Germination Responses of *Acacia Cyclops And A, Victoriae* Seeds to Different Scarification Treatments

Mahmood B. Shanta, Ibrahim A. Eshkab, and Hisham N. Alwaer

Abstract—This study was carried out to assess the effects of different scarification treatments on seed germination of *Acacia cyclops (A. Cunn.. ex G. Don)* and *Acacia victoriae* (Benth).The results of the study show that H_2SO_4 acid scarification was superior in enhancing seed germination percentage (G %), germination rate (GR), and germination index (GI) of both acacia species. Interestingly, *A. cyclops* seeds treated with H_2SO_4 required 90 minutes to attain the highest germination percentage, while *A. victorria* obtained its highest germination percentage at the minimum assigned time of 30 minute, possibly reflecting differences in seed coat thickness. Although both boiling water and dry heat treatments didn't improve the germination % of *A. victoriae*, these treatments were effective in enhancing all the measured parameters of *A. cyclops* seeds. It was also found that the germination of *A. victoriae* dramatically reduced with increasing time of exposure to boiling water. The results of this study confirmed that seeds of *A. cyclops and A. victoriae* possess a physical dormancy and will not germinate satisfactorily unless treated.

Keywords—*Acacia cyclops; Acacia victoriae;* Seed scarification; Germination percentage (G %), germination rate (GR); germination index (GI).

I. Introduction

ACACIA is a genus of shrubs and trees belonging to the subfamily Mimosoideae of the family Fabaceae [45]-[52]-[62]-[44]. They are robust, wide-ranging plants that have proved to be well adapted to harsh environments such as arid and semi-arid zones [40]. Acacias exhibit many desired attributes [25]-[15]-[40]-[38]. They grow relatively quickly and are a source of nitrogen in forest ecosystems. Many have considerable value as emergency fodder and in dry zones play an important role as browse plants. The wood is useful for fuel, fence posts and poles, while the trees provide shade and shelter [42] and are important in soil conservation. To benefit from these desirable products and services, increased attention has been paid to the use of Acacia tree species in afforestation and restoration activities programmes in Libya [26]. Attempts are made to choose species that can be used for various purposes and can endure the prevailing unfavourable environmental conditions such as drought, salinity and infertile soils. However, propagation of Acacia seedlings for tree-planting programmes is hampered by slow and difficult seed germination caused by physical dormancy [30].

According to [6] physical dormancy is caused by an impermeable seed coat where embryo growth is physically restricted due to a hard enclosing structure. Seed dormancy causes germination failure to an intact viable seed even under favourable conditions [10]-[28]-[33]. However, this phenomenon has two main functions; 1) matching germination with optimal conditions in attempt to guarantee growth and survival of young seedlings [16], 2) adopting the bet-hedging strategy by spreading the germination of a fraction of seeds over time so that a distress after germination (e.g., late frosts, drought and herbivore pressure) does not cause the death of all offspring of a plant [24]-[27]. Thus, seed dormancy is a mechanism to prevent germination during unsuitable ecological conditions, when the probability of seedling survival is low [12].

Acacia victoriae is much-branched, usually glabrous shrubs or small trees 1.5-3.5 m tall, best suited to arid and semi-arid climates. It is adapted to a range of soil types, including alkaline soils and cracking clays and is moderately salt tolerant [35]-[36]. It has been successfully used to remediate mine sites. *A. victoriae* has potential as a source of fuel-wood and charcoal although on some sites it has shown relatively slow growth [35]. Their phyllodes have moderate palatability and digestibility and have potential as a source of stock fodder.

Acacia cyclops is native to coastal districts of Australia. It is a dense, evergreen, bushy shrub, often multi-stemmed or small tree growing usually 1-4 m tall, but occasionally reaching up to 7 m in height [48]. *A. cyclops* grows well on calcareous sand or limestone and prefers well drained, sandy or quartzitic soils, but can survive on drier sites such as dune crests [40]. It produces large quantities of litter which leads to increased soil nitrogen content [61], It has been employed as a stabiliser of coastal sand dunes as it is tolerant of wind, salt spray, sand blasts and soil salinity [40]. Grazing animals browse the phyllodes and seed may be suitable for cattle feed after it is crushed [40].

