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Effect of different pre-sowing seed treatments on the germination of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana* (L.)

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Abstract *Leucaena leucocephala* and *Acacia farnesiana* are tree species used for several agricultural purposes in the Mediterranean region. The seeds of these species exhibit dormancy, causing delayed germination. Two experiments were conducted to investigate the effect of pre-sowing treatments (scarification, hot water, or soaking) on seed germination of *L. leucocephala* and *A. farnesiana*. In one experiment, seeds were exposed to three pre-sowing treatments: control, sandpaper scarification, or soaking in 70°C water for 4, 8, 12, 16, 20, or 24 min. In another experiment, seeds were soaked in 70°C water for 20 min, and then soaked in water at room temperature for an additional 24, 48, or 72 h or blade scarified. In general, soaking the seeds of the two species in hot water was more effective in breaking seed dormancy than scarification. Sandpaper scarification was not effective for either species. Blade scarification increased *A. farnesiana* seed germination to 56%, indicating that seed dormancy was mainly a consequence of hardseededness. *L. leucocephala* seeds collected from Jordan University of Science and Technology (JUST) site and soaked in 70°C water for 20 min and then soaked for 24, 48, or 72 h had germination rates above 97%. Our results suggest that blade scarification of *A. farnesiana* seeds and soaking of *L. leucocephala* seeds in 70°C water for 20 min are effective treatments to break seed dormancy and enhance seed germination of these vital species.

Keywords *Leucaena leucocephala* · *Acacia farnesiana* · Seed germination · Seed pre-treatment · Hardseededness

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Introduction

Leucaena leucocephala (Lam.) and *Acacia farnesiana* (L.) are multipurpose legume tree species that are widely distributed around the world (Siegler et al. 1986; Brewbaker and Sorensson 1990) and are often grown in arid and semiarid regions of Jordan. Both species have high nutritional value and are used as feed for livestock (Graham and Vance 2003), to improve soil characteristics (Arowolo 2007), in agroforestry systems (Graham and Vance 2003), and as fuel wood. *A. farnesiana* is also used in traditional medicines (Parrotta 2001). Both species have the potential to be used for pasture improvement, especially in semi-arid regions (Meloni et al. 2000), and are important for agriculture in a wide range of climates (Webb et al. 1980; Brewbaker et al. 1985). Because *L. leucocephala* can fix nitrogen, it can survive in dry regions with poor-quality soils (Cronk and Fuller 1995).

The impermeable seed coat is considered the main problem in establishing forests of legume species (Smith et al. 2003). Seed coat–imposed dormancy, known as “hardseededness”, is an ecological mechanism that allows the seed to germinate only when conditions are favorable for supporting seedling growth; however, it represents a limitation when prompt and high-level germination is required (Argel and Paton 1999). This phenomenon generally applies to leguminous forest trees or shrubs such as *Leucaena*, *Parkia*, *Faidherbia*, *Albizzia*, *Prosopis*, and *Acacia* (Albrecht 1993; Grubb and Coomes 1997). Therefore, seeds require pre-treatments before sowing to obtain rapid, uniform, and high germination rates (Teketay 1996; Teketay and Tigabu 1996; Schutz and Rave 1999; Yang et al. 1999; Huang and Gutterman 2000).

