

RESPONSES OF SEEDS OF *VACHELLIA ERIOLOBA* (E. MEY.) P.J.H. HURTER IN BOTSWANA TO DIFFERENT PRE-SOWING TREATMENT METHODS

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ABSTRACT

Vachellia erioloba (E. Mey.) P.J.H. Hurter is a multipurpose tree that provides goods and services and confronted with over-exploitation regular bush fires, habitat change and global warming. Germination of seeds of this species is not well understood. They are characterized by hard seed coats impermeable to water and gaseous exchange. A study was conducted at Botswana University of Agriculture and Natural Resource to evaluate the responses of *Vachellia erioloba* to different pre-sowing treatments in a completely randomized design (CRD) with ten treatments; control, mechanical scarification (nicking), hot water, boiling water (1, 3 and 5 minutes) and 98% concentrated sulphuric acid (15, 30, 45, 60 minutes) from January to February 2018. The results indicated that seed germination was significantly ($p = 0.00001$) affected by pre-sowing treatment methods. The highest germination percentages were recorded in nicking, seeds subjected to sulphuric acid and hot water for 24h. Soaking seeds in boiling water (1, 3 and 5 minutes) resulted in no germination. Nicking and soaking seeds in hot water for 24 hours are simple technique that can be recommended for use in nurseries and by smallholder tree growers. Although the concentrated sulphuric acid improved germination in this work, it is not suitable for use in nurseries because of its high cost and the danger it poses to workers.

Key-words: Pre-sowing treatments; seed dormancy, seed germination; *Vachellia erioloba*, indigenous trees, Southern Africa

INTRODUCTION

The genus *Vachellia* is widely distributed in arid and semi-arid regions (Aref *et al.*, 2011). The genus is the most important tree component of the flora of southern Africa (Timberlake, 1980). Species from the *Vachellia* genus have attracted great interest in recent years because they are drought tolerant (Oba *et al.*, 2001), provide numerous goods (fodder, wood gums, resins and medicine) and services (shade and live fencing) to local communities (Timberlake, 1980). Some of the species have been reported to maintain soil fertility through atmospheric nitrogen fixation (Belsky *et al.*, 1989).

Vachellia erioloba (E. Mey.) P.J.H. Hurter formerly *Acacia erioloba*, is a multipurpose species that belongs to the pod bearing family (Fabaceae), subfamily Mimosaceae (Venter and Venter, 1996). It has a wide distribution range in southern Africa from Angola, Botswana, Mozambique, Namibia, South Africa, Zambia, Zimbabwe (Hyde *et al.*, 2018). It is large evergreen to semi-deciduous tree growing up to a height of 16-26 m (Timberlake, 1980; Storrs, 1995; Venter and Venter, 1996; Palgrave, 2002). It grows on Kalahari sands in areas receiving rainfall of 40 to 900 mm yr⁻¹ and tolerates hot summer temperatures and severe frosts. The tree has a rounded or umbrella shaped crown (Timberlake, 1980; Storrs, 1995; Venter and Venter, 1996) spreading up to 18 m (Timberlake, 1980). It is widely used for poles and firewood (Timberlake, 1980). The wood resists borers and termite and has been used for mine props and wagon building in the past (Palgrave, 2002). *Vachellia erioloba* produces quality gum eaten by people and a variety of animals (Timberlake, 1980; Venter and Venter, 1996; Palgrave, 2002). Pods and leaves provide nutritious fodder for livestock and game. Pods can be milled for livestock fodder during drought periods (Venter and Venter, 1996). The species has also been used in traditional medicine to treat gonorrhoea (Timberlake, 1980) and burnt and powdered bark treat headaches, while powdered pods are said to be effective in treating infected and discharging ears (Venter and Venter, 1996; Palgrave, 2002). Mature trees provide shade for people and animals during the hot drier season (Venter and Venter, 1996).