Acacia species usually exhibit hard seed coat imposed by dormancy which may be due to the impermeability of testa to water [51]. Scarification treatments with concentrated acid, hot water and dry heating can be effective in overcoming physical dormancy [22]-[55]. This work examines the effect of some common scarification methods on seed germination parameters

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of *A. cyclops (A. cunn. . ex G. Don)* and *A. victoriae* (Benth) investigated under laboratory conditions.

II. Materials And Methods

a. Experimental Design

Seeds of the studied species were collected from trees grown in Tripoli area - Libya, during 2013. They were tested for viability by floating them in distilled water prior to the experiments; those that floated were discarded and those that sank and settled at the bottom were used in the trials. Seeds were sown in Petri dishes lined with double-layer of filter papers. Dishes were moistened and distilled water was added whenever necessary throughout the duration of the experiments. Petri dishes were sealed with a strip of Parafilm to reduce water loss. Germination tests were carried out in April 2014 under Laboratory conditions at the Range and Forestry Department, Faculty of Agriculture, University of Tripoli, Libya. Germinated seeds whose radicle was at least 2 mm long were counted and removed daily for a period of three weeks. Germination percentage (G %), germination rate (GR) and germination index (GI) were determined as follows:

1. Germination percentage (G %): the number of germinated seeds as a percentage of the total number of tested seeds given as; G% = (germinated seeds/total tested seeds) × 100 %
2. Germination rate (GR) = ($1/t_{50}$), where GR = germination rate and t_{50} is the time needed to achieve 50% of the final germination percentage.
3. Germination index (GI) was calculated for each treatment according to [3].

$$GI = \frac{\text{No. of germinated seeds}}{\text{Days of frest count}} + \frac{\text{No. of germinated seeds}}{\text{Days of second count}} + \ldots + \frac{\text{No. of germinated seeds}}{\text{Days of final count}}$$

b. Scarification Treatments

Seed Scarification is a method that used to break seed dormancy by cutting or softening the hard wall of a seed [56]. A process accomplished by chemical, mechanical, heat, or moisture treatment allowing water and oxygen into the seed, thereby helping to improve germination in some species. The scarification treatments employed were immersing seeds in concentrated sulphuric acid (98%) for 30, 60, and 90 minutes; dipping seeds in boiling water (on-source) for 30, 60 and 90 seconds; and oven dry- heating at ~100 ºC for 30, 60 and 90 seconds. Un-scarified seeds were used as a control. The experimental design consisted of a completely randomized design (CRD) with three replications and 20 seeds per each. In the chemically-scarified treatment: seeds were divided and put into three 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. The seeds in the three beakers were left for the assigned times in concentrated sulphuric acid and stirred occasionally with a glass rod. When the allotted time was finished, seeds were removed and the acid drained off into another beaker. Seeds were thoroughly washed and rinsed in distilled water to remove all the acid. They were then surface-dried to be ready for germination. For the dry heating treatment (DH), seeds were divided and placed in three shallow containers in a preheated oven according to the prescribed temperature and duration. Finally, the boiling water treatment (BW), seeds were divided and put into three 100 mL heat resistant glass beaker and dipped in boiling water (on source) depending on the assigned time of exposure.

c. Statistical Analysis

Data was subjected to analysis of variance (ANOVA). Significant differences between treatments were considered at the $P<0.05$ level of significance using Tukey's Honestly Significant Difference Test (HSD**).** The germination percentage data were arcsine-transformed before statistical analysis.

III. Results

A. Acacia cyclops

Variations in germination percentage (G %), germination rate (GR) and germination index (GI) of *Acacia cyclops*, across different scarification treatments are depicted in figure (1, 2 and 3, respectively). Generally, all treatments increased germination percentage compared to untreated control. The P value was <0.001. Moreover, there was a significant difference between treatments. Seeds of *Acacia cyclops* treated with H_2SO_4 especially for 60 and 90 min and those treated with dry-heat (DH) for 90 s gave the highest germination percentage (74 %, 73% and 68%, respectively). The germination percentage of seeds treated with dry heat was increased as exposure time extended. In contrast, the germination percentage of boiling water treated seeds (BW) decreased with increasing time of exposure to temperature. The highest germination capacity of boiling water treated seeds was observed in seeds exposed to wet heat for 30 s (45%).