Several pre-sowing treatments have been used to overcome hard seed coat–imposed dormancy (Meyer et al. 1990; Ferasol et al. 1995; Broncano et al. 1998; Goslan and Gutterman 1999; Rachel and Galatowitsch 1999) and to increase the permeability of the seed coat to water. Different pre-sowing treatments such as cold stratification, mechanical disruption, or acid and hot water treatments are widely used because they can improve germination within a relatively short period. Hot water treatment can enhance germination of dormant hard-coated seeds by elevating the water and O_2 permeability of the seed coat (Msanga and Maghembe 1986; Teketay 1998; Hermansen et al. 1999; Aydın and Uzun 2001). Many treatments have been proposed for overcoming hardseededness and improving germination rate in legume species (Ibanez and Passera 1997; Sy et al. 2001). Nicking is an effective method to mitigate seed dormancy in *Acacia tortilis* (Forsk.), *A. seyal* (Del), *Albizia gummifera* (J. F. Gmel.), *Brachystegia spiciformis* (Benth.), *Delonix elata* (L.), *Faidherbia albida* (Delile), *L. leucocephala* (L.), *Maesopsis eminii* (Engle.), and *Terminalia* spp. (Wolf and Kamondo 1993; Msanga 1998). Hot water treatment is another frequently used method to overcome hardseededness, whereby the seeds are soaked in water at 40–100°C (depending on the species and seed coat thickness) for a specific period or until the boiling water cools to ambient temperature. For *A. nilotica* (L.) and *Tamarindus indica* (L.), pouring 80°C water over seeds in a container, followed by soaking for 24 h, was found to be an effective method for breaking seed dormancy (Albrecht 1993). Pouring 100°C water over seeds of *Adansonia digitata* (L.), *Calliandra calothyrsus* (Meissn), and *Sesbania sesban* (L.), with continued soaking in water for 24 h is also effective in breaking seed coat dormancy (Albrecht 1993). Boiling in water for 1 min followed by scarification produced the highest germination in seeds of *Aacaia cochliacantha* (H. & B.) and *A. pennatula* (Schltdl. & Cham.), although the germination rates were low (Cervantes et al. 1996).

There is little information about techniques for breaking seed coat–imposed dormancy of *L. leucocephala* and *A. farnesiana*. For *Leucaena* seeds, dormancy can be overcome by soaking in hot water for 5–15 min, followed by soaking in water or acid (Macklin et al. 1989; Roshetko 1995). Argel and Paton (1999) also reported a range of temperatures and boiling periods used to enhance *Leucaena* germination. Padma et al. (1994) found that germination of *L. leucocephala* seeds could be improved by soaking the seeds in 80°C water for 5 min, by grindstone scarification, or by nicking. The geographical location where seeds are produced can affect germination owing to moisture and temperature variation, which affect seed dormancy and hardseededness (Bewley and Black 1982). Thus, in the present study we investigated the germination response of *L. leucocephala* and *A. farnesiana* seeds to different pre-sowing treatments. Understanding these factors is crucial for successful regeneration and recruitment of these species in forestry nurseries as well as for direct planting in arid and semiarid regions.

Materials and methods

Seed collection and preparation

Seeds of *L. leucocephala* were collected from two locations in Jordan: (1) northern Jordan (32°32′44″N, 35°51′26″E; 350 mm annual rainfall, 650 m altitude); and (2) Jordan University of Science and Technology (JUST) campus arboretum (32°28′44″N, 35°59′6″E; 250 mm annual rainfall, 520 m altitude). Seeds of *A. farnesiana* were collected only from the JUST campus arboretum. Seeds were extracted from the pods by manually cracking and cleaning the seeds. The seeds were then stored at room temperature (20–22°C) at the Seed Science Laboratory at JUST for 1 month until the experiments were initiated. Before treatment, seeds of each species were randomly mixed, and aborted or misshaped seeds were separated out.

Pre-sowing seed treatments

Two experiments were conducted to evaluate the effects of pre-sowing treatments on breaking seed dormancy and enhancing seed germination of *L. leucocephala* and *A. farnesiana*. In the first experiment, seeds were exposed to one of the following pre-sowing treatments: (1) untreated (control); (2) sandpaper scarification; (3) soaking in 70°C water for different periods (4, 8, 12, 16, 20, or 24 min). In the second experiment, the seeds were exposed to one of three treatments: (1) untreated (control); (2) blade scarification; (3) soaking in 70°C water for 20 min (selected based on the results of the first experiment) followed by soaking in water for 24, 48, or 72 h at room temperature (20–22°C).