Indigenous shrubs and trees in arid and semi-arid environments are threatened by over-exploitation for their goods such as poles, firewood etc. In addition, they face threats from regular bush fires, habitat change and global warming. Various indigenous trees and shrubs in arid and semi-arid environments including the *Vachellias* could be fast growing and better producers of biomass, though little has been done to include them in planting programs

despite their importance. Arid and semi-arid trees and shrubs are characterized by hard seed coats impermeable to water and gaseous exchange (Baskin and Baskin, 1998; Walters *et al.*, 2004; Liu *et al.*, 2011). The hard seed coats exert physical exogenous dormancy (Holmes *et al.*, 1987) which prevents seeds from germinating at a time when the completion of germination or survival of the seedlings is unlikely, even though conditions would be temporarily favourable for germination (Baskin and Baskin, 2000; Kheloufi *et al.*, 2017). Germination of seeds with physical dormancy may extend over months or years and therefore pre-treating prior to sowing is necessary to maintain maximum and rapid germination (Aref *et al.*, 2011). Seed dormancy is the major problem encountered by foresters and other tree growers in their attempt to propagate seedlings of indigenous tree and shrubs in arid and semi-arid environments (de Faria *et al.*, 2010).

Vachellia erioloba is a one of the important multipurpose tree species which has not been incorporated into planting programs probably due to poor seed germination caused by physical dormancy. Consequently, seeds require pre-treatments to break the physical dormancy in order obtain a fast and a uniform germination (Azad *et al.*, 2013). Several physical or chemical treatment methods that include stratification, mechanical, acid, cold, hot and boiling water, organic solvents, alcohols, dry heat and fire have been used to break hard seed coats of many shrubs and tree species to render them permeable (Teketay, 1996, 1998; Ren and Tao, 2004; Uniyal *et al.*, 2000; Okunomo and Bosah, 2007; Aref *et al.*, 2011; Botsheleng *et al.*, 2014; Fredrick *et al.* 2016; Mojeremane *et al.* 2017, 2018). Information on techniques suitable for softening or breaking the hard seed coat of *V. erioloba* is scanty. Therefore, the objective of this study was to evaluate the suitability of various techniques for breaking dormancy in *Vachellia erioloba* seeds.

MATERIALS AND METHODS

Study Site

The study was conducted at the laboratory of the Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources (BUAN). BUAN is located at Sebele (23°34' S and 25°57' E, altitude of 994 m above sea level) 10 km from the centre of Gaborone, the Capital City of Botswana along the A1 North-South highway. Pods of *Vachellia erioloba* were collected from Maun (19°59'S and 023°25'E, elevated 927 m above sea level), Botswana. Maun is administrative centre of the Ngamiland District and the fifth largest town in Botswana. Pods were transported to BUAN in black plastic bags and stored in a refrigerator awaiting seed extraction and commencement of the study. Seeds were extracted and screened prior to commencement of the experiment and those with visible signs of insect damage were discarded. Healthy seeds were subjected to viability test through the floating method, in which the floated were considered unviable and discarded. The experiment was carried out from January to February 2018.

Experimental design and treatments

Ten pre-sowing treatments methods were laid out in a completely randomised design (CRD) as follows; 1) control (untreated seeds), 2) Mechanical scarification (nicking), 3) seeds immersed in hot water for 24 hours, 4) seeds immersed in boiling water for 1, 3 and 5 minutes respectively, 5) seeds immersed in 98% concentrated acid (H₂SO₄) for 15, 30, 45 and 60 minutes. Each treatment had a total of 100 seeds (four replicates each with 25 seeds).

Description of pre-sowing treatments

For the control, untreated seeds were germinated to compare the effects of no pretreatment on seed germination. In the mechanical scarification treatment, seeds were nicked using a pair of scissors to remove approximately 1-2 mm of the seed coat at the distal end before sowing in petri-dishes. For hot water treatment, seeds were immersed in the water which was left too cool for 24 hours. For boiling water treatments, seeds were immersed in boiling water for 1, 3 and 5 minutes, respectively. After each boiling time, seeds were removed from the pot and immersed in a small bucket containing cold distilled water to cool them down for few minutes before sowing. In the 98% concentrated acid, four replications of 25 seeds of each treatment were put into heat resistant non-corrosive glass beakers, after which the acid was added slowly to a level covering all the seeds and occasionally shaken. After each immersion time (15, 30, 45 and 60 minutes), seeds were sieved out using an acid resistant sieve and the acid drained off simultaneously into the beaker. Seeds were thoroughly washed in running tap and distilled water to remove all the acid for safe handling. Seeds were germinated in petri-dishes lined with cotton wool kept moist by continuous spraying with distilled water. Ambient air temperature in the laboratory varied between 25 and 30°C.

