Effect of seed size, pre-germination treatment and sowing depth on germination, establishment and seedling growth of *Acacia saligna*

Zeineb yahya Elmghadmi and P.J.C. Harris

Abstract

Acacia saligna is grown widely along the Mediterranean coast of Libya where it is used both for erosion prevention and as forage for a range of species. The main problem encountered using *Acacia* species as forage for range programmes is the poor seed germination and seedling establishment. Experiments were carried to investigate the effect of dormancy breaking treatments and sowing depth on germination and seedling establishment of *A. saligna*, comparing large (8 x 3 mm), medium (5 x 2.5 mm) and small (3 x 2 mm) seeds. For dormancy breaking, Seeds were treated by pouring 200 ml of boiling water onto 400 seeds and leaving them to cool. Boiling water was applied one, two, three or four times at 30 min intervals. Final germination was determined in a Petri dish germination test and establishment assessed after sowing at 30 mm depth in sandy soil in pots in a greenhouse. In a second greenhouse experiment, dormancy of seeds of each of the size classes was broken by three applications of boiling water at 30 min intervals. Thereafter, imbibed seeds of each size class were sown at 10, 20, 30 or 40 mm depth. Seedling establishment was assessed after 7 weeks as well as the shoot and root lengths and dry weights of surviving seedlings. Treatment of *A. saligna* seeds of all sizes with boiling water three times gave the greatest seed germination. Large seeds germination earlier and achieved greater germination than smaller seeds, and greater seedling establishment from 30 mm depth sowing with all boiling water treatments. No or very few seeds emerged and survived from 40 mm depth. Sowing *A. saligna* seeds at 30 mm depth generally

gave the greatest seedling growth from large and medium seeds whereas 20 mm was more suitable for small seeds.

These results may be useful as a guide for carrying out some nursery operation, regarding *Acacia* species and cold enable avoiding the most complex hazard one.

Introduction

Acacia saligna is grown widely along the Mediterranean coast of Libya where it is used both for erosion prevention and as forage for a range of species. *A. saligna* shrubs are one of the most important introduced shrubs that can suit the environmental conditions of the northern coast of Libya. The main problem encountered using *Acacia* species as a forage for range programmes is the poor germination of their seeds. This is due to their water impermeable testas, which exert a physical exogenous dormancy (Holmes *et al*. 1987). *Acacia*, therefore, will not germinate promptly when placed under conditions that are normally regarded as suitable for germination. To overcome seed dormancy artificially and obtain rapid and synchronous germination, the seed must be subjected to some physical or chemical treatment before sowing. The methods which are recommended are mechanical scarification or abrasion of the integument and scalding the seeds in boiling water. Seed size may be an important factor in seedling survival as it is likely to be affected by the quantity of metabolic reserves in the seed. A difference in seed size may in turn affect any of the following: seed distribution; seed water relation; persistence in the soil bank; seedling establishment; and plant fitness (Bonfil 1998). Small seeds are characteristic of species that have persistent, dormant, seed banks in the soil. Small size may facilitate burial, allow escape from predation, assist in dispersal and enhance germination rate. Small seeds tend to have a higher surface-volume ratio than larger once (Wulff 1986). One of the most effective adaptations for ensuring successful seedling establishment is the possession of large seeds. Seedlings from large seeds draw on greater metabolic reserves in the embryo and endosperm and thus can attain a larger initial seedling size (Westoby, Jurado, and Leisman 1992). The larger reserve in heavier seeds may allow more pre-photosynthetic growth of the seedling. Large seeds have the capacity to contain large embryos and substantial food reserves, which enable a seedling to achieve more growth of both root and shoot before it becomes dependent on it own photosynthesis. Enhanced nutrient reserves in large seeds and their translocation from cotyledons to elsewhere in the seedling during early growth can reduce the reliance of the seedling on external supplies of nutrients, a distinct advantage in infertile soil (Vaughton and Ramsey 2001). Black (1955) found that seed size determination the initial area of cotyledons; the area of the cotyledons determines the extent of the difference in the early vegetative growth stage between plants from different seed sizes. One aim of the study was to development a better understanding effect of seed size on germination.

