Seed dormancy overcoming and seed coat structure change in *Leucaena leucocephala* and *Acacia nilotica*

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**ABSTRACT**

*Leucaena leucocephala* and *Acacia nilotica* are two arboreal legumes with several uses in agriculture, nutritious forage and livelihoods, fence posts, wood production, biofuel production, charcoal, firewood, shading, hedges, windbreaks, and improvement of soil fertility. The seed dormancy is common in *Leucaena* and *Acacia* species, and it creates difficulties in seed testing and planting. The aim of this study is to determine the effective methods of sulfuric acid and hot water due to overcoming dormancy in seeds of those species. Most pretreatments significantly (*p* < 0.05) reduced hard seed content and enhanced germination percentage when compared to nontreated seeds. Effectiveness of water and acid pretreatments increased when increasing in duration time from 3 to 60 min. The best recommended time pretreatment of sulfuric acid 60 min for *A. nilotica*, and 3 min of hot water for *Leucaena*. These treatments all reduced hard seeds to 0% and did not cause damage to the seeds during the germination. Further, the lens and hilum both were identified as primary sites for water uptake into the seeds of *Leucaena*. Whereas, the lens was recognized as the original site of water intake in *A. nilotica*. In addition, the seed coat anatomy identified that the palisade epidermal layer was dense, and thickness tissue had been prevented imbibition of water in *Leucaena* and *A. nilotica*.

**Introduction**

*Leucaena* and *Acacia* species are fast-growing leguminous trees distribution from northern Central America, Southern Mexico, and Africa. Currently, they have been adopted throughout the subtropics and tropics regions. *Leucaena* and *Acacia* species had high competition, plays a critical dynamic trait in promoting sustainable forestry and drought tolerance in arid and semi-arid zones. These species produce nutritious forage and livelihoods, Arabic gum, medicine, human food, timber, firewood, shade, improving soil fertility, highlighting in replantation of the degraded region, represented a source of livelihood and economic value. Presently, they are used for green manuring, fence posts, poles, cellulose, biofuel, plywood, phytoremediation, palatable, digestible, animal feed particularly ruminant feeding similar to alfalfa (Gutteridge and Shelton 1994; Danthu et al. 2003; Osechas et al. 2008; Kassa et al. 2010; Balehegn et al. 2015; Rusdy 2016).

Further, *Leucaena* and *Acacia* are suitable species to include in Agroforestry systems in the semi-arid region (Faye et al. 2011). Therefore, those species are fast-growing and high content proteins and minerals (Walker 2012; Crawford et al. 2015). As well, *Leucaena* and *Acacia* have high seed production, and production of seedlings, also, is high if dormancy is broken, seeds are made water permeable (Alamgir and Hossain 2005; Drumond and Ribaski 2010). Even more, *Leucaena* has excellent potential as a pasture’s species providing sources of crude protein and other essential nutrients for livestock production. *Leucaena* is economically a vital leguminous plant with its ability to grow in different ecological in the tropics (Aganga and Tshwenyane 2003).

However, the physical obstruction, for example, dormancy, raises the existence of those species, which tolerates seeds to preserve viability for the along with the time (Nesi et al. 2016). In many legume seeds, dormancy is due to the impermeability of the seed coat to water (Drumond and Ribaski 2010). Unless the seed coat made the water-permeable, low germination percentage, and irregular timing of seedling emergence results (Martins and Lago 1996). Sowing seeds of the *Leucaena* without breaking dormancy caused a germination index less than 50%. Consequently, the development of pretreatments to break dormancy is needed to standardize and accelerate seed germination (Teles et al. 2000; Paulino et al. 2004). The best way to break dormancy and make seeds of legumes water-permeable is to use sulfuric acids (Rebouças et al. 2012) or die in hot water (Araújo et al. 2012). Moreover, effective
methods to break dormancy and promote seed germination are critical for the production of plants for reforestation programs. Therefore, the enhance seed germination by using hot water treatment distressing with many factors, seed coat permeability to gases, water exchange, and the release of inhibitory (Warrag and Eltigani 2005; Rusdy 2017).

The present study was intended to evaluate the effect of concentrated sulfuric acid and hot water pretreatments on germination of seeds from *Leucaena leucocephala* and *Acacia nilotica* and identify the seed coat structure change and the original site of water entry during imbibition.