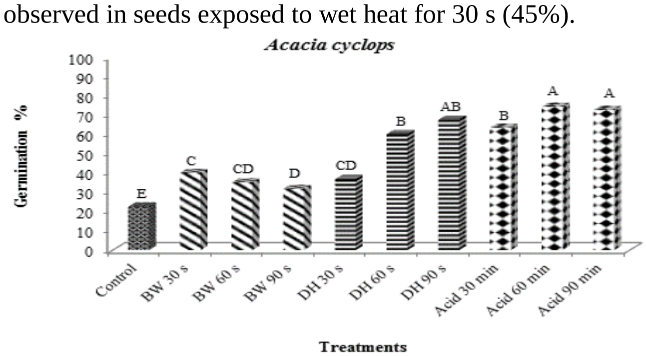

Fig. 1 Germination percentage (G%) of Acacia cyclops seeds under different scarification treatments. Means with the same letter are not significantly different at the p < .05 level.

The rate of germination was also affected by scarification treatments (P <0.001). Compared to control, all treatments except seeds treated with H_2SO_4 for 30 min. significantly increased the germination rate. The highest germination rate was observed in seeds treated with acid for 90 min. It is also evident that the germination rate of boiling water treated seeds did not change with boiling time increment, whereas, dry heat treatment significantly improved the germination rate with increasing time of exposure.

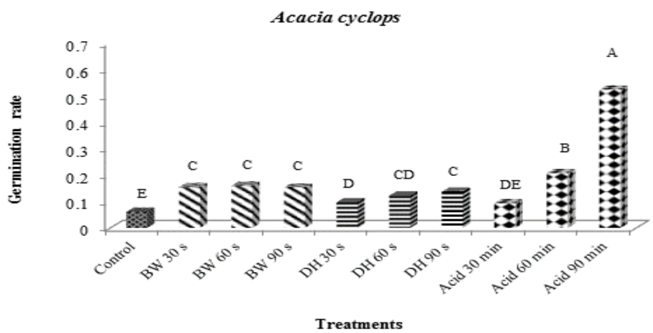

Fig. 2 Germination rate (GR) of *Acacia cyclops* seeds under different scarification treatments. Means with the same letter are not significantly different at the $p < .05$ level.

With regard to germination index (GI) of *A. cyclops*; Analysis of variance shows that scarification treatments also influenced the index significantly. The P value was < 0.001. The highest GI was recorded in seeds treated with H_2SO_4 for 90 and 60 min, respectively. The germination index of dry heat treatment for 60 and 90 seconds were also significantly greater than that of control. Whereas, the germination index of boiling water treated seeds for 30 was the only treatment resulted in GI greater than that of untreated seeds. Additionally, although, there was a reduction in GI with increasing time of exposure, this decrease was not significant.

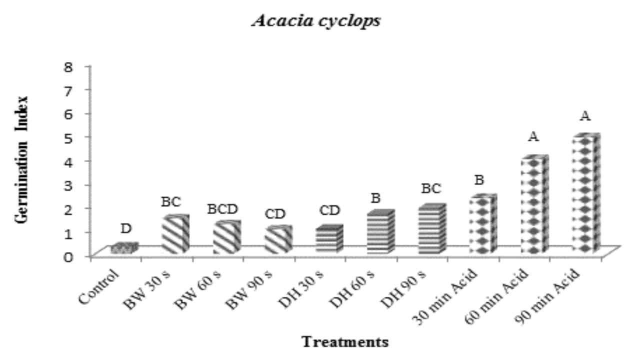

Fig. 3 Germination Index (GI) of Acacia cyclops seeds under different scarification treatments. Means with the same letter are not significantly different at the $p < .05$ level.

B. Acacia victoriae

The effect of scarification treatments on germination percentage (G %), germination rate (GR) and germination index (GI) of *Acacia victoriae* is presented in figure (4, 5, and 6), respectively. Analysis of variance indicates that there were significant effects of scarification treatments on germination percentage. The P value was < 0.001. Results show that H_2SO_4 acid treatments distinctively increased germination percentage greater than all treatments including untreated control. The germination percentage of seeds treated with acid for 30, 60 and 90 min was 78, 82, 84%, respectively, as compared to 20% recorded in untreated control. Compared to un-scarified seeds, neither boiling water nor dry heat treatments could increase G% significantly. It was also noted that the germination percentage of boiling water treatment dramatically decreased with increasing time of exposure to reach 0% at 90 s. Moreover, the germination percentage of dry heat scarified seeds decreased with increasing time of exposure to temperature. However, this reduction was not significant. Compared to untreated seeds, the germination percentage of dry heat treated seeds remained unaffected.

