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# PRESOWING SEED TREATMENT METHODS TO OVERCOME DORMANCY IN SEEDS OF *VACHELLIA REHMANNIANA* SCHINZ

## SUMMARY

A germination experiment of *Vachellia rehmanniana* seeds was conducted in the laboratory of the Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources from November to December 2016. Mature *Vachellia rehmanniana* seeds were collected from healthy erect trees at Butale Village, Botswana to investigate the effect of different pre-sowing treatment methods on seed germination. The experiment was laid out in a completely randomized design (CRD) with five treatments (control, mechanical scarification, boiling water, hot water and concentrated sulphuric acid (98.8%)). Boiling water had three different levels of time exposure (1, 3 and 5 min) whereas, concentrated sulphuric acid had four different levels of time exposure (15, 30, 45 and 60 min). The results revealed that seed germination percentage, germination mean time and germination index were significantly ($P < 0.01$) affected by pre-treatment methods. The highest significant cumulative germination percentages were recorded in seeds subjected to boiling water for 3 and 5 min, sulphuric acid for 45 and 60 min, and mechanical scarification. Based on the findings mechanical scarification and boiling water techniques are recommended for use in nurseries and by farmers because sulphuric acid is expensive and need to be handled by trained individuals. It is recommended that future research should target increasing the exposure time over five and 60 minutes for boiling water and sulphuric acid treatments, respectively for this species to increase the cumulative germination percentage.

**Keywords:** *Vachellia rehmanniana*, pre-treatment, seed dormancy, germination percentage.

## INTRODUCTION

The *Vachellia*, formerly known as *Acacia* (Kyalangalilwa *et al*., 2013) is a genus of shrubs and trees that belong to the subfamily Mimosoideae of the family Fabaceae (Palgreaves, 1983). They are widely distributed in arid and semi-arid

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regions where they evolved to withstand harsh climatic and environmental conditions (Timberlake, 1980). The genus *Vachellia* is one of the most important tree components of the flora of southern Africa (Timberlake, 1980). *Vachellia rehmanniana* Schinz is very important source of construction wood, firewood (Timberlake, 1980; Hanaoka *et al.*, 2014; Missanjo *et al.*, 2014) and non-wood products (Kassa *et al.*, 2010), such as medicines (Timberlake, 1980; Ali *et al.*, 2012; Missanjo *et al.*, 2014), edible gum and fodder (Hanaoka *et al.*, 2014; Missanjo *et al.*, 2014). In addition, some are nitrogen fixers (Missanjo *et al.*, 2014) and have been used in agroforestry systems (Azad *et al.*, 2011) and soil conservation projects.

*Vachellia rehmanniana* Schinz, commonly known as silky thorn (Timberlake, 1980; Palgrave, 1983), is a small thorny tree or shrub that grows to 5 m high (Timberlake, 1980), occasionally reaching 10 m (Timberlake, 1980; Palgrave, 1983). It is deciduous with a flat spreading crown (Palmer and Pitman, 1972; Timberlake, 1980). Leaves are yellow-green, neat slender, composed of 15-40 pairs of slender pinnae, each with many small leaflets (Palmer and Pitman, 1972; Palgrave, 1983). Flowers are in round greenish-white, fragrant balls grouped at the ends of young branches (Palmer and Pitman, 1972; Timberlake, 1980; Palgrave, 1983) appearing before or at the same time with leaves between October and December (Timberlake, 1980). Fruits are straight, dehiscent, aligned and twisted hairless flat pods (Timberlake, 1980; Palgrave, 1983), greyish to olivine in colour (Palgrave, 1983). The species occurs in southern Zambia, Zimbabwe, north eastern Botswana and South Africa, particularly in the Limpopo province (Timberlake, 1980). It grows in wooded grasslands, along river banks and on termite mounds (Timberlake 1980; Palgrave, 1983). It is a drought tolerant multipurpose tree species which provides firewood, construction wood, many non-timber products that include fodder and several ecosystem services. The leaves, young shoots, flowers and pods are eaten by both livestock and wildlife (Tsumele *et al.*, 2007).

The most common and cheap method of raising a large number of plant seedlings is through seeds (Kim *et al.*, 2008). Seeds of many leguminous tree species have hard seed coat impervious to water (Teketay, 1996, 1998; Walters *et al.*, 2004), which exerts a physical exogenous dormancy (Falemara *et al.*, 2014). The hard seed coats hinder germination and limit their propagation (Karthika *et al.*, 2016) as well as their use in afforestation programmes (Botsheleng *et al.*, 2014). Seed germination is an important stage in the life cycle of plant species (Evren and Kucukoduk, 2012) because it determines their survival (Yang *et al.*, 2008). The hard seed coat of many forest species have evolved to withstand unfavourable conditions, such as intense heat from sunlight, dispersing animals, severe drought and physical damage (Tadros *et al.*, 2011). Seed dormancy is an adaptation strategy that ensures seeds germinate only when environmental conditions are suitable for the survival of germinates (Falemara *et al.*, 2014).