Germination experiment

Seeds were germinated by placing seeds on moistened filter papers (Albet 150, Albet LabScience, Germany) in glass Petri dishes (11.5 cm diameter), and then incubating in a dark germination chamber at 20°C for a total of 21 days. Petri dishes were re-arranged randomly every 2 days to eliminate any effect of position within the germination chamber. The pre-sowing seed treatments consisted of four replicates for a total of 160 seeds (40 seeds per replicate) for each species. At the end of each experiment, the number of normal seedlings, abnormal seedlings, dormant seeds, and dead seeds was recorded. Seedlings were considered normal if they had a vigorous primary root or a secondary root system, intact undamaged hypocotyls and/or epicotyls, at least one attached cotyledon, and attached terminal buds (AOSA 1986). Abnormal seedlings had no primary root and a weak secondary root system, a lesion in the conducting tissues, more than one cotyledon missing, or had damaged terminal buds (AOSA 1986). Dormant seeds were imbibed seeds that remained firm and apparently viable at the end of the test period (ISTA 2004). Dead seeds had decayed tissues at the end of the test (seeds that were neither hard nor fresh and did not produce seedlings) as determined by pressing a finger onto the seed (ISTA 2004). Germination count was recorded at 3, 5, 8, 10, and 12 days after incubation and was expressed as a percentage. Germination rate was expressed as the germination rate index (GRI) according to Maguire (1962):

$$\mathrm{GRI} = \sum \frac{\text{No. of Germinated Seeds}}{\text{No. of Days}}$$

Experimental design and statistical analysis

The experiment had a completely randomized design with four replications. The collected data were analyzed with a general linear model using SAS software version 8.2 (SAS 2001). Means were analyzed according to the Least Significant Difference at probability level $P < 0.05$.

Results

The germination of *L. leucocephala* and *A. farnesiana* seeds was affected by pre-sowing treatment. In the first experiment, soaking the seeds in 70°C water for 20 min resulted in the highest germination percentage and GRI of *A. farnesiana* seeds from JUST, whereas soaking for 20-24 min significantly increased the germination percentage and GRI of *L. leucocephala* seeds collected from JUST (Table 1). However, soaking for just 12 min in 70°C water was the best pre-sowing treatment for *L. leucocephala* seeds collected from Irbid (66% germination). Sandpaper scarification did not overcome the hardseededness in either species (Table 1; Fig. 1). For *A. farnesiana*, the 81% germination after 20 min of soaking did not differ significantly from the 16-min treatment compared to *L. leucocephala* under the same conditions (Fig. 1). *L. leucocephala* seeds collected from Irbid had the highest germination and GRI with a 12-min soaking period compared to the seeds collected from JUST (20 min). The highest germination observed in the first experiment was 81%, achieved with *A. farnesiana* seeds after 20 min of soaking that was not significantly different from the 74% for the 16-min treatment (Table 1; Fig. 1). Compared with *L. leucocephala* seeds collected from JUST showed a maximum germination percentage and GRI at 20 min of soaking, while those from Irbid had their highest germination and GRI with just a 12-min soak (Fig. 1).

In the second experiment, scarification was more effective when using a blade rather than sandpaper for both species in terms of germination percentage and GRI (Tables 1; 2). For *A. farnesiana*, blade scarification resulted in a higher germination percentage and GRI compared with the other treatments, whereas the *L. leucocephala* seeds responded almost equally to all pre-sowing treatments (Table 2; Fig. 2). Generally, soaking the seeds of *L. leucocephala* in 70°C water for 20 min followed by soaking in room water temperature (20–22°C) for 24 or 48 h resulted in the highest germination percentage and GRI compared to the control (Table 2; Fig. 2).

