Influence of Treatments to Break Seed Dormancy of *Acacia saligna*

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Abstract

Seeds of *Acacia* species are known to have hard coats that completely prevent the imbibition of water and exchange of gases, such physical seed-coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons such as that of Northern Libya. To accelerate germination of *Acacia* seeds, various pre-treatment methods have been assayed including soaking in boiling water and scarification of the seed coat. Both boiling water treatment and scarification had a clear positive impact on germination. Pre-treatment increased germination of *Acacia spp.* up to 90%. Immersion of seeds in boiling water may stimulate germination by causing rupture of the lens tissue; the results further, indicated that treatment of *Acacia saligna* by boiling water more than three times is not worth while. Seeds made water- permeable by boiling water treatment germinated at a much slower rate than those made water- by mechanical scarification. Manual chipping of the seed gave larger improvements in germination rate, and the seeds began to germinate faster than those given any boiling water treatment. Chemical (H_2SO) and mechanical (with a sand paper scarified). Scarification improved germination rate response to all treatment. However, the boiling treatments are easy and safe to treat large number of seeds and higher germination percentage (90%) obtained in this way. Although ttreatment by scarification was also successful and gave 90 % germination of seeds, it is hard to work and somewhat impractical with a large amount of seed.

The results of this study suggest that treatment of *Acacia saligna* by boiling three times gave the best seed germination.

Introduction

Aforestation in Libya has been drawing considerable attention for several decades. Efforts have been made to choose tree species that can be used for a range of purposes and can cope with the prevalent harsh environmental conditions. An important problem encountered in using *Acacia* species in aforestation programmes is the poor germination of their seeds if untreated. This is due to their water impermeable testas, which exerts a physical exogenous dormancy (Holmes, McDonald, and Juritz 1987). Seeds of *Acacia* species are known to have hard coats that completely prevent the imbibition of water and exchange of gases, thus preventing initiation of the germination process (Khasa 1993). *Acacia* seeds, therefore, will not germinate promptly when placed under conditions that are normally regarded as suitable for germination. Such physical seed coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons. The seeds usually have a fleshy outgrowth called an aril where the seed attaches to the

pod. The arils may be white or brightly coloured and are often attractive to ants or birds that help disperse the seed (Entwistle *et al.* 1996). To accelerate germination of *Acacia* seeds, various pre-treatment methods have been assessed including soaking in boiling water and sulphuric acid scarification (Doran and Genn 1987). The proportion of hard-coated seeds in a sample may be influenced by environmental conditions during the growth of the plant, the degree of the maturation of the seeds when collected, and duration and type of seed storage (Willan 1985). One of the simplest and most direct methods is to cut, drill or file a small hole in the seed coat before sowing. This was done on *Acacia* seeds in Honduras (Willian 1985). In other work soaking the seed in cold water for many hours gave an effective treatment for *A. farnesiana* (Doran and Gunn 1987). However, it is vital to practise efficient, easily applied seed pre-treatment methods that can be used if large numbers of plants are to be established uniformly and cheaply. The aim of this study was to establish efficient methods for removing hard seed coat dormancy of *A. saligna*.

Materials and methods

Seeds of *Acacia saligna* (Labill.) H.L. Wend. were supplied from Setropa BV, Troelstralaen 4, 1272 JZ Huizen, The Netherlands in 1 kg quantities in air-tight aluminium foil bags. This experiment was carried out from November to December 2007 in a laboratory at Coventry University England.

Mechanical and chemical

Seeds were divided into two groups, with arils and without arils. Germination of unscarified seeds with and without arils was tested without the application of dormancy breaking methods to assess the effects of arils. Seeds without arils were scarified by mechanical and chemical means to break their hard seed coat dormancy. Mechanical scarification was achieved by removing a small section of the seed coat at either the aril end of the seed or the opposite end. Sand paper scarification was carried out using coarse sand paper (aluminium oxide (45-PG). Seeds were abraded by rubbing the side opposite the embryo between two sheets of sand paper for 10, 20, 30, 40 or 50 min. Chemical scarification involved the immersion of seeds in an excess of 98% sulphuric acid for 20, 40, 60, 80 or 100 min. Seeds were immersed in approximately 100 ml of sulphuric acid per 100 seeds, in 250 ml beakers at room temperature. The seeds were stirred occasionally to prevent them sticking together and to ensure contact with the acid. After scarification, the seeds were removed from the acid by pouring through a plastic sieve and were then washed for 10-20 min under running tap water. When all traces of acid had been removed, the seeds were blotted dry and allowed to air dry at room temperature.