**Materials and methods**

**Seed collection and preparation**

Seeds of *L. Leucaena* were harvested in Hainan, Southern China, and those of *A. nilotica* was collected from Western Sudan and brought to the Key Laboratory of Grassland Agroecosystems, College of Pastoral, Agricultural Sciences and Technology, Lanzhou University, China. In December 2018, healthy, uninfected seeds of almost uniform size were selected for each species. These seeds were stored in plastic boxes at 5 °C and were used for evaluating different treatment.

**Sulfuric acid (H₂SO₄) treatment**

Seeds of *Leucaena* were soaked in concentrated sulfuric acid (98%) for 0, 1, 3, 6, 10, 20, 30, and 40 min, and those of *Acacia* was, soaked in concentrated sulfuric acid (98%) for 0,10, 20, 30, 40, 50, and 60 min; the control treatment contained untreated seeds.

**Hot water treatment**

To examine the impact of boiling water in physical dormancy breaking, seeds of *Leucaena* and *Acacia* were soaked in boiling water (100 °C) for 0, 3, 6, 9, and 20 min.

**Point of water entry during imbibition**

To evaluate the original site of water entry into the seed, 400 seeds of *Leucaena* were scarified in concentrated H₂SO₄ and hot water for 0, 3, 10 min and 400 seeds of *A. nilotica* were, they are soaked in concentrated sulfuric acid and hot water for 0, 20, 60 min. The experimentation contained four class groups of 100 seeds for each treatment, as included: (1) control, no blockage applied, (2) Vaseline applied to the hilum area, (3) Vaseline applied to the lens area, (4) Vaseline applied to the seed coat area. For each hot water and sulfuric acid blockage treatment, four replicates of 25 seeds were used. The acid-treated seeds were thoroughly washed in running tap water to remove all residues of sulfuric acid; all treated seeds were incubated at 20 °C in the light-dark. A seed was considered to have germinated when the radical extension was at least 1.5 mm. Seeds were evaluated according to the ISTA Rules (ISTA 2010), and the final germination percentage was determined at 21 days for each species. The research experiments were laid out in a completely randomized design.

**Changes in seed coat structure**

Changes in seed coat features were evaluated using scanning electron microscopy (SEM). *Leucaena* seeds were acid scarified by immersing them in concentrated sulfuric acid (98%) for 0, 10, and hot water for 3 min. Moreover, seeds of *Acacia* were, soaked in concentrated sulfuric acid (98%) and hot water for (control), 20, 60 min. The seeds were then dried at room temperature for 24 hours. Three seeds were chosen at random from each treatment and the control. All seeds were coated with gold and examined with a (JSM-6380LV (JEOL, Japan) scanning electron microscope at 20 kV. On each examined seed, two regions of the seed coat could be distinctly identified hilum and lens.

**Seed germination**

Seed germination was recorded for all experiments when the seeds emerge from the paper surface. Germination percentage (GP), germination rate (GR), hard seed reduction (HS), abnormal seeds (AS), and dead seeds (DS) were calculated using the following equations:

\[ \text{Germination percentage, } \% = \frac{\text{Number of germinated seeds}}{\text{total number of seeds}} \times 100 \]

\[ \text{Hard seed reduction, } \% = \frac{\text{number of seeds not imbibed}}{\text{total number of seeds}} \times 100 \]

\[ \text{Abnormal seed, } \% = \frac{\text{number of abnormality seeds}}{\text{total number of seeds}} \times 100 \]

\[ \text{Dead seed, } \% = \frac{\text{number of deadening seeds}}{\text{total number of seeds}} \times 100 \]

**Statistical analysis**

Germination performance of seeds of *Leucaena* and *Acacia* across different seed pretreatment methods was compared using one-way ANOVA in SPSS version 19.0. Significant differences were sorted out using the Duncan multiple range test (DMRT). All data were expressed as means.

**Results**

**Effect of sulfuric acid and hot water on dormancy-break and germination**

The sulfuric acid pretreatments had the highest effect in hard seeds reduction compared to nontreated control seeds (Figure 1(a,b)). Furthermore, the 6 and
60 min of sulfuric acid pretreatment displayed a hard seed reduction from 89 to 0.5% for Leucaena and from 82 to 3% for Acacia. The sulfuric acid treatments ultimately improved the germination of Leucaena and Acacia seeds. Moreover, the highest germination 91% was noted when seeds soaked in sulfuric acid for 6 min, followed with soaking in sulfuric acid for 10, 20, and 30 min. Further, the germination percentage decreased gradually as the pretreatment period increased to 40 min (Figure 1(a,b)).