Table 1 Percentage of normal and abnormal seedlings, hard and dead seeds, and germination rate index (GRI) in standard germination test for *A. farnesiana* and *L. leucocephala* seeds exposed to different pre-sowing treatments

Treatments	Normal (%)	Abnormal (%)	Hard (%)	Dead (%)	GRI
Acacia farnesiana (JUST)[a]					
Control	3 C	0 C	93 A	4 B	0 D
Scarification (sandpaper)	0 C	2 BC	91 A	7 B	0 D
Hot water 4 min	56 B	5 B	18 B	21 AB	12 C
(70°C) 8 min	62 B	4 BC	13 B	21 AB	15 BC
12 min	68 AB	4 BC	10 B	18 AB	17 AB
16 min	74 AB	6 AB	6 B	14 B	18 AB
20 min	81 A	3 BC	6 B	10 B	20 A
24 min	53 B	10 A	2 B	35 A	13 C
LSD (0.05)	17	5	25	19	4
Leucaena leucocephala (JUST)[a]					
Control	0 C	0 C	100 A	0 C	0 D
Scarification (sandpaper)	3 C	1 B	96 A	0 C	0 D
Hot water 4 min	52 B	5 AB	39 B	3 BC	11 BC
(70°C) 8 min	52 B	9 A	28 BC	11 AB	11 BC
12 min	45 B	9 A	28 BC	18 A	10 C
16 min	62 AB	7 AB	18 CD	13 AB	14 AB
20 min	68 A	9 A	9 D	14 AB	16 A
24 min	67 A	8 A	10 CD	15 AB	16 A
LSD (0.05)	14	7	19	15	4
Leucaena leucocephala (Irbid)[b]					
Control	0 C	1 C	96 A	3 D	0 C
Scarification (sandpaper)	1 C	0 C	99 A	0 D	0 C
Hot water 4 min	43 B	12 B	4 B	41 A	10 B
(70°C) 8 min	48 B	13 AB	2 B	37 AB	11 B
12 min	66 A	11 B	2 B	21 C	16 A
16 min	53 AB	17 AB	2 B	28 BC	12 B
20 min	43 B	15 AB	2 B	40 A	10 B
24 min	41 B	19 A	2 B	38 AB	9 B
LSD (0.05)	14	7	4	12	4

[a] Seeds were collected from Jordan University of Science and Technology (JUST) Campus

[b] Seeds were collected from Irbid

Discussion

Similar to other forage legume species (Van Assche et al. 2003), the two species examined in this study have very low germination (<3%, due to hardseededness) if not treated before sowing. The hard seed coats of many forest species have evolved to withstand unfavorable conditions such as intense heat from sunlight, dispersing animals, severe drought, and physical damage. Germination requires rupture of the seed coat and subsequent absorption of water by the seed (Freas and Kemp 1983; Baskin and Baskin 1998; Silvertown 1999).