Sowing depth is the mean vertical distance (cm) of seeds below the soil surface, after the seedbed has settled (Sylvester-Bradley, Grylls, and Roebuck 1985). Soil depth will influence aeration as well as penetration of water and light. Sowing depth may affect yield through its effect on date of emergence and percentage of plants emerging. Sowing deeper than necessary exposes the seeds to possible anaerobic conditions during wet periods and inevitably extends the pre-emergence growth period (Perry 1984). Heydecker (1956) suggested, however, that increased depth of sowing might increase root anchorage and moisture availability.

Materials and Methods

Seeds of *Acacia saligna* (Labill.) H.L. Wend was supplied from Setropa BV, Troelstralaen 4, 1272 JZ Huizen, The Netherlands in 1 kg quantities in air-tight aluminium foil bags.

Seeds were measured with Vernier calipers and graded into large (length 8 mm, width 3 mm), medium (length 5 mm, width 2.5 mm), and small (length 3 mm, width 2 mm) size.

Dormancy breaking test

A germination test was carried out to investigate the effect of seed size and boiling water treatment on germination. Seeds were treated by pouring 200 ml of boiling water onto 400 seeds and leaving them to cool. Boiling water was applied one, two, three and four times at 30 min intervals. Seeds treated once with cold water for 30 min were used as a control. 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 in each dish and four replicate dishes where used for each treatment in a completely randomized design. Seeds were incubated in the dark at a constant temperature of 15°C. The seeds were observed daily and scored as germinated when approximately 2 mm of radical 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.

For the greenhouse experiment seeds of each seed size x dormancy breaking treatment were germinated as described above, but after 24 h the seeds were checked to see if they imbibed water, as indicated by swelling of the seed, and imbibed seeds were sown at 30 mm depth in sandy soil. The greenhouse experiment was carried out at Coventry University, England during February and March 2007. The temperature during the entire growth period was maintained at 25°C and the relative humidity ranged from 40 to 60%. Tall profile pots with a diameter of 150 mm were used. The bottom of the pots contained a piece of filter paper cut to size so as to prevent the soil running out when dry. Sandy soil was added to the partly fill the pots, leaving sufficient room to bury the seeds at 30 mm depth. Twenty imbibed seeds were sown in each of four replicate pots for each seed size x dormancy breaking treatment. The pots were arranged on a bench in the greenhouse in a completely randomized design. Pots were watered as required to maintain a moist soil. Seedling establishment was assessed after 7 weeks as the number of plants which has emerged and survived until the end of the experiment.

Effect of sowing depth and seed size on seedling establishment and growth

For the second greenhouse experiment, dormancy of seeds of each of the size classes was broken by three applications of boiling water at 30 min intervals. Seeds were germinated as described above and after 24 h the seeds were checked to see if they imbibed water, as indicated by swelling of the seed. Thereafter, 80 imbibed seeds of each size class were selected for each of four sowing depth treatments, 10, 20, 30 or 40 mm depth. Twenty imbibed seeds were sown in each of four replicate pots for each of the four sowing depth treatments. Thus this part of the experiment comprised three seeds sizes, four sowing depths and four replicates of 20 seeds for each size x depth combination. The pots were arranged on a bench in the greenhouse in a completely randomized design. Seedling establishment was assessed after 7 weeks as the number of plants which has emerged and survived until the end of the experiment. The shoot and root lengths of surviving seedlings were measured when harvested after 7 weeks. Shoot and root dry weight per plant was measured after drying in an oven at 80°C for 48 h and cooling in a desiccators. Seeds that had not germinated and seedlings that had died were excluded from the dry weight estimates. This experiment was carried out twice once with irrigation with distilled water and once with tap water.

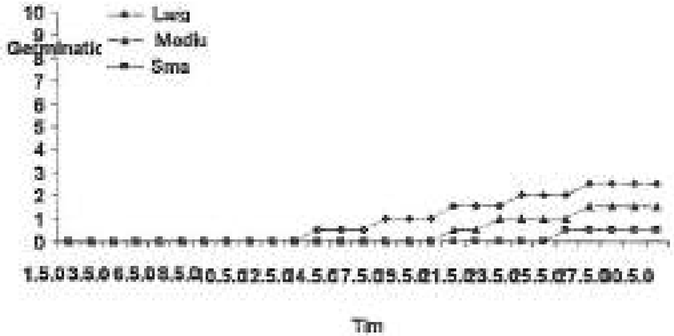
Statistical Analysis

The significance of differences between means was tested by one way analysis of variance and the calculation of a least significant difference for all pair's comparisons using Tukey's test. Germination and survival data were arcsin transformed before analysis of variance

