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Effect of Seed Pre-sowing Treatment on Germination of Three Acacia Species Indigenous to Botswana

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Authors' contributions

This work was carried out in collaboration between the authors. Authors TM and WM designed the study and author LR carried out laboratory work. Authors LR and WM managed the literature searches and TM managed statistical analysis. Authors TM and WM managed the manuscript. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Effectiveness of selected immersion-based seed pre-sowing treatments (cold water, hot water and concentrated sulphuric acid) on the germination of three *Acacia* species (*A. tortilis*, *A. erioloba*, and *A. nigrescens*) was studied between December 2012 and January 2013. For each species, four treatments (including the control) were replicated four times in a Completely Randomized Design. Percentage germination, germination mean time (GMT) and germination index (GRI) were calculated and the data were subjected to analysis of variance (ANOVA). Significant differences in germination were observed across the treatments. Concentrated sulphuric acid significantly ($P < 0.01$) increased the germination percentages of *A. erioloba* (87%) and *A. nigrescens* (30%) while the control, cold water and hot water treatments significantly ($P < 0.01$) reduced their germination percentages (5%). However, treating *A. tortilis* with hot water for 9 min significantly ($P < 0.01$) enhanced its germination percentage (30%) compared to the other treatments. As expected the control had the highest GMT, although this was not significant for *A. tortilis*. The GRI revealed similar trend as germination percentages across the treatments for the three *Acacia* species. Based on these results, we recommend concentrated sulphuric acid and hot water as suitable seed pre-treatments for enhancing the germination of the three

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<i>Acacia</i> species.

Keywords: *Acacia tortilis*; *Acacia erioloba*; *Acacia nigrescens*; seed pre-treatment; germination mean time and germination index.

1. INTRODUCTION

The use of indigenous tree species in afforestation programmes has received considerable attention in Botswana in recent years. Nowadays efforts are directed towards choosing indigenous tree species that can be used for different purposes and have the capability to cope with prevalent harsh environmental conditions of Botswana. *Acacias* are multipurpose trees that have proved to survive harsh environmental conditions. *Acacia* is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae [1,2]. The *Acacia* species are widely distributed in arid and semi-arid regions [3] and are the most successful survivors in these regions because they have characteristics required to withstand harsh climatic and environmental conditions [4,5]. *Acacias* are drought tolerant multipurpose species that provide local communities with fodder, wood and non-wood products as well as services such as shade and live fencing [6].

The main problem encountered in propagating *Acacia* seedlings for afforestation programmes in arid and semi-arid areas is poor seed germination caused by the water-impermeable seed coat [7,8], which exerts physical dormancy [9]. Seed dormancy hinders the completion of germination of an intact viable seed under favourable conditions [3,10,11]. Seed dormancy has evolved differently across species through adaptation to the prevailing environment, to allow germination to take place when conditions are likely to be favourable for a new plant to establish itself [3,10,11,12,13]. Seed germination is a complex process and incorporates events that start with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis [14]. Due to water-impermeable seed coat of most *Acacia* seeds do not germinate promptly when subjected to conditions normally regarded as suitable for germination. This makes it impossible to obtain uniform germination in the nursery and at times it may take *Acacia* seeds up to 6 months to germinate.

To break dormancy and obtain rapid germination, the seeds of *Acacia* species must be subjected to some physical or chemical treatment if the species is to be successfully included in tree-planting programmes. Seed pre-sowing treatments break the impermeable seed coat and allow the embryo to imbibe water. Several artificial methods have been used to break the dormancy of tree seeds with a hard, impermeable seed coat [15]. Mechanical or chemical (sulphuric acid and hot water) treatments are the method most often used to break seed coat dormancy to obtain uniform and rapid germination [7,9,16,17,18,20,21,22,23]. Lack of knowledge of the germination requirements of indigenous trees species including *Acacias* is an obstacle for their artificial regeneration in Botswana. This present study was carried out to compare the effects of some recommended seed pre-sowing treatments (including, control, cold water, hot water and sulphuric acid) on germination parameters of three *Acacia* species grown in Botswana.

2. MATERIALS AND METHODS

Three experiments were conducted with seeds of three *Acacia* species (*A. tortilis*, *A. erioloba* and *A. nigrescens*) under laboratory conditions at the Department of Crop Science

and Production, Botswana College of Agriculture, Botswana, Africa, between December 2012 and January 2013. The College is on latitude 23°34'S and longitude 25°57'E with an altitude of 994 m above sea level, located at Sebele, 10 km from Gaborone along Gaborone to Francistown highway. The seeds for the experiment were obtained from the National Tree Seed Centre, Ministry of Environment, Wildlife and Tourism, Gaborone, Botswana.