Boiling water

Seeds were treated with boiling water to break their hard seed coat dormancy. Seeds were placing in boiling water and cooled to room temperatures. The volume

of boiling water was approximately 100 ml of water per 100 seeds, in 250 ml beakers at room temperature. The treatments applied were. Treatment 1: Control treated by adding 100 ml of tap water to the seeds, Treatment 2: Addition of 100 ml boiling water to seeds and seeds left to cool in water for 30 min, Treatment 3: As 2 but after 30 min the boiling water was replaced by another 100 ml of boiling water, Treatment 4: As 3 but after 60 min the boiling water was replaced by a further 100 ml of boiling water, Treatment 5: As 4 but after 90 min the boiling water was replaced by another 100 ml of boiling water.

Seed germination

Seeds were germinated in 9 cm plastic Petri dishes containing two Whatman No 1 filter papers and 10 ml of distilled water. Twenty seeds were sown per dish and five replicate dishes were used for all treatments. The design adopted was a completely randomized design. Seeds were incubated in the dark at a constant 15°C. The seeds were observed daily and scored as germinated when approximately 2 mm of radicle had emerged through the testa. Germinated seeds were removed from the Petri dishes daily. Final germination was calculated as the maximum germination obtained when no further germination took place for several days. Germination rate was recorded as $1/t_{50}$ where t_{50} is the number of days required to reach 50% of the final germination.

Statistical analysis

The significance of differences means was tested by one-way analysis of variance followed by the calculation of a least significant difference for all pairs comparisons using Tukey's test at $p \leq 0.05$. Final germination data was arcsin transformed before analysis.

Results

Final germination percentage

The analysis of variance revealed that germination percentage and germination rate of *A. saligna* was affected significantly by the pre-germination treatments applied. Figure 1 shows that mechanical scarification by cutting the testa with nail clippers significantly increased seed germination compared with untreated seeds. There was no significant different in germination between seeds cut at the aril end or the opposite end. There was no significant difference in germination between uncut seed with or without arils.

Figure 2 shows that treating *A. saligna* once with boiling water significantly improved seed germination compared with the control. Boiling water applied twice (60 min) improved germination compared with once. Increasing the frequency of boiling water treatments to three times (90 min) substantially increased germination and led to all seeds germinating at the end of experiment. However, treatment of seeds with boiling water four times (120 min) was less effective than three times. Figure 3 shows that scarification using sulphuric acid increased germination compared with the untreated control. Germination increased significantly with

increasing time of treatment up to 60 and 80 min but decreased again when treatment was extended to 90 min. Figure 4 shows that sand paper treatment significantly increased germination percentage. Germination increased with increasing time of treatment up to 30 min but did not different significantly between 30, 40 or 50 min.

Germination rate

Figure 5 shows that mechanical scarification by cutting the testa with nail clippers significantly increased germination rate compared with untreated seeds. There was a significant difference in germination rate between seeds cut at the aril end or the opposite end. There was no significant difference in germination rate between uncut seed with or without arils. Figure 6 shows that treating *A. saligna* once (30 min) or twice (total = 60 min) with boiling water did not significantly improve germination rate compared with the control. However, increasing the frequency of boiling water treatments to three times (total = 90 min) increased germination rate but treatment of seeds with boiling water four times (total = 120 min) was less effective than three times. Figure 7 shows that scarification using sulphuric acid for all times tested increased germination rate compared with the control. There was no difference in germination rate between seeds treated for 20, 40, 60 or 80 min but germination rate decreased again when treatment was extended to 90 min. Figure 8 shows that sand paper treatment had a significant effect on germination rate. Sand paper significantly increased germination rate. Germination increased with increasing time of treatment up to 30 min but did not different significantly between 20, 30, 40 or 50 min.

Discussion

Seeds of *Acacia* species are known to have hard coats which are considered to be one of several strategies for survival in the spatially and temporally variable environment. Hard coats can completely prevent the imbibition of water and exchange of gases, thus preventing initiation of the germination process (Khasa 1993). Such physical seed coat dormancy occurs most frequently in species adapted to alternating dry and wet seasons such as that of Northern Libya. To accelerate germination of *A. saligna* seeds, various pre-treatment methods were assayed, including soaking in boiling water, mechanical and chemical scarification of the seed coat. The simplest and most direct physical method is to cut, drill or file a small hole in the seed coat. This has been found to be successful. Sand paper is also used to reduce seed coat thickness by abrasion, especially on hard coated species. Mechanical scarification is reported to one of the most effective dormancy breaking treatments of *A. saligna* but cannot used to treated large amount of seeds as manual treatment of individual seeds, although safe and effective is very slow.