Seed germination counts

Germination was defined as the emergence of the radicle. It was recorded daily for a period of 30 days. Counted germinated seeds were discarded after recording and those that had not germinated at the end of the experiment were tested for viability using a cutting test.

Evaluation of seed size and weight

To assess the seed characteristics of *V. erioloba*, the three dimensions of the seeds, namely length, width and breadth of seeds were measured using an electronic digital caliper (0-150 mm), and their thousand seed weight were determined by weighing the seeds using an electronic analytical balance (Model: PW 124). Five replications of 10 seeds and ten replications of 100 seeds were used to determine the mean dimensions and thousand seed weights of the seeds, respectively. The thousand seed weight of the seeds was, then, computed from the mean seed weight of the 100 seeds.

Data analyses

Data collected on the germinating seeds were used to calculate, (1) germination percentage (GP) for each treatment using the equation: $GP (\%) = (\text{number of germinated seeds}/\text{total number of seeds}) \times 100$ and (2) mean germination time (MGT) using the equation: $MGT (\text{days}) = \sum T_i N_i / S$ (Scott *et al.*, 1984). Data was analyzed using both descriptive statistics and One-Way ANOVA using Statistix Software, Version 10 (Statistix 10, 2013). All data percentage sets were arcsine transformed prior to statistical analysis to meet the requirement of normality assumption for analysis of variance (Zar, 2010). Significant differences of means were tested using Tukey's Honestly Significant Difference (HSD) Test at the significance level of $P \leq 0.05$.

RESULTS

Seed size and weight

Seed size is an important characteristic of seed quality that plays a crucial role in plant seedling propagation. The *V. erioloba* seed length, diameter and breadth recorded in this study was *erioloba* 10.3 ± 0.21 , 7.9 ± 0.13 and 4.3 ± 0.06 mm, respectively. The mean seed length, diameter and breadth of *V. erioloba* used in the present study were 10.3 ± 0.21 (range 9.7-10.9), 7.9 ± 0.13 (range 7.4-8.1) and 4.3 ± 0.06 (range 4.1-4.5) mm, respectively. The mean mass of a single seed and the 1000 seed weight of *V. erioloba* were 0.27 ± 0.01 and 26.6 ± 0.4 g, respectively.

Table 1. Means of the cumulative germination and germination time of seeds of *V. erioloba* subjected to different pre-sowing seed treatments.

Treatment	Germination (%)	
	Mean \pm Standard Error	MGT
Control	56 ± 5.2^b	17.90 ^a
Nicking	98 ± 1.2^a	3.05 ^c
Water (24 hours)	95 ± 1.0^a	15.20 ^a
Boiling Water (1 minute)	0.00 ± 0^c	0.00 ^d
Boiling Water (3 minutes)	0.00 ± 0^c	0.00 ^d
Boiling Water (5 minutes)	0.00 ± 0^c	0.00 ^d
Sulphuric Acid (15 minutes)	97 ± 1.9^a	2.90 ^c
Sulphuric Acid (30 minutes)	94 ± 1.2^a	2.34 ^c
Sulphuric Acid (45 minutes)	98 ± 1.2^a	2.16 ^c
Sulphuric Acid (60 minutes)	98 ± 2.0^a	2.19 ^c

Means separated using Tukey's Honestly Significant Difference (HSD) Test at $P \leq 0.05$. Means within columns followed by the same letters for each species are not significantly different. MGT= mean germination time.

Seed germination

The results indicated that the seeds treated with mechanical, sulphuric acid and boiling water scarification had significantly higher mean germination percentages than the controls [One Way ANOVA: ($F_{(9, 39)} = 218, P = 0.00001$) (Table 1). The results of this study showed that there were significant differences ($P < 0.05$) in germination among pre-sowing treatments (Table 1). The highest germination was given by mechanical scarification; soaking in 98% concentrated H_2SO_4 for 45 and 60 minutes respectively followed by soaking seeds in 98% concentrated H_2SO_4 for 15 minutes, hot water for 24 hours and H_2SO_4 for 30 minutes. There were no statistical differences observed among these treatments. Untreated seeds exhibited 56% germination which was not consistent and spread of a long period of time. This result infers that *V. erioloba* seeds are characterized by physical dormancy related to the hard seed coat. The contents in Table 1 clearly showed that soaking *V. erioloba* seeds in boiling water for 1, 3 and 5 minutes did not aid germination. The cutting test conducted at the end of the experiment revealed that all seeds in the boiling water treatments were rotten. The test also showed that the majority of the untreated seeds were intact.