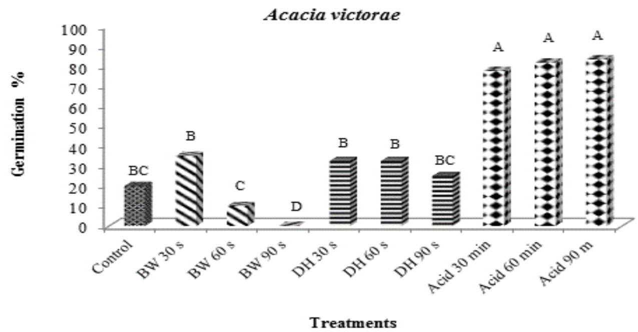

Fig. 4 Germination percentage (G%) of *Acacia victoriae* seeds under different scarification treatments. Means with the same letter are not significantly different at the $p < .05$ level.

Scarification treatments have also significantly influenced the germination rate of of *A. victoriae*. In addition, the results indicate that the germination rate of *A. victoriae* seeds significantly differ among treatments. The P value was < 0.001. The highest germination rate was recorded in seeds immersed in H_2SO_4 for 90 min. However, the value is statistically similar to seeds scarified with acid for 60 minutes and with those treated with boiling water for 30 and 60 seconds. In respect to dry heat treatments, the germination rate remained unchanged compared to untreated seeds.

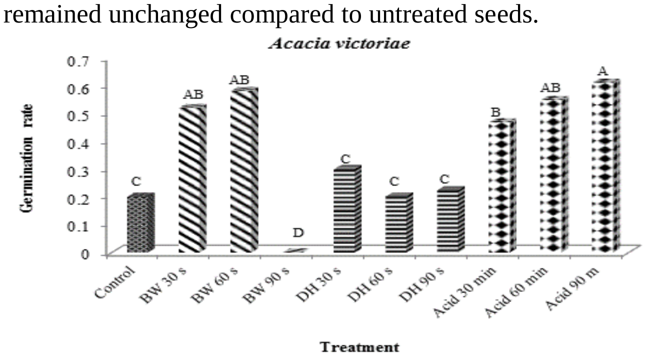

Fig. 5 Germination rate (GR) of *Acacia victoriae* seeds under different scarification treatments. Means with the same letter are not significantly different at the $p < .05$ level.

Analysis of variance clearly indicates that there was a significant influence of treatments on GI of *A. victoriae* seeds and there were significant differences between treatments. The P value was < 0.001. Concentrated H_2SO_4 acid treatments for 90 and 60 minutes achieved the highest GI. The germination index of dry heat treated seeds for 30 s was significantly higher than of control. However, the index decreased significantly with increasing time of exposure, but this reduction did not differ significantly with untreated seeds. Similar pattern was observed with boiling water treated seeds.

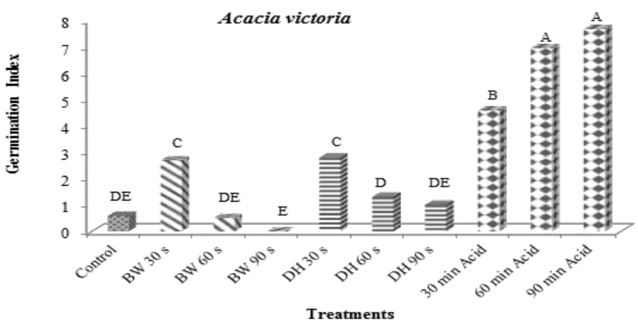

Fig. 6 Germination index (GI) of *Acacia victoriae* seeds under different scarification treatments. Means with the same letter are not significantly different at the $p < .05$ level.