2.1 Experimental Design

The experiments were laid out in a completely randomized design (CRD) with four replicates. In both experiments, treatments were as follows; non-treated seeds (control), immersing seeds in cold water, immersing seeds in concentrated sulphuric acid (98%) (3, 6, 9 and 12 min) and immersing seeds in hot water (3, 6, 9 and 12 min). A total of 1200 seeds (400 seeds for each species of *Acacia*) were used, and they were tested for viability by floating them in distilled water prior to the experiment. Only those that sank and settled at the bottom were deemed viable and hence used for the experiment. Ten (10) seeds were sown in forty (40) glass petri dishes as per the treatments containing moistened germination paper for each species (thus giving 400 seeds). Germination papers were kept moistened by adding distilled water whenever necessary throughout the duration of the experiments.

2.2 Pre-sowing Treatments

Untreated seeds (control) were germinated in petri dishes, whereas cold water-treated seeds were soaked in distilled water whose temperature was about 25°C for 24 hours, after which they were germinated in petri dishes. As for chemical scarification, a method described by [24] was used for this treatment: seeds were divided and put into four 100 ml heat-resistant non-corrosive glass beaker and concentrated sulphuric acid (98%) was poured slowly on the side of the beaker to a level where all the seeds were covered (50 ml). The seeds in the four beakers were left for different times; 3, 6, 9 and 12 min in concentrated sulphuric acid, after which they were removed and the acid drained off into another beaker. Seeds were thoroughly washed and rinsed in tap water and distilled water, respectively, to remove all the acid. Seeds were then soaked in distilled water to be ready for sowing after 24 hours. Finally, hot water-treated seeds were divided and put into four 100 ml heat-resistant glass beaker. The seed in the four beakers were soaked in hot water whose temperature was about 98.8°C and left for different times; 3, 6, 9 and 12 min, after which the seeds were removed. Seeds were then soaked in distilled water for 24 hours.

2.3 Germination Parameter

The following germination parameters were determined daily, however the results were cumulatively presented in four days intervals;

Germination percentage (GP) - the number of germinated seeds as a percentage of the total number of tested seeds given as;

$$GP = (\text{germinated seeds}/\text{total tested seeds}) \times 100 \% \quad (1)$$

Germination mean time, was given according to [25] as;

$$(GMT \text{ days}): = \Sigma Ti Ni/S \quad (2)$$

Where T_i is the number of days from the beginning of the experiment, N_i the number of seeds germinated per day and S is the total number of seeds germinated.

Germination index (GRI) - was calculated for each treatment using the following equation;

$$GRI = (G1/1) + (G2/2) + \dots + (Gx/x), \quad (3)$$

Where G is the germination day 1, 2..., and x represents the corresponding day of germination [26].

2.4 Data Analysis

The data collected was subjected to analysis of variance (ANOVA) using the STATISTIX-8 program. Treatment means were separated using Tukey's Studentized Range (HSD) Test at $P \leq .05$.

3. RESULTS

3.1 *Acacia erioloba*

Immersing *A. erioloba* seeds in concentrated sulphuric acid (98%) for 3 min significantly ($P < .01$) increased the seed germination compared to the control and hot water for the first four days (Table 1). Similar germination trend was observed after 8, 12 and 16 days. However, germination was slightly higher in seeds immersed in sulphuric acid after 12 and 16 days. The control had the highest germination mean time (GMT) ($P < .01$) of 12 days. No significant differences in GMT were observed across the other treatments (Table 1). Treating *A. erioloba* seeds with sulphuric acid for 6 min significantly ($P < .01$) increased the germination index (GRI) but the control, cold water and hot water treatments significantly ($P < .01$) reduced the GRI (Table 1).

3.2 *Acacia tortilis*

A. tortilis seeds immersed in hot water for 9 min significantly ($P < .01$) increased the germination as compared to the control 4, 8, 12 and 16 days after sowing (Table 2). However, the germination percentages were very low with a maximum of 30% after 16 days. Treatments had no significant ($P > .05$) effect on the GMT of *A. tortilis* seeds (Table 2). In addition, immersing *A. tortilis* in hot water for 9 min significantly ($P < .01$) increased the GRI as compared to the control (Table 2).

Table 1. Effect of seed pretreatment on mean germination parameters of *A. erioloba*