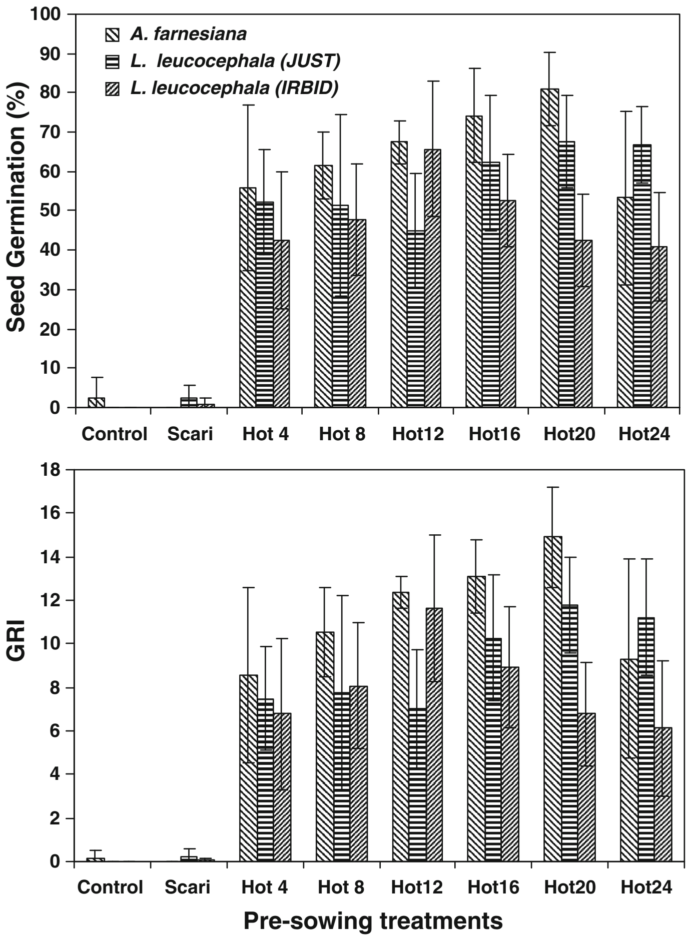

Fig. 1 Seed germination percentage (%) and germination rate index (GRI) of *Leucaena leucocephala* and *Acacia farnesiana* seeds exposed to different pre-sowing treatments: (1) Control; (2) sandpaper scarification (Scari); (3) soaking in 70°C water (Hot) for different periods (4, 8, 12, 16, 20, or 24 min). The *error bars* represents the standard deviation of the means ($n = 4$)

Our results showed that soaking the seeds in 70°C water was the most effective pre-sowing treatment in both species compared to the control or the sandpaper scarification. The use of 70°C water for 12–24 min increased seed germination of *L. leucocephala* to 66–68%, but this was not as effective as the procedure reported by Gosling et al. (1995), which entailed soaking *L. leucocephala* seeds in 100°C water for 4 s, resulting in 82% germination. In contrast, Argel and Paton (1999) reported that *L. leucocephala* seeds were successfully scarified and then immersed in 60°C water for 15–30 min or at 80–100°C for 1–3 min without affecting seed viability and seedling vigor.

For *A. farnesiana*, the hot water treatment was only effective with 20 min of soaking. In contrast, Tietema et al. (1992) reported that 4 min of soaking in hot water was effective in breaking seed dormancy in other *Acacia* species such as *A. burkei*, *A. karroo*, and *A. nilotica*. Bowen and Eusebio (1981) reported that *A. mearnsii* and *A. melanoxylon* seeds had increased seed germination after 1 min of soaking in 90°C water and only 30 s soaking

Table 2 Percentage of normal and abnormal seedlings, hard, dead and dormant seeds, and germination rate index (GRI) in standard germination test for *Acacia farnesiana* and *Leucaena leucocephala* seeds exposed to different pre-sowing treatments

Treatments	Normal (%)	Abnormal (%)	Hard (%)	Dead (%)	Dormant (%)	GRI
Acacia farnesiana (JUST)[a]						
Control	3 C	2 AB	95 A	0 B	0 B	1 C
Scarification (blade)	56 A	4 A	28 C	9 A	3 AB	16 A
70°C for 20 min, soaking 24 h	19 B	0 B	74 AB	2 B	5 A	5 B
70°C for 20 min, soaking 48 h	14 BC	0 B	85 AB	0 B	1 B	3 BC
70°C for 20 min, soaking 72 h	16 B	1 B	80 AB	2 B	1 B	3 BC
LSD (0.05)	13	3	22	7	4	4
Leucaena leucocephala (JUST)[a]						
Control	0 B	0 A	100 A	0 A	0 A	0 C
Scarification (blade)	93 A	0 A	0 B	0 A	7 A	25 B
70°C for 20 min, soaking 24 h	99 A	0 A	0 B	0 A	1 A	31 A
70°C for 20 min, soaking 48 h	99 A	0 A	0 B	0 A	1 A	28 AB
70°C for 20 min, soaking 72 h	93 A	0 A	0 B	0 A	7 A	24 B
LSD (0.05)	8	0	0	0	8	4
Leucaena leucocephala (Irbid)[b]						
Control	0 A	0 A	100 A	0 A	0 A	0 C
Scarification (blade)	97 A	0 A	0 B	0 A	3 A	30 A
70°C for 20 min, soaking 24 h	99 A	0 A	0 B	0 A	1 A	31 A
70°C for 20 min, soaking 48 h	99 A	0 A	0 B	0 A	1 A	30 A
70°C for 20 min, soaking 72 h	97 A	0 A	0 B	0 A	3 A	22 B
LSD (0.05)	5	0	0	0	4	3