The speed of germination was faster for seeds soaked in 98% concentrated H_2SO_4 for 45 minutes (MGT= 2.16 days), 60 minutes (MGT= 2.19 days), 30 minutes (MGT = 2.34 days) and 15 minutes (MGT= 2.90 days) as well as those scarified mechanically (MGT= 3.05 days) (Table 1 and Fig. 1). The seeds exposed to the hot water and the control exhibited slower germination with MGT from 15.20 to 17.90 days.

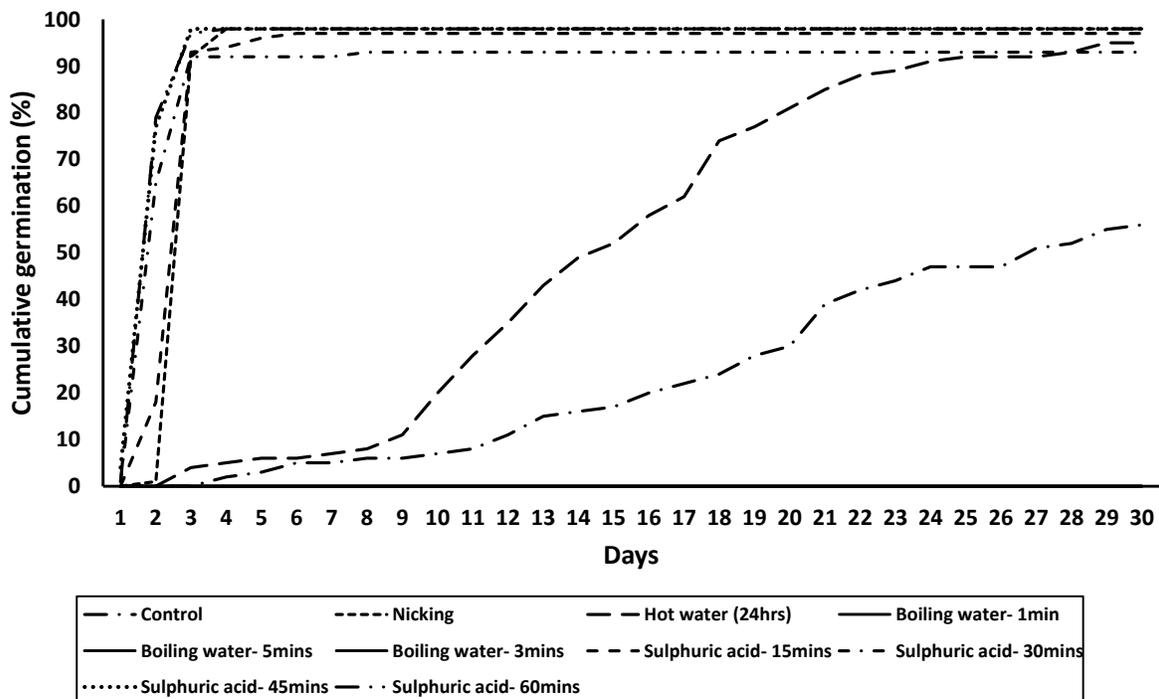


Fig. 1. Speed of germination of *V. erioloba* seeds subjected to different pre-sowing treatments.

DISCUSSION

Most leguminous species including the *Vachellia* are characterized by a hard seed coat (physical dormancy) which is water-impermeable (Baskin and Baskin, 1998; 2000; Walters *et al.*, 2004; Liu *et al.*, 2011; Venier *et al.*, 2012). Seeds with a hard seed coat will not germinate promptly when placed under conditions normally regarded as suitable for germination. A hard seed coat is a very common adaptive strategy that enables plants to survive in unpredictable and harsh environment (Aref, 2000; Jurado and Flores, 2005). Results of the present study show that manual scarification improved seed germination (98%) compared with untreated/control seeds which recorded 56% germination. Improved germination was accomplished by nicking 1-2 mm of the seed coat at the distal end, thus removed water impermeable palisade cell layers that form a physical barrier for water entry (Baskin and Baskin 1998; Baskin *et al.*, 2000) and gaseous exchange. Improved germination in mechanically scarified *V. erioloba* seeds is consistent with results of other studies conducted on different plants elsewhere (Mackay *et al.*, 1995;