Treatments	Mean germination percentages				GMT	GRI
	Day 4	Day 8	Day 12	Day 16		
Control	0.0 ^b	2.5 ^b	2.5 ^b	7.5 ^b	12.0 ^a	0.1 ^b
Cold water	5.0 ^b	5.0 ^b	5.0 ^b	5.0 ^b	1.0 ^b	0.0 ^b
Hot water						
Hot water (3 min)	2.5 ^b	2.5 ^b	2.5 ^b	5.0 ^b	3.8 ^b	0.0 ^b
Hot water (6 min)	10.0 ^b	12.5 ^b	12.5 ^b	12.5 ^b	1.9 ^b	0.1 ^b
Hot water (9 min)	12.5 ^b	12.5 ^b	12.5 ^b	12.5 ^b	1.5 ^b	0.1 ^b
Hot water (12 min)	2.5 ^b	5.0 ^b	5.0 ^b	5.0 ^b	1.8 ^b	0.0 ^b
Sulphuric acid (98%)						
Sulphuric acid (3 min)	80.0 ^a	85.0 ^a	85.0 ^a	85.0 ^a	2.7 ^b	0.5 ^a
Sulphuric acid (6 min)	72.5 ^a	80.0 ^a	87.5 ^a	87.5 ^a	3.7 ^b	0.6 ^a
Sulphuric acid (9 min)	65.0 ^a	75.0 ^a	75.0 ^a	75.0 ^a	3.2 ^b	0.5 ^a
Sulphuric acid (12 min)	67.5 ^a	67.5 ^a	75.0 ^a	75.0 ^a	3.2 ^b	0.5 ^a
Significance	**	**	**	**	**	**
HSD	21.46	22.99	21.80	21.91	5.98	0.14

** Highly significant at $P < .01$. Means separated using Tukey's studentized range (HSD) test at $P \leq .05$, means within columns followed by the same letters are not significantly different. GMT is germination mean time and GRI is germination index

Table 2. Effect of seeds pretreatment on mean germination parameters of *A. tortilis*

Treatments	Mean germination percentages				GMT	GRI
	Day 4	Day 8	Day 12	Day 16		
Control	0.0 ^d	5.0 ^{cd}	5.0 ^{bc}	5.0 ^b	3.3	0.0 ^b
Cold water	8.0 ^{bc}	15.0 ^{abc}	17.5 ^{ab}	17.5 ^{ab}	5.4	0.1 ^{ab}
Hot water						
Hot water (3 min)	0.0 ^d	5.0 ^{cd}	5.0 ^{bc}	5.0 ^b	3.5	0.0 ^b
Hot water (6 min)	7.5 ^{bc}	7.5 ^{bcd}	7.5 ^{bc}	7.5 ^b	1.0	0.0 ^b
Hot water (9 min)	20.0 ^a	25.0 ^a	30.0 ^a	30.0 ^a	3.0	0.2 ^a
Hot water (12 min)	2.5 ^{cd}	5.0 ^{cd}	5.0 ^{bc}	5.0 ^b	2.5	0.0 ^b
Sulphuric acid (98%)						
Sulphuric acid (3 min)	0.0 ^d	0.0 ^d	2.5 ^c	5.0 ^b	5.8	0.0 ^b
Sulphuric acid (6 min)	2.5 ^{cd}	17.5 ^{ab}	17.5 ^{ab}	17.5 ^{ab}	5.8	0.1 ^{ab}
Sulphuric acid (9 min)	10.0 ^b	17.5 ^{ab}	17.5 ^{ab}	17.5 ^{ab}	3.7	0.1 ^{ab}
Sulphuric acid (12 min)	10.0 ^b	17.5 ^{ab}	17.5 ^{ab}	17.5 ^{ab}	3.9	0.1 ^{ab}
Significance	**	**	**	**	ns	**
HSD	7.28	12.45	13.57	13.75	ns	0.09

** Highly significant at $P < .01$, ^{ns} non-significant at $P > .05$. Means separated using Tukey's studentized range (HSD) test at $P \leq .05$, means within columns followed by the same letters are not significantly different. GMT is germination mean time and GRI is germination index

3.3 *Acacia nigrescens*

Immersing *A. nigrescens* seeds in sulphuric acid for 3 min significantly ($P < .01$) increased the germination percentage (30%) from 4 to 16 days as compared to the control and cold water (Table 3). As would be expected the control had the highest GMT of 6.5 days (Table 3). The highest GRI was recorded in *A. nigrescens* seeds that had been immersed in sulphuric acid for 3 min (Table 3).

Table 3. Effect of seeds pretreatment on mean germination parameters of *A. nigrescens*