Boiling water treatment and scarification had a clear positive impact on germination. Immersion of seeds in boiling water may stimulate germination by causing rupture of the lens tissue, thereby allowing water to enter the seeds as

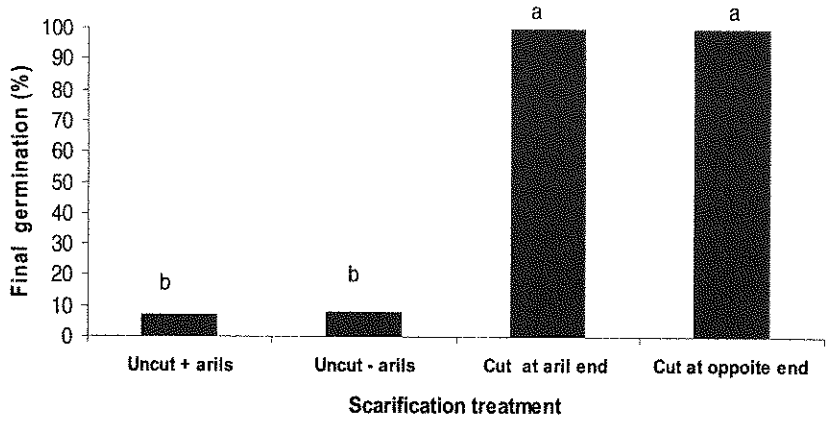
reported by Willan (1985) and Cavanagh (1987). The results further indicated that treatment of *A. saligna* by boiling water more than three times for a total of 90 min is not worthwhile. This finding agrees with that reported by Omori (1993). Seeds made water permeable by boiling water treatment germinated at a slightly slower rate than those made water permeable by cutting the seed coat. However, the boiling treatments are easy and it is safe to treat large number of seeds in this way.

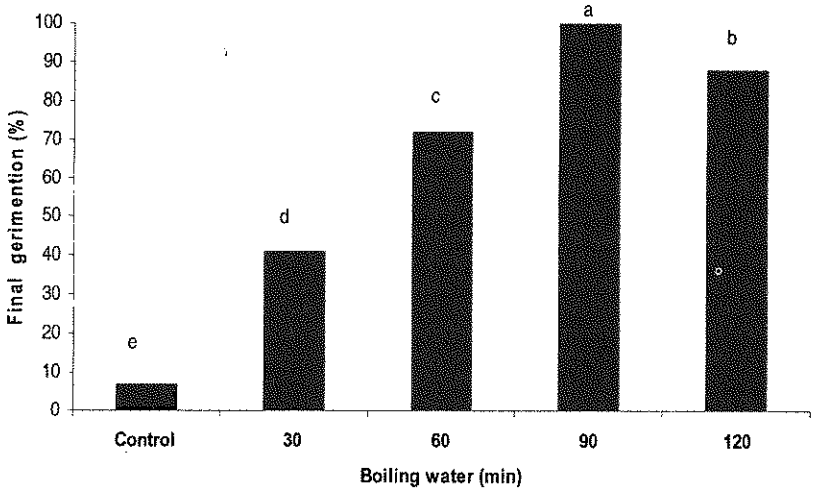
Although treatment by clipping the seed or sand paper abrasion was also successful and gave high germination percentage and rate, it is hard work and somewhat impractical with a large amount of seed. The results in this experiment, treating seeds with boiling water three times, can be compared with Omori (1993) who also obtained the best results with boiling water three times, and are further comparable to those of Youssef, Heikal, and Shaker (1991) who compared species of *Acacia* and found *A. saligna* gave the highest germination after boiling water treatment. Khasa (1993) investigated different methods of overcoming seed coat dormancy of *A. auriculiformis*. Of the water pre-treatments tested, soaking seeds in boiling water (heat source removed) gave the best germination (77.5% after 20 days of germination). Immersing the seeds in boiling water for 1 min gave the second highest result for water pre-treatments (51.0%). A number of methods have been used in Sabah, Malaysia, to break the dormancy of *A. mangium* seeds caused by the hard seed coat.