[a] Seeds were collected from Jordan University of Science and Technology (JUST) Campus

[b] Seeds were collected from Irbid

in boiling water, which was sufficient to overcome seed coat dormancy in *A. mangium* seeds. Several studies have reported that soaking seeds in hot water enhances seed germination in many *Acacia* species. For example, Matias et al. (1973) reported that seeds soaked 2–48 h in water improved seed germination of many tropical tree species such as *A. mearnsii*, *A. melanoxylon*, *A. nilotica*, *Adenanthere mirosperma*, *Albizia amara*, *A. procera*, *Grevillea robusta*, *Trewia nidiflora*, and *Pinus caribaea*.

The differences in seed germination percentage and GRI of *L. leucocephala* collected from the two locations in our study may reflect different environmental conditions and irrigation regimes, which may affect seed coat structure and hardseededness. The requirements for overcoming seed dormancy may differ depending on the local ecology and time of harvest from different locations (Koller 1962; Bewley and Black 1982).

In our second experiment, blade scarification was the most effective treatment for *A. farnesiana*, attaining 56% germination, compared to the hot water treatments (Table 2; Fig. 2). Cervantes et al. (1996) reported that *A. farnesiana* seeds had their highest germination after sandstone scarification compared to other tested *Acacia* species. In contrast, scarification was not an effective treatment for increasing germination in some crops such as *A. farnesiana* (Gill et al. 1986), *Cassia fistula*, and *C. glauca* (Todaria and Negim 1992) as well as *L. leucocephala* (Foroughbakhch 1989).

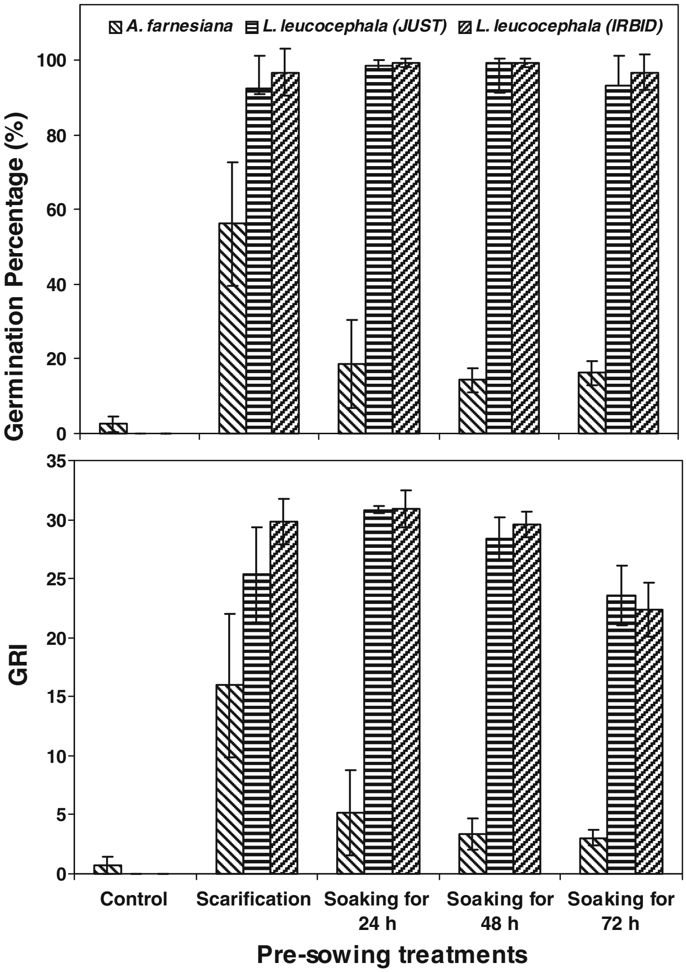

Fig. 2 Seed germination and germination rate index (GRI) of *Leucaena leucocephala* and *Acacia farnesiana* seeds exposed to different pre-sowing treatments: (1) Control; (2) blade scarification; (3) soaking in 70°C water for 20 min followed by soaking in water for 24, 48, or 72 h at room temperature (20–22°C). The *error bars* represents the standard deviation of the means ($n = 4$)