Treatments	Mean germination percentages				GMT	GRI
	Day 4	Day 8	Day 12	Day 16		
Control	2.5 ^b	5.0 ^b	7.5 ^c	12.5 ^c	6.5	0.1 ^{bcd}
Cold water	7.5 ^b	12.5 ^b	17.5 ^{abc}	17.5 ^{abc}	5.6	0.1 ^b
Hot water						
Hot water (3 min)	7.5 ^b	7.5 ^b	10.0 ^{bc}	10.0 ^c	3.8	0.1 ^{cd}
Hot water (6 min)	7.5 ^b	7.5 ^b	7.5 ^c	7.5 ^c	1.0	0.1 ^d
Hot water (9 min)	12.5 ^b	17.5 ^{ab}	22.5 ^{ab}	27.5 ^{ab}	5.8	0.2 ^a
Hot water (12 min)	10.0 ^b	15.0 ^b	15.0 ^{bc}	17.5 ^{abc}	5.0	0.1 ^b
Sulphuric acid (98%)						
Sulphuric acid (3 min)	30.0 ^a	30.0 ^a	30.0 ^a	30.0 ^a	1.7	0.2 ^a
Sulphuric acid (6 min)	7.5 ^b	7.5 ^b	7.5 ^c	7.5 ^c	0.8	0.1 ^d
Sulphuric acid (9 min)	15.0 ^b	15.0 ^b	15.0 ^{bc}	15.0 ^{bc}	1.0	0.1 ^{bc}
Sulphuric acid (12 min)	7.5 ^b	7.5 ^b	7.5 ^c	7.5 ^c	0.8	0.1 ^d
Significance	**	**	**	**	ns	**
HSD	12.65	13.57	14.27	12.65	ns	0.08

** Highly significant at $P < .01$, ^{ns} non-significant at $P > .05$. Means separated using Tukey's studentized range (HSD) test at $P \leq .05$, means within columns followed by the same letters are not significantly different. GMT is germination mean time and GRI is germination index

4. DISCUSSION

Results of the present study show that immersing *A. erioloba* seeds in concentrated sulphuric acid for 3 and 6 min enhanced germination by breaking the seed coat. In addition, the germination of *A. nigrescens* seeds was enhanced by immersing them in concentrated sulphuric acid for 3 min. These results are supported by studies conducted elsewhere using seeds of different plant species [19,27,28,29,30,31]. Immersing seeds in concentrated sulphuric acid breaks the seed coat which hinders the germination of an intact viable seeds placed under conditions suitable for germination [3,10,11]. Breaking seed coat dormancy by scarification breaks the seed coat and exposes the lumens of the macrosclereids cells, allowing the seed to imbibe water. Imbibition of water after breaking the seed coat is known to trigger germination [27], whereas water may not be available to the embryo in the untreated seeds. The duration of exposure of the seeds to the concentrated sulphuric acid is critical since long soaking periods can result in excessive burning of the seed coat, thereby causing damage to the embryo as observed in studies conducted elsewhere [29,32,33].

Soaking *A. tortilis* seeds in hot water also enhanced the germination. These results are in agreement with those of other authors who reported that immersing dry seeds of other tropical tree species in boiling water enhanced seed germination [28,34,35]. However, the germination percentages observed in the hot water treatment were lower compared to figures reported in some of the above mentioned studies, probably due to short soaking periods adopted in the present study. Boiling water softens the seed coat, allowing water to enter the seed and induce physiological changes that subsequently lead to the germination of the embryo. Hot water did not significantly increase the germination of the other two *Acacia* species. It could be that *A. erioloba* and *A. nigrescens* seeds require longer soaking periods in hot water to soften the seed coat.

The results of this study show that *Acacia* species are characterised by physical dormancy (seed coat dormancy). Poor seed germination is caused by the water impermeable seed coat [7,8], and this exerts physical dormancy [9]. This is the main problem encountered when propagating *Acacia* seedlings for afforestation programmes in arid and semi-arid areas. Physical dormancy is most often caused by a modification of seed covering, especially the outer integument layer of the seed that may become hard, fibrous, or mucilaginous during dehydration and ripening [24]. For the seeds to germinate, physical dormancy is often broken by mechanical and chemical scarification [7,15,16,17,18,19,20,21,22,23,24]. Concentrated sulphuric acid and hot water broke the impermeable seed coat and allowed the embryo to imbibe water in the present study, hence the observed rapid and enhanced germination of the *Acacia* species.

In general, seeds soaked in cold water showed the lowest germination percentages. This is in agreement with Amusa [36] who observed that soaking seeds in cold water reduced the germination of *Azelia africana* seeds due to oxygen deficiency. *A. nigrescens* treated with cold water had more germinated seeds as compared to *A. tortilis* and *A. erioloba*, and this probably suggests that *A. nigrescens* possesses a weak seed coat. Haper [37] stated that the cause of seed coat impermeability is not fully understood, but that under natural conditions and after most treatments, the first site at which water penetration occurs is the stophioles. This is the weakest and the least reinforced area of the seed coat and it is seen as the small raised area close to the hilum but on the side opposite the micropyle [38].

5. CONCLUSION

Based on the results presented, it is clear that the seeds of *Acacia* species need pre-sowing treatment to enhance germination. The results also suggest that the type of seed dormancy in *Acacia* species is physical dormancy (seed coat dormancy). Concentrated sulphuric acid or hot water can be used to pre-treat *Acacia* seeds in order to scarify or break the impermeable seed coat and allow the embryo to imbibe water, which is a way of ensuring rapid and enhanced germination of the seeds of *Acacia* species.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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