*Vachellia* species are characterized by a hard, seed coat which hinders uptake of water and air by the seed (Teketay, 2005; Aref *et al.*, 2011) to trigger

germination. For germination to take place, it requires the seed coat to rupture and allow the absorption of water by the seed. Seeds with a hard seed coat require some pre-treatments before sowing to break dormancy and obtain rapid and synchronous germination. Seed dormancy is referred to as a temporary failure of a mature viable seed to germinate when subjected to favourable germination conditions (Ibiang *et al.,* 2012). Numerous techniques have been used to break dormancy in many species with hard coats including species of *Vachellia* (Teketay, 1998, 2005; Botsheleng *et al.,* 2014; Rasebeka *et al.,* 2014). Different pre-sowing techniques, such as stratification, mechanical, acid and hot water scarification as well as tap water have been widely used (Teketay, 1996, 1998, 2005; Aref *et al.,* 2011; Tadros *et al.,* 2011; Missanjo *et al.,* 2014; Rasebeka *et al.,* 2014; Fredrick *et al.,* 2016) because they can improve germination within a relatively short period of time (Tadros *et al.,* 2011). As with the other leguminous species, seeds of *V. rehmanniana* have hard seed coats, which require pre-sowing treatment to overcome dormancy. However, there is complete lack of information about the pre-sowing methods for overcoming the seed coat-imposed dormancy in *V. rehmanniana*. The objective of this study was, therefore, to investigate the effects of different pre-sowing treatment methods on the germination of seeds of *V. rehmanniana.*

## MATERIALS AND METHODS

### Experimental site

The experiment was carried out in the laboratory at the Botswana University of Agriculture and Natural Resources (BUAN) from November to December 2016. BUAN is located at Sebele, 23°34' S and 25°57' E with an altitude of 994 m above sea level, 10 km from the centre of Gaborone City, the Capital of Botswana along the A1 North-South highway.

### Seed collection and processing

The pods *of V. rehmanniana* were collected directly from different healthy, erect and mature mother trees at Butale Village (North East District), eastern Botswana, in September 2016. Subsequently, the pods were placed in a paper bags and transported to the laboratory in BUAN where the seeds were extracted and mixed together. In the laboratory, seeds were screened to remove those showing some signs of damage, and about 4% of the seeds showed some signs of insect damage. The seeds were kept in a tightly sealed bottle and stored in a cool place awaiting commencement of the study. Prior to the experiment, seeds were immersed in distilled water, and only those that sank and settled at the bottom were used for the experiment. The seeds that floated, which represent unfilled and dead seeds, were discarded.

### Seed characteristics

To assess the seed characteristics of *V. rehmanniana,* 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.

**Experimental design and germination experiment**

The experiment was laid out in a completely randomized design (CRD) with five treatments, i.e. mechanical scarification, boiling water, hot water, concentrated sulphuric acid (98.8%) and control. The boiling water treatment had three different levels of exposure time (1, 3 and 5 minutes) whereas the concentrated sulphuric acid treatment had four different levels of exposure time (15, 30, 45 and 60 minutes). Each treatment had 100 seeds replicated four times with 25 seeds in each replication. The seeds were germinated in petri dishes lined with cotton wool, which was kept continuously moist with distilled water.

**Pre-sowing seed treatment procedures**

In the mechanical scarification treatment, the seeds were scarified by carefully nicking the seed coat at the distal end by hand with a nail cutter. In the boiling water treatment, first seeds were enclosed in coffee filter papers and clipped tightly to avoiding any of them falling out and, then, immersed in boiling water for 1, 3 and 5 minutes. They were then removed from the boiling water and left to cool down on a table. Before the hot water treatment, water was boiled to up to approximately 100°C. Then, the simmering hot water (about 98.5 °C) was poured in a 100 ml heat resistant glass beaker containing the seeds, which was left to gradually cool for 24 hours at room temperature. For the sulphuric acid treatment, the method described by Botsheleng *et al.* (2014) was followed. Four hundred seeds were counted from the seed lot and then divided into batches of 100 seeds. The seeds were, then, put into four 100 ml heat resistant non-corrosive glass beakers and concentrated sulphuric acid (98.8%) was added slowly on the side of the beakers to a level where all seeds were covered (about 50 ml). Seeds in the four beakers were left in the sulphuric acid for different time periods (15, 30, 45 and 60 minutes), and they were stirred regularly to ensure equal exposure of the seeds to the acid. After each soaking period, the sulphuric acid was drained off, and the seeds were repeatedly rinsed in running tap water until they were considered safe to handle. The control consisted of seeds that did not receive any treatment.

**Germination assessment**

The counting of germinated seeds was done daily, and a seed was considered germinated when the radicle reached about 2 mm. The experiment was terminated after 21 days.

**Data analyses**

Data collected on the germination of seeds were used to calculate germination percentage, germination mean time and germination index for each treatment using the equations below.

Germination percentage (GP), which represents the number of germinated seeds as a percentage of the total number of tested seeds, was computed using the following formula:

GP (%) = (germinated seeds/total tested seeds) × 100

Germination mean time (GMT) and germination index (GI) were calculated as follows (Botsheleng *et al.*, 2014):

GMT (days) = $\Sigma T