Mapongmetsem *et al.*, 1999; Chisha-Kasumu *et al.*, 2007; Lopes *et al.*, 2015; Galindez *et al.*, 2016; Elazazi *et al.*, 2017; Frederick *et al.*, 2017; Bodede *et al.*, 2018). Mackay *et al.* (1995) reported 100% germination in mechanically scarified *Lupinus havardii* seeds. Khan and Sahito (2017) reported great enhancement in germination of *Acacia stenophylla* in nicked seeds. The speed of germination in the nicked *V. erioloba* seeds was faster (3.05 days). This technique is safer and more practical for small farmers because it is a simple and effective in promoting rapid and uniform germination. However, it can be laborious and time-consuming, especially when large numbers of seeds are required (Himanen *et al.*, 2012).

Scarification *V. erioloba* seeds in hot water for 24 hours significantly improved germination (95%) compared with the control/untreated seeds. The improve germination could indicate that hot water was effective in softening the hard seed coat of *V. erioloba* which resulted in uptake of water and gaseous exchange. This result concurs with others who reported improved germination in seeds of several other tree species following soaking in hot water (Sajeevukumar *et al.*, 1995; Aref, 2000; Mwase and Mvula, 2011; El-Sayed *et al.*, 2013; Das, 2014). Mwase and Mvula (2011) reported hot water softened hard seed coats and leached out chemical inhibitors making them permeable to water and gaseous exchange. Seeds soaked in hot water imbibe and swell as the water cools. A study conducted by Sajeevukumar *et al.* (1995) found that hot water increased germination in two species of *Albizia*. Soaking seeds in hot water is a simple and reliable technique that could be used effectively on large numbers of seeds (Ortega-Baes *et al.*, 2002; Hopkinson and English, 2004; Himanen *et al.*, 2012).

Soaking seeds in 98% concentrated H_2SO_4 for 15, 30, and 45 and 60 minutes improved germination compared to the control/untreated seeds (Table 1). The effectiveness of concentrated H_2SO_4 in improving germination revealed that *V. erioloba* has a seed coat imposed dormancy which creates a barrier to water uptake. The speed of germination in seeds soaked in 98% concentrated H_2SO_4 treatments was fast (2.2-2.8 days). This finding is in agreement with results of others studies that have reported physical dormancy to be common in the Fabaceae (Baskin and Baskin, 1998) which are characterized by water impermeable seed coats (Baskin and Baskin, 1998; Walters *et al.*, 2004; Teketay, 2005; Liu *et al.*, 2011). This results demonstrated that the hard seed coat of *V. erioloba* seeds can be broken by using concentrated H_2SO_4 as reported for other leguminous plant species in other studies (Khan *et al.*, 1984; Aref, 2000; McDonald and Omoruyi 2003; Nadjafi *et al.*, 2006; Keshtkar *et al.*, 2008; Aref *et al.*, 2011; Pipinis *et al.*, 2011; El-Sayed *et al.*, 2013; Das, 2014; Mojeremane *et al.*, 2017; 2018; Teketay *et al.*, 2018). Concentrated H_2SO_4 soften the seed coat to expose the lumens of the macrosclereids cells and allow imbibition (Amusa, 2011) and gaseous exchange. The effectiveness of concentrated H_2SO_4 in breaking hard seed coats depends on the species and the time seeds are exposed to the acid (Biruel *et al.*, 2010). Although concentrated H_2SO_4 has proved to be effective in improving seed germination in most leguminous species, it cannot be used by farmers because of costs and danger it poses to them (Danthu *et al.*, 1992).

Soaking seeds in boiling water for 1, 3 and 5 minutes did not aid germination. The 0% germination observed in *V. erioloba* seeds in the boiling water treatments could be attributed to the high level of heat transmitted from the boiling water through the seed testa to the cotyledon and the embryo. The rotting revealed by the cutting test could be an indication that the boiling water destroy and denature enzymes in the seed. The failure to germinate following soaking in boiling water has been demonstrated for other leguminous species by several earlier studies (Gill *et al.*, 1990; Obiazi, 2015; Kahaka, 2017; Mojeremane *et al.*, 2018).

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