The hot water treatment has a positive effect on dormancy break in Leucaena and negative consequences on dormancy break in Acacia. The percentage of hard seed reduction diminished gradually as the pretreatment duration time. The 3 min of hot water pretreatment reduced a hard seed percentage from 98 to 1.5. The germination percentage was enhanced gradually as the pretreatment period increased from 3 to 20 min. The highest germination (98%) results were obtained 3 min (Figure 1(a,b)).

**Point of water entry during imbibition seeds**

In most treatments, the percentage of imbibed seeds increased lens blockage compared to the hilum and seed coat both in sulfuric acid and hot water treatment (Figures 2–5). In contrast, the percentage of imbibed seeds of Leucaena and Acacia exposed in 3, 10, and 20, 60 min for acid scarification were significantly improved
by blockage of the seed coat (Figures 2 and 4). Furthermore, the primary site of water entry into Acacia seeds was the lens (Figures 4 and 5). For Leucaena seeds, water entered through the hilum and lens (Figures 2 and 3).

**Seed coat structure**

Three structures could be observed for the examined seeds receiving treatments. Hilum was located in the middle of the micropyle, and the lens was on the opposite side of the hilum (Figures 6(a–c) and 7(a–c)). In the control seeds, the hilum and lens remained intact with visible cracks under 200 magnification (Figures 6 and 7(a)). After acid scarification for 10 and 20 min, the hilar groove appeared fuller than in the controls, and seed cracks were found in eroded areas visible in the lens, hilum, and seed coat area (Figures 6 and 7(b)). The sulfuric acid and hot water treatments to remove the waxy layer of the seed coat (Figure 6(b,c)). However, after the hot water treatment area for 3 and 60 min, the number of cracks in the lens and hilum increased, and most of the lens appeared destroyed the water gap was open (Figures 6 and 7(c)). Moreover, the different cellular features of the seed coats displayed the same general structures. Palisade epidermal layer showed a compact, and thick tissue that had been penetrated by water, the hypodermis was linked with the light line and a distinct number of sclerified parenchyma layers under it (Figures 6 and 7(d)).

**Discussion**

This study demonstrated the effectiveness of concentrated sulfuric acid pretreatments in breaking dormancy in Leucaena and Acacia species. The effectivity of the pretreatments declined with the decrease in the pretreatment period. The hard seed content reduction of Leucaena showed from 89 to 0% and Acacia from 82 to 3%. This outcome supports the conclusion of (Duguma et al. 1988) that sulfuric acid scarification is
Figure 4. Imbibition seeds after 14-day incubation at 20 °C, when different areas of the seed coat were blocked with Vaseline following by sulfuric acid treatments. NON B: non-blocked; SA: sulfuric acid; H: hilum; L: lens; SC: seed coat.

Figure 5. Imbibition seeds after 14-day incubation at 20 °C, when different areas of the seed coat were blocked with Vaseline following by hot water treatments. NON B: non-blocked; HT: hot water; H: hilum; L: lens; SC: seed coat.

Figure 6. Scanning electron micrographs of seed coat structure changes and anatomy of the L. leucocephala, following different concentrated sulfuric acid and hot water treatments. (a), (b), (c) and (d): treated for 0, 10, and 3 min, respectively. H: hilum; L: lens open with destroyed palisade layers; PL: palisade layer; Y: hypodermis; CU: cuticle; LL: light line arrow; SP: sclerified parenchyma layers.
the most significant efficient way of promoting the seed coat permeability of *Leucaena*. Also, sulfuric acid 98% have been reported to improve seed coat permeability of *Acacia* (Harvey 1981). However, stated in other plants, for instance, *Tamarindus indica* (Muhammad and Amusa 2003), *Prosopis koelziana* (Agbogidi et al. 2007), *Leucaena diversifolia* (Dos Santos Carvalho et al. 2007), *Prosopis juliflora* (Zare et al. 2011), *Centrosema pubescens* (Rusdy et al. 2015), *L. Leucaena* (Rusdy 2017) and *Adenanthera pavonina* (Mantoan et al. 2012). Therefore, most studies consider that the cuticle is the site of thickness, and impermeability has implicated in the grade of impermeability in some species (Kolattukudy 1981).

Sulfuric acid is assumed to disturb the seed coat and the palisade epidermal layer, imbibition of water, which activates the release of less sugar that can be used intended for protein synthesis, thus encouraging germination (Jackson 1994). Also, the higher effectiveness associated with acid scarification compared to hot water in *Leucaena* seeds, particularly in seeds tested for hot water for a longer time, which probably killed the embryo and decreased germination about 30%, compared to sulfuric acid treatment. Therefore, the seed germination of 97.3% treated with sulfuric acid for 10, 15, and 20, approving the effectiveness of the way in restraining the seed dormancy of *Leucaena* (Masamba 1994; Teles et al. 2000).