Soaking seeds of *A. nilotica* and *Tamarindus indica* in 80°C water followed by another 24 h of soaking was effective for increasing germination (Albrecht 1993). Moreover, seed coat dormancy was overcome when *Adansonia digitata*, *Calliandra calothyrus*, and *Sesbania sesban* seeds were soaked in 100°C water for 1-3 min and then soaked in room temperature water for 24 h (Albrecht 1993). Similarly, Oakes (1984) suggested that water temperature has a greater effect on hardseededness breakdown than immersion time, and reported a high germination after immersion of *Leucaena* seeds in 80°C water for 2–5 min or in 100°C water for 2–5 s. In contrast, soaking seeds in hot water for 20 min followed by prolonged soaking in water at room temperature (20–22°C) had adverse effects on *A. farnesiana* seed germination and GRI compared to scarification (Table 2). *L. leucocephala* seeds responded positively to all pre-sowing treatments and attained more than

96% germination (Table 2). The excessive effect of hot water treatment reduces the number of normal seedlings that significantly increases the number of dead and abnormal seedlings, and can rupture the seed coat by ejecting the strophiolar plug and cracking the testa (Argel and Paton 1999). For this reason, water temperature and immersion durations should be adjusted to minimize seed damage that could result in abnormal seedlings or dead seeds. The scarification treatment of *L. leucocephala* resulted in 97% seed germination, which was not significantly different from the soaking treatments (Table 2). These results are similar to previous studies of other legume seeds (Hardegree and Emmerich 1991; Thanos et al. 1992; Ibanez and Passera 1997; Uzun and Aydin 2004). The scarification treatment can be achieved either manually for small seed quantities used for laboratory testing or research purposes, or by a machine (Albrecht 1993; Poulsen and Stubsgaard 1995; Msanga 1998). Padma et al. (1994) reported that grindstone scarification and seed nicking in *Leucaena* increased the germination percentage. Also, with small seed quantities, mechanical scarification is an effective and efficient method for many tropical seeds (Poulsen and Stubsgaard 1995; Msanga 1998).

Sandpaper scarification may not have facilitated the water imbibitions or the permeability of the seed coat to water and oxygen. Hence, sandpaper was not an effective means to overcome the physical barrier to germination. Although there are several physical causes of seed dormancy, the sandpaper was not an effective tool. This is supported by the fact that blade scarification improved germination percentages and GRI in both species, as demonstrated by experiment two (Table 2; Fig. 2). Consequently, the observed effect of the treatments on hardseededness is likely due to the alteration of the physical properties of the seed coat rather than a direct effect on the physiological processes underlying dormancy. Results of the second experiment suggest that seeds of *A. farnesiana* are more vulnerable to “oxygen deficiency”, resulting from longer soaking periods, compared with seeds of *L. leucocephala* (Argel and Paton 1999; Smith et al. 2003).

Conclusion

To overcome seed dormancy in *A. farnesiana*, soaking seeds in 70°C water for 20 min is recommended. Blade scarification or soaking seeds in 70°C water for 12–24 min followed by 24–48 h of soaking in water at room temperature is recommended for *L. leucocephala*. Our results will assist forestry nurseries to reduce the time and labor needed to overcome seed dormancy, especially for these two species. Knowledge of the most effective and lowest-cost pre-sowing treatment can be used to increase seedling viability and enhance field establishment for greater production and profit.

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