difference with the other treatments studied. In fact, the seeds of Black Wattle have tegument dormancy due to waterproofing in the tegument, so it did not respond significantly to sowing without treatment.

For the variables evaluated at two months after sowing, it was verified that the treatment of immersion in hot water at 90 ºC for 5 minutes, followed by resting in water

at room temperature for 16 hours (T2) was the one that demonstrated greater development of the root length of the seedlings, with 161.53 mm of root.

Figure 2 – Mean data of the germination speed index (IVG) of Acacia mearnsii De Wild seeds submitted to pre-germination treatments. Means followed by distinct letters differ from each other by the t test at the 5% probability level. Coefficient of variation (CV) of 4.62%



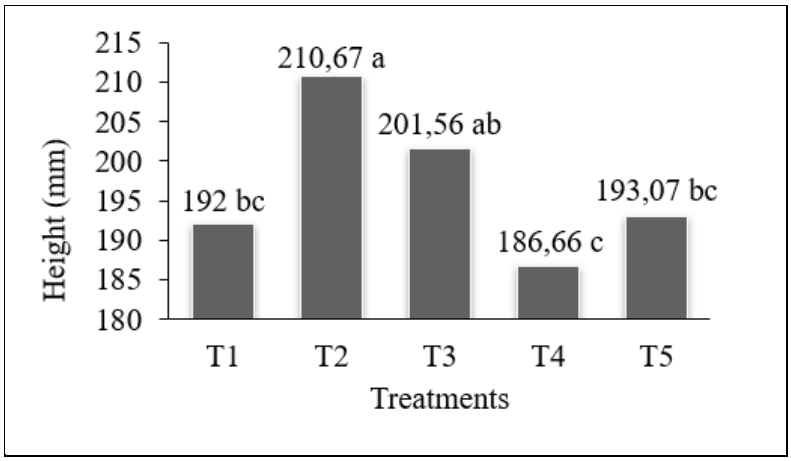
The second best response was observed with T3, with 153.25 mm (Table 2). This, however, did not differ statically from the witness that presented 149.17 mm, and the witness, in turn, did not differ from the worst treatments observed: T4 and T5, with 144.64 and 146.38 mm, respectively.Analyzing the Table 2, it can be affirmed that better results for aerial part length were also obtained with water immersion treatments at 90 ºC for 5 and 10 minutes, followed by resting in water at room temperature for 16 hours (T2 and T3), with 49.14 and 48.31 mm, respectively. However, there was no statistically significant difference between these treatments and the others. The highest average total length was also reached by treatment in hot water for 5 minutes: 210.67 mm. This, reached approximately 9.0 cm more than the second best average (T3), according to Figure 3.

Table 2 – Results of the variables root length, aerial part length and total length analyzed under seedling nursery conditions on Black Wattle seedlings at two months after sowing

Treatments	Root length (mm)	Length of the aerial part (mm)
T1	149,17 bc	42,83 a
T2	161,53 a	49,14 a
T3	153,25 b	48,31 a
T4	144,64 c	42,02 a
T5	146,38 c	46,69 a
	CV(%) = 8,43	CV(%) = 10,01

Means followed by distinct letters in the column differ from each other by the t-test at the 5% probability level. T1 - Witness; T2 - Immersion in water at 90 °C for 5 minutes, followed by standing in water at room temperature for 16 hours; T3 - Immersion in water at 90 °C for 10 minutes, followed by standing in water at room temperature for 16 hours; T4 - Immersion in H_2SO_4 for 3 minutes; T5 - Immersion in H_2SO_4 for 5 minutes.

Figure 3 – Average height of Black Wattle seedlings evaluated under different treatments of dormancy at 60 days. Means followed by distinct letters in the column differ from each other by the t-test at the 5% probability level. Coefficient of variation (%) = 2.07



With a total length of 186.66 mm, the treatment that favored the growth was the immersion in sulfuric acid for 3 minutes, which reinforces the study by Bianchetti and Ramos (1982) where they affirm that to promote dormancy of Black Wattle the seeds should be immersed for times of 5 to 20 minutes.

In the case of stem diameter, the control (T1) presented the best results, as shown in Table 3, with 0.98 mm, followed by the treatment T2, with 0.91 mm, but for this variable it was not statistically significant difference was observed among the five evaluated treatments. The worst treatment for the variable stem diameter was T4, with 0.67 mm.

Table 3 – Results of the variables stem diameter and green mass weight analyzed under seedling nursery conditions on Black Wattle seedlings at two months after sowing

Treatments	Stem diameter (mm)	Weight of the Green Mass (g)
T1	0,98 a	0,428 a
T2	0,91 a	0,451 a
T3	0,85 a	0,537 a
T4	0,67 a	0,413 a
T5	0,73 a	0,483 a
	CV(%) = 16,32	CV(%) = 20,06

Means followed by distinct letters in the column differ from each other by the t-test at the 5% probability level. T1 - Witness; T2 - Immersion in water at 90 ºC for 5 minutes, followed by resting in water at room temperature for 16 hours; T3 - Immersion in water at 90 °C for 10 minutes, followed by standing in water at room temperature for 16 hours; T4 - Immersion in H_2SO_4 for 3 minutes; T5 - Immersion in H_2SO_4 for 5 minutes. As observed, for green mass weight there was also no significant difference, but T3 and T5 were the best, with 0.537 and 0.483 g, respectively. The treatment that promoved the smallest increase in mass was T4, with 0.413 g.

4 CONCLUSIONS

The treatment of hot water immersion at 90 ºC for 5 minutes followed by rest in water at room temperature for 16 hours exceeded the rest of the methods during the evaluations in the seedling nursery, where it presented germination of 81.29% with an average time of 18.23 days.

In the evaluation of the initial development parameter the same treatment was also the one that showed more uniformity of the physical characteristics of the seedlings, with greater root length and aerial part. The treatment that least satisfied the characteristics desired for initial development was the method of immersion in H_2SO_4 for 3 minutes, which presented, for all variables, the worst means.

This study allows to conclude that for seedling nursery workers this is a good result, since immersion in hot water is a simple, low-cost, easy to handle, low-run and safe method to promote the germination of *Acacia mearnsii* De Wild.

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