The most common and practical pre-treatment method now in use in almost all nurseries for *A. mangium* seeds is the hot water treatment. Larsen (1962) reported large increases in germination rates after the following procedure: seeds were dropped into ten times their volume of heated water for 30 min, and then immersed in 20 times their volume of cold water, where they imbibed for 18 h. Ninety-one percent of seeds of *A. mangium* pre-treated with boiling water for 30 min germinated. In laboratory trials in Sweden, sandpaper scarification followed by a 3 h cold water soak was the most effective treatment for *A. farnesiana* (Baskin and Cordell 2004). Using sulphuric acid as a seed coat softener, on the other hand, would be difficult in nursery conditions and is a hazardous method.

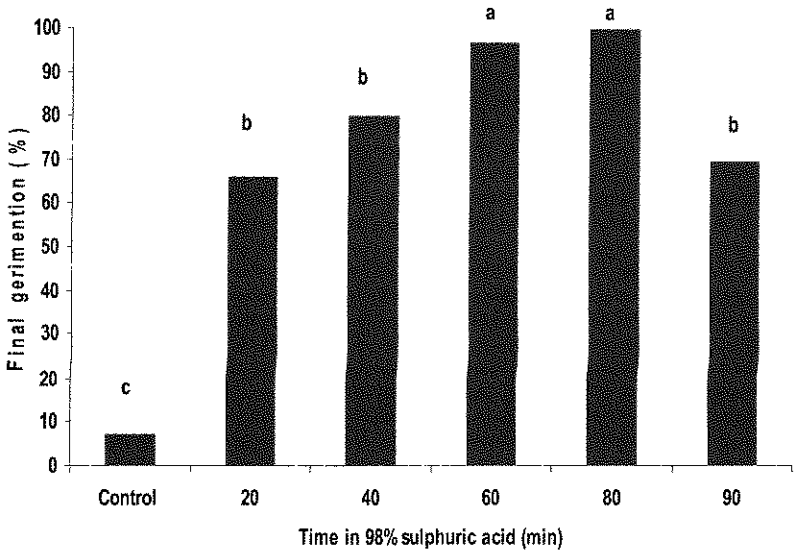
The germination rate of the seed of *A. saligna* was also improved by treatment with boiling in agreement with the results of Larsen (1962) who found that treatment of *A. senegal* with boiling water improved germination rate. The results confirm from the present experiment emphasize the necessity of treating *Acacia* seeds before sowing in seedbeds to promote a high germination percentage and to produce uniform seedling and improve the germination rate.

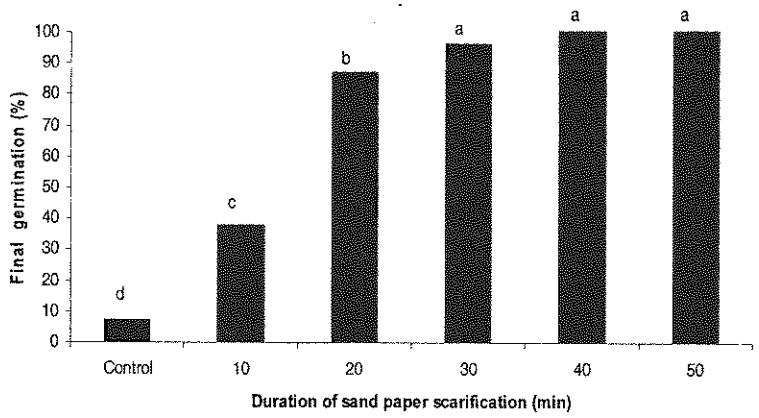
Treatment of *A. saligna* seeds with boiling water three times gave the best seed germination. It is easy and safe to treat large number of seeds in this way. These results may be useful as a guide for nursery operations leading to the successful establishment of *A. saligna* seedlings

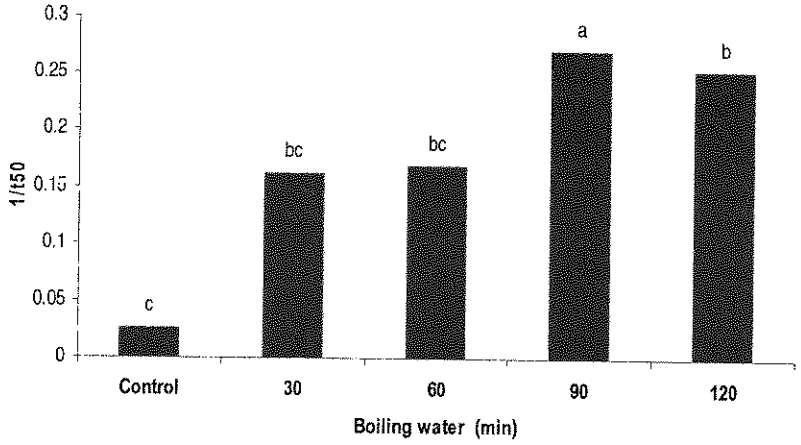

Final germination (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
b
Uncut + arils
Uncut - arils
Cut at aril end
Cut at oppoite end
Scarification treatment