In contrast, the hot water pretreatment duration to breaking seed dormancy in *L. Leucaena* was 5 min at 80 °C (Teles et al. 2000). Additionally, seed germination in *Leucaena* was highest when the seeds were exposed in hot water 80 °C for 10 min.

Therefore, all blockage treatment had significant effects on seed imbibition. Our result is in line with previous studies concerning the site of water entry. Imbibition of *Acacia* seeds was initially reported to be restricted by the small diameter of the lens (Hanna 1984; Barbosa et al. 1999). In estimating water entry and water gap in *Delonix regia*, dormancy was reported to be relieved in 18%, and 24% of seeds stored dry and watered, resulting in the lifting of palisade layers in the lens region to form a circular lid-like opening water gap (Jaganathan et al. 2017). The study concluded that in blocking experiments, the site of water entered only through the lens with no secondary water entry point. (Graziela et al. 2017), reported that the lens was the only water entry point in *Paulillo dubium* seeds after temperature treatments at 40, 50 °C, whereas, in *Mimosa* seeds, the primary of water uptake was the lens and micropyle. The lens is identified to be the first site of water uptakes in Fabaceae seeds after physical dormancy (Baskin et al. 2000) and was reported in many Fabaceae species, including *Sesbania punicea* (Manning and Van Staden 1987) and *Albizia lophanta* (Dell 1980).

Several studies have reported that the seed coat impermeability is generally induced by a substance primary lignin current in the epidermal palisade-like tissue that prevents water intake (Wilson and Witkowski 1998; Baskin et al. 2000; Baskin 2003; Ma et al. 2004). Indeed, suggested in several species, differences in lignification grade of palisade-like layers may render seeds, some non-permeable, and permeable to water (Kelly et al. 1992). Therefore, Baskin et al. (2000) recommended that the mere facing of a palisade-like tissue layer in the seed coat, even though the cells were lignified, that the seed is impermeable to water. Further, the results reported that in *Leucaena* seed, the lens acts as the site of primary water entry, while the

Figure 7. Scanning electron micrographs of seed coat structure changes and anatomy of *A. nilotica*, following different concentrated sulfuric acid and hot water treatments. (a), (b), (c) and (d): treated for 0, 20, and 60 min, respectively. H: hilum; L: lens open with destroyed palisade layers; PL: palisade layer; Y: hypodermis; CU: cuticle; LL: light line arrow; SP: sclerified parenchyma layers.
micropylar region and the palisade layer tolerate water to pass later, in specific, for the micropyle (Serrato-Valenti et al. 1995).

Conclusion
This study presented that the hot water and sulfuric acid scarification were affected significantly promoted seed germination and the reduced hard seed of *Leucaena* and *Acacia*. The best recommended time pretreatment of sulfuric acid 60 min for *A. nilotica* and 3 min of hot water for *Leucaena*. Therefore, the lens and hilum were primary sites for water uptake into the seeds of *Leucaena*, whereas the lens was the original site for water entry in seeds of *A. nilotica*. Even more, seed coat structure demonstrated that the *A. nilotica* and *Leucaena* showed the palisade epidermal layer more compact and thickness tissue had been water penetrated.

Our outcomes raised the most important trait of the anatomical seed coat efficiency in breaking seed dormancy in *Leucaena* and *Acacia* species to be resulting in the future to promote sustainable forest, livelihoods, and environmental restoration programs in arid and semi-arid regions. We also noticed that the disparity observed in the seed coat structure among the studied species could be related to different regenerative responses to environmental conditions. *Leucaena* and *Acacia* species seem to build a more classification necessarily. Also, it would be much better to carry more studies using molecular data, which may be contributing to the open field of research to identify genotypic differences and dormancy behavior, which are genetically controlled.

Acknowledgments
The authors are grateful to all Staff of Laboratory of Pastoral, Agricultural Sciences, and Technology, particularly Dr. Li Ya Jie and Yu-Ling for providing competent technical assistance. I am also thankful to Qibo Tao for his help in the data analyses. Finally, I would like to thank Prof. Carol Baskin and Baskin for their advice and valuable comments on this manuscript that have helped to enrich it.

Disclosure statement
No potential conflict of interest was reported by the authors.

Funding
This research was funded by the Chinese Government Scholarship for Foreigner Students and the “111” Project (B12002).

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