IV. Discussion

Acacia victoriae, and *A. cycolps* species are multipurpose trees with diverse economic and ecological significance. However, seed dormancy and low germination percentage seem to be problematic for their usage in afforestation or restoration activities. Germination involves several stages that begin with water uptake by dry seed and end with the elongation of the embryonic axis [11]. Nonetheless, even under favourable conditions, water absorption is prevented in seeds of many species characterized with hard impermeable seed coat causing physical dormancy [8]. In order to improve germination capacity of these species, such type of dormancy must be overcome. Thus, several artificial scarification methods were applied.

The results of this study indicate that acid scarification is an effective treatment in enhancing seed germination percentage (G %), germination rate (GR), and germination index (GI) of both acacia species. It has been widely reported that acid scarification is an important and quite satisfactory treatment to overcome physical dormancy particularly for species with very hard seed coats including *Acacia* species. For instance, [49] reported that germination percentage, and germination rate index of *Acacia erioloba* were improved when treated with concentrated sulphuric acid. Positive influence of acid on seed germination of *Acacia nilotica,* and *A. auriculiformis* seeds has also been found by [53-[43], respectively.

Interestingly, *A. cyclops* seeds treated with H_2SO_4 required 90 minutes to attain the highest germination percentage, while *A. victorria* obtained its highest germination percentage at the minimum assigned time of 30 minute. This suggests that the seed coat of *A. cyclop*s might be thicker than that of *A. victoriae*. Therefore, the effectiveness of this treatment is influenced by duration of treatment [17].

The positive effect of sulphuric acid scarification on germination parameters detected in this current investigation could be due to the ability of acid to break hardseededness simply by corroding away layers of the seed coat [17], hence inducing permeability and leading to water uptake and oxygen diffusion, thereby re-activating the physiological and biochemical activities required for seed germination [11].

Acid scarification has many advantages and is considered as one of the most effective methods for improving germinability, especially in seeds with very hard coats. In addition, it has been claimed that acid scarified seeds can be stored for several months after treatment [13]. However, there are some disadvantages of acid scarification technique including high cost, serious safety hazard to workers and the environment and risk of seed damage caused by overtreatment [22].

Although both boiling water and dry heat treatments didn't improve the germination % of *A. victoriae*, these treatments were effective in enhancing all the measured parameters of *A. cyclops* seeds. The beneficial effect of boiling water on the germination of impermeable seeds has been observed by [9]-[29]-[34]-[57]-[46]-[47]-[8]-[5]-[19]-[4]-[1]-[41].

The effectiveness of boiling water treatment in enhancing germination has been attributed to the release of physical dormancy from hard seeded species by causing ruptures in the seed wall thereby allowing imbibition, O_2 diffusion and germination to occur [23]-[37]-[58].

It was also observed that the germination percentage of *A.cyclops* and *A. victoriae* reduced with increasing time of exposure to boiling water. Similar pattern of reduction was observed with *A. salicina* seeds, [50] with *A. terminalis* [18] and with *A. origena* and *A. pilispina* [58]. The decline of germination could be attributed to embryo damage caused by wet heating [50] or probably due to low O_2 availability at high temperature which resulting in destruction of certain enzymatic components [60]. Moreover, in the case of *A. victoriae*, the severe decrease of germination suggests that the species is very sensitive to wet heating suggesting that time of exposure is critical.

The present investigation revealed that dry heat treatment was productive with *A. cyclops*. Several researchers have found that dry heat treatment is effective in rendering *Acacia* seeds permeable and consequently increasing germinability. For instances, dry heat effectively improved the germination of *Acacia. longifolia* and *A. sophorae* [59], *A. longifolia* [39] *A. saligna* [3]. The mechanism through which dry heat overcomes physical seed dormancy is by modifying the seed coat mechanically. After this, the ruptured seed coat allows further imbibition and hence germination processes initiate [14].

This study also suggests that species may respond differently to treatments. Varied response of different species to scarification treatments has been observed by [31]-[32]**.** This variable response of the tested species could be attributed to different thickness of seed coats [54]. Generally, there are several factors that may influence the hardness of seed coat including growth location, year of production, seed moisture content [2].

V. Conclusion

The results of various treatments in this study confirmed that seeds of *A. cyclops* and *A. victoriae* possess a physical dormancy and will not germinate properly unless treated. Moreover, although boiling water and dry heat treatments are more practical and often recommended to overcome physical dormancy of many species, concentrated H_2SO_4 was proved to be more effective. Thus, it is advisable to implement this method for the studied species providing that precautionary measures are taken.

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