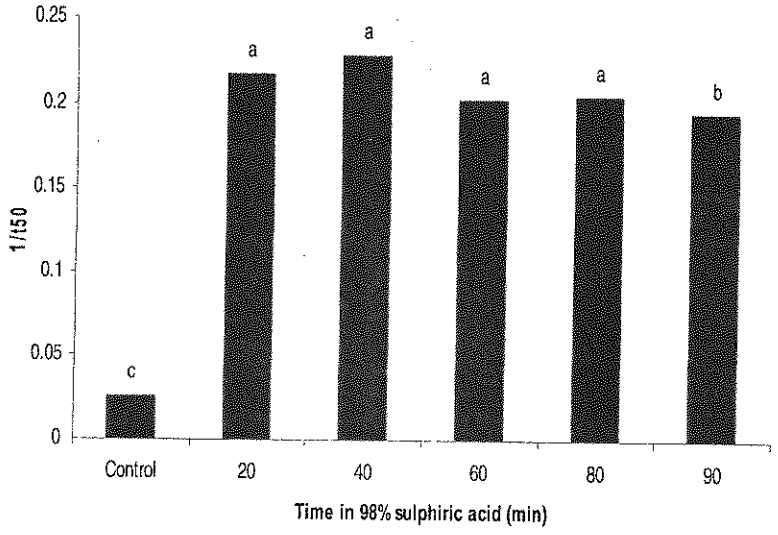

Final gerimention (%)
100
90
80
70
60
50
40
30
20
10
0
a
b
c
d
e
Control
30
60
90
120
Boiling water (min)

Influence of Treatments to Break Seed Dormancy






0.3
0.25
0.2
0.15
0.1
0.05
0
1/t50
c
bc
bc
a
b
Control
30
60
90
120
Boiling water (min)


0.25
0.2
0.15
0.1
0.05
0
1/t50
c
a
a
a
a
b
Control
20
40
60
80
90
Time in 98% sulphiric acid (min)

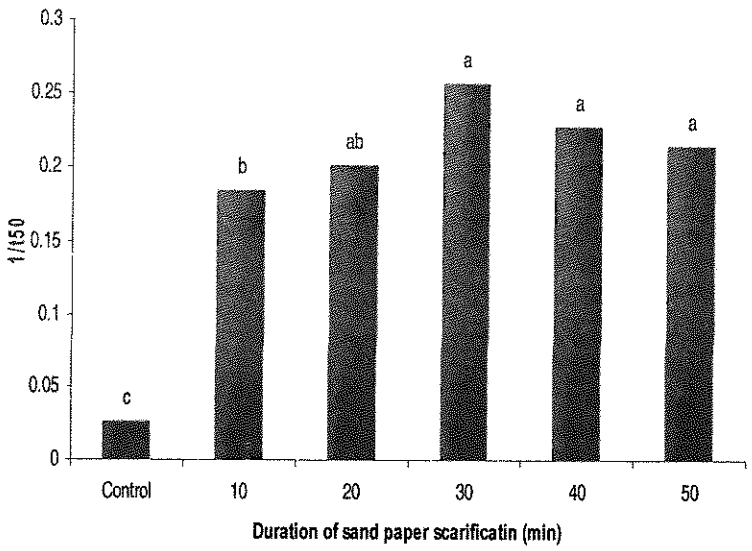

0.3
0.25
0.2
0.15
0.1
0.05
0
1/150
c
b
ab
a
a
a
Control
10
20
30
40
50
Duration of sand paper scarificatin (min)

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Figure 1: Effect of cutting on seed germination. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test on arcin transformed data.

Figure 2: Effect of boiling water treatment on seed germination. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test on arcin transformed data.

Figure 3: Effect of H_2SO_4 treatment on seed germination. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test on arcin transformed data.

Figure 4: Effect of sand paper treatment on seed germination. Means without a letter in common differ significantly at $p < 0.005$ using Tukey's test on arcin transformed data.

Figure .5: Effect of cutting on germination rate. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test.

Figure 6: Effect of boiling water treatment on germination rate. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test.

Figure 7: Effect of H_2SO_4 treatment on germination rate. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test.

Figure 8: Effect of sand paper treatment on germination rate. Means without a letter in common differ significantly at $p < 0.05$ using Tukey's test.