Results

Figure 1A shows that with the control treatment (cold water only) large seeds started to germinate after 12 days. After 1 month, 25% of large, 15% of medium and only 5% of small seeds had germinated (Table 1). Treating *Acacia* seeds once with boiling water, improved germination somewhat compared with the control (Figure 1B, Table 1). Both large and medium seeds germination earlier than small and germination reached 40%, 30% and 20% for the large, medium and small seeds, respectively. Treating *Acacia* seeds with boiling water twice, increased large seed germination to 60% compared with 40% after one boiling water treatment (Figure 1C, Table 1). Increasing the number of boiling water treatments to three substantially reduced the time to the onset of germination and led to 95% of large seeds germinating after approximately one month (Figure 1D, Table 1). Treating *Acacia* seeds with boiling water four times had a similar effect to three times except for medium size seeds where final germination was significantly lower with four than with three treatments (Figure 1E, Table 1). With all dormancy breaking treatments tested, the final germination of large and medium size seeds was significantly great than that of small seeds (Table 1).

A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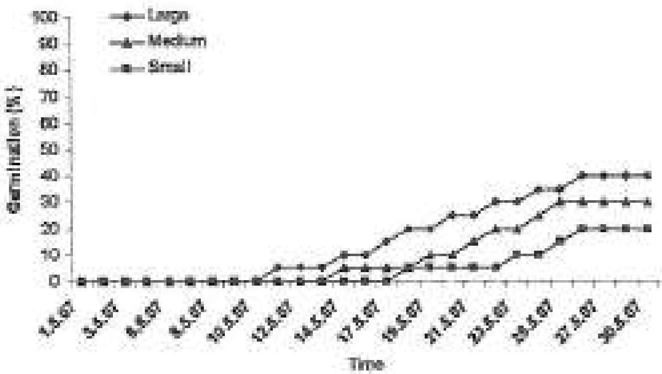
Large
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Germination (%)
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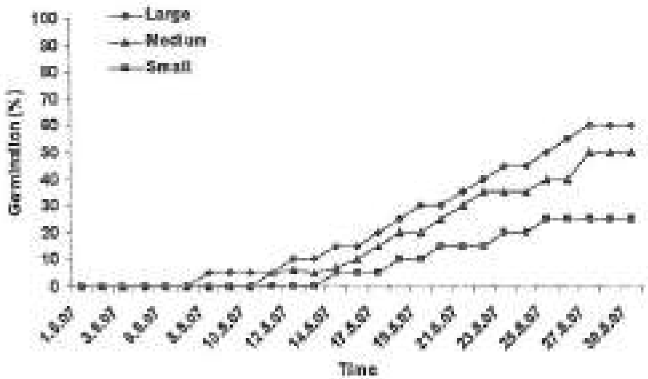
Large
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Germination (%)
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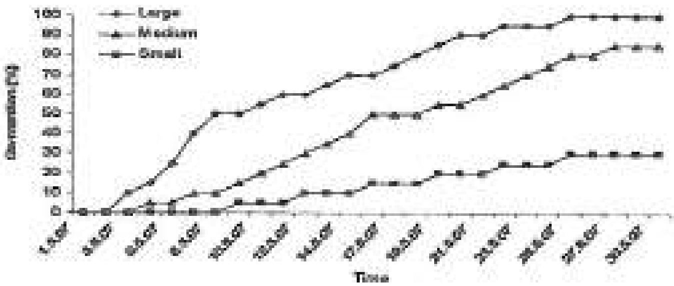

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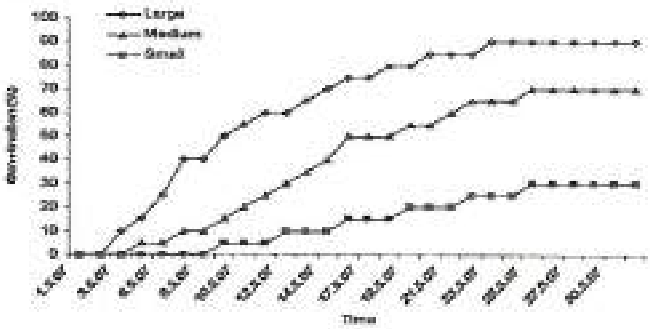


Figure 1: The effect of seed size on germination of *Acacia saligna* seeds cold water treated
or treated with boiling water (B) once. (C) twice, (D) three times, and (E) four times

Table 1: Effect of frequency of boiling water treatment on germination of large,
medium and small seeds after 35 days

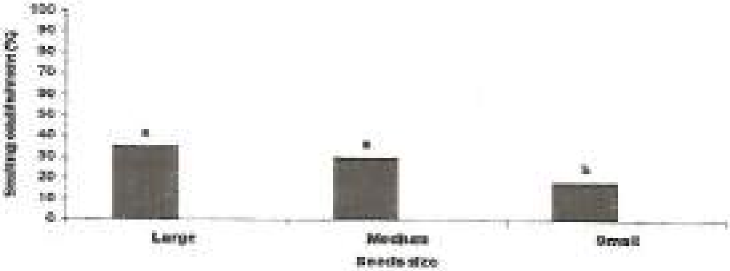
Number of boiling water treatments	Germination (%)		
	Small	Medium	Large
0	5 e	15d	25 d
1	20 d	30 c	40 c
2	25 d	45 c	60 b
3	30 c	85 a	95 a
4	30 c	70 b	90 a

Mean not followed by the same letter differ significantly at $P < 0.05$

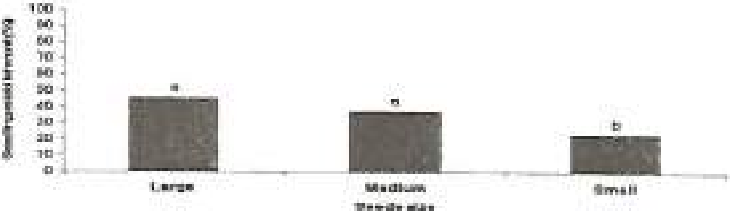
Figure 2 shows the establishment of seedlings, as measured by the number of seedlings which emerged and survived after 7 weeks, the end of the greenhouse experiment. As shown in the laboratory germination test above, establishment increased with increasing number of dormancy breaking boiling water treatments, reaching a maximum with three treatments. With all dormancy breaking treatments, establishment of seedlings was significantly greater from large than from small seeds, with seedling establishment from medium size seeds being lower than from large seeds with the control and higher than the from the small seeds with one or two boiling water treatments.

A

B


a
a
b
Large
Medium
Small
Seeds size

C


a
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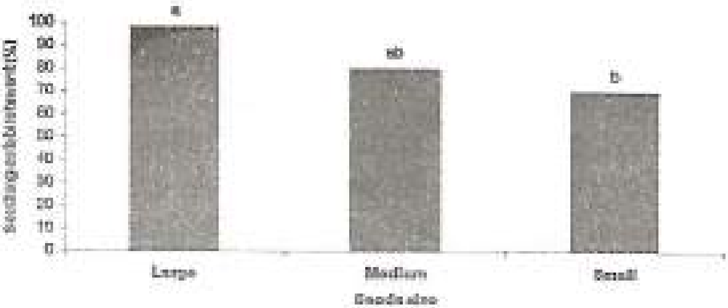
D


a
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Seeds size

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a
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Figure 2: The effect of seed size on seedling establishment of *Acacia saligna* seedlings from seeds (A) cold water treated or treated with boiling water (B) once. (C) twice, (D) three times, and (E) four times and sown at 30 mm depth. Columns without the same letter differ significantly at P < 0.05.

Table 2: Effect of seed size on length of root and shoot of *Acacia saligna* seedlings sown at 10, 20, 30 or 40 mm depth

Seed size		Length (cm)			
		10 mm	20 mm	30 mm	40 mm
Large	Root	2.88 b	3.61 a	3.69 a	-
	Shoot	2.52 b	2.94 b	3.46 a	-
Medium	Root	2.35 b	3.57 a	3.51 a	-
	Shoot	1.12 c	2.90 b	3.89 a	-
Small	Root	3.76 a	3.66 a	2.76 b	-
	Shoot	1.71 c	2.59 b	0.61 d	-

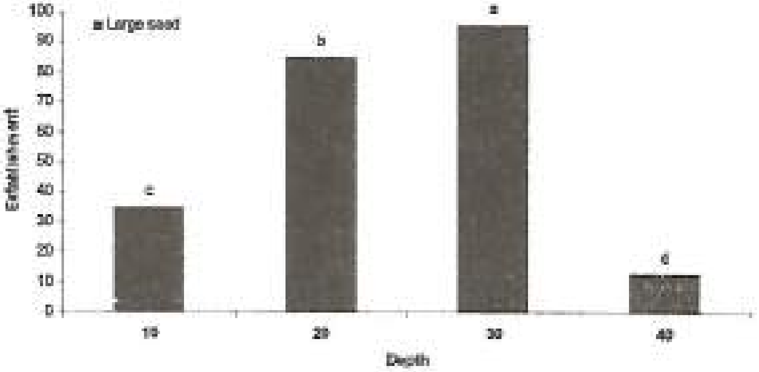
Means for root or shoot not followed by the same letter differ significantly at P < 0.05

When pots were watered with tap water (Figure 3) a few seeds of each size emerged and survived from 40 mm depth, but when watered with distilled water (Tables 2 and 3) no seeds emerged and survived when sown at 40 mm. With large seeds, 30 mm gave significantly greater seedling establishment than 20 or 10 mm sowing depth (Figure 3), while for medium and small seeds 20 or 30 mm gave greater establishment than 10 mm depth.

For large and medium seeds there was no significant difference in length of roots between 20 and 30 mm sowing depth (Table 2), and this was higher than at 10 mm. However, for shoot growth of large and medium seeds length was significantly higher when seeds were sown at 30 mm, followed by 20 m and then 10 mm. In contrast, the roots of seedlings established from small seeds were longest when the seeds were sown at 10 mm depth the shoots longest when the seeds were sown at 20 mm depth. The root of seedlings from larger seeds were longer than those from small seeds when sown at 30 mm, but shorter when sown at 10 mm. However, the shoot of seedlings from large seeds was longer with both 10 and 30 mm sowing depth.

With large and medium size seeds, dry weight of shoots of seedlings was significantly greater at 20 and 30 than at 10 mm sowing depth (Table 3), but with small seeds greater shoot dry weight was obtained with sowing at 20 than at 10 or 30 mm depth. At all sowing depths, large seeds produced seedlings with root and shoot dry weights higher than those from small seeds.

A



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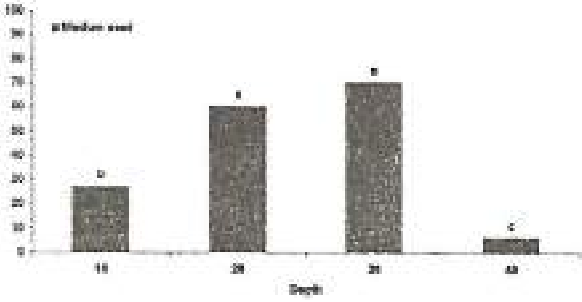

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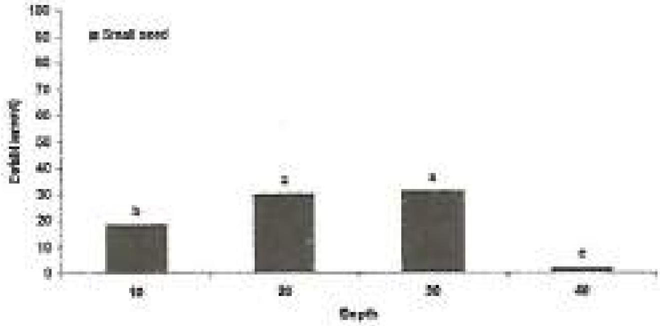

Figure 3: The effect of seed size on establishment of *Acacia saligna* seedlings from (A) large seeds, (B) medium seeds and (C) small seeds treated three times with boiling water and sown at 10, 20, 30 or 40 mm. Columns without the same letter differ significantly at P < 0.05.

Table 3: Effect of seed size on dry weight of root and shoot of *Acacia saligna* seedlings established from seeds sown at 10, 20, 30 or 40 mm

Seed size		Dry weight (g/pot)			
		10 mm	20 mm	30 mm	40 mm
Large	Root	0.056 b	0.088 a	0.087 a	-
	Shoot	0.52 b	0.81 a	0.94 a	-
Medium	Root	0.040 c	0.054 b	0.059 b	-
	Shoot	0.38 c	0.48 b	0.58 b	-
Small	Root	0.032 c	0.039 c	0.019 d	-
	Shoot	0.38 c	0.59 b	0.25 d	-

Means for root or shoot not followed by the same letter differ significantly at P < 0.05

Discussion

Most *Acacia* species have a hard coat, which is considered one of several strategies for survival in the spatially and temporally variable environment. *Acacia* seeds, therefore, will not germinate promptly when placed under conditions that are normally regarded as suitable for germination. Such seeds are said to be dormant. Seed dormancy aids the survival of *Acacia* populations by preventing out of season emergence, and spreading the germination over many weeks or even years. The hard coat of *Acacia* seeds can be broken by mechanical or chemical methods (Edwards 1973, Clements, Jones, and Gilbert 1977, Sadhu and Kaul 1989, Danthu *et al.* 1992). However, manual scarification cannot be guaranteed to promote the germination of all viable seeds because it may have unexpectedly detrimental effects on germination and it is unsuitable for large scale usage (Clements, Jones, and Gilbert 1977). Using sulphuric acid as a seed coat softener, on the other hand, would be difficult in nursery conditions and is a hazardous method (Danthu *et al.* 1992). In the present study, treating *Acacia* seeds with boiling water resulted in a high germination percentage, a result that concurs with that of Magnani *et al.* (1993) who found a positive effect of boiling the seeds of seven *Acacia* species on their germination percentage. The germination rate of the seeds of *A. senegal* and *A. lineata* also improved when soaked in boiling water (Larsen 1962). The results in this experiment, treating seeds with boiling water three times, can be compared with Omori (1993) who found best results with boiling water three times, and these results are further comparable to that of Youssef, Heikal, and Shaker (1991) who compared species of *Acacia* and found that *A. saligna* gave the highest germination after boiling water treatment. Boiling waters somehow breaks or reduces the effect of the hard coat, which allows the seed to germinate. The results further, show that treatment of *A. saligna* more than three times is not worthwhile. Large seeds germinate earlier and achieve greater final germination than medium and smaller seeds, and this has been found in many species. Example includes pines, e.g. *Pinus radiate* and *P. toeda*. In the present study (Table 3) there were large differences in the dry matter accumulated in seedlings developed from large and small seed-size classes and sowing depth. It is generally agreed that large seeds tend to produce larger seedlings (Schaal 1980). Patterns of absolute growth rate of seedlings have shown that consistently greater absolute growth of seedlings from large seeds than from small seeds is maintained until maturity (Schaal 1980, Stanton 1984) and although the initial size advantage of seedlings from large seeds may disappear with time because of higher relative growth rate of seedlings from small seeds (Westby *et al*. 1996, Bonfil 1998, Khurana and Singh 2000). There was no significant difference in dry weight of root and shoot of seedlings from large and medium seeds sown at 20 and 30 mm depth. However, when small seeds were used, root and shoot dry weights were significantly decreased by deeper sowing. Erickson (1946) disclosed that both germination and vigour of seedlings are directly associated with seed size, as seedling vigour was decreased by increasing the planting depth of small alfalfa seeds, and, conversely, a higher percentage of vigorous seedlings resulted when large seeds were planted deep. Bolton (1983) and Johnson (1983) suggested that the large seeded cereal crops should be seeded at a maximum 5 to 6 cm (Anderson 1974 cited in Johnson 1983). These advantages could be offset by inferior aeration, increased the mechanical obstruction and the possibility of increased microbial attack on longer hypocotyls. The greatest seedling growth of small seeds was obtained when seeds were sown near the soil surface and this may reflect a general survival strategy adopted by *Acacia* spp. and suggests that small seeded crops should be seeded at 2.5 cm or less.

Conclusion

The results obtained from the present experiment emphasize the necessity of treating *Acacia saligna* seeds before sowing in seedbeds to promote a high germination percentage and to produce uniform seedling. Treatment of *A. saligna* seeds of all sizes with boiling water three times gave the greatest seed germination. Large seeds germination earlier and achieved greater germination than smaller seeds. Seed size my also affect the size of resulting seedlings because large seeds contain more mineral nutrients. Seed size is very important for the germination and growth of seedlings of *A. saligna* and could be of adaptive significance in establishing and maintaining the populations, because seedlings from large seeds are able to survive hazards including coastal dunes stresses such as sand burial, drought, and few mineral nutrients. Sowing *A. saligna* seeds at 30 mm depth generally gave the greatest seedling growth from large and medium seeds whereas 20 mm was more suitable for small seeds. These results may be useful as a guide for carrying out some nursery operation, regarding *Acacia* species and cold enable avoiding the most complex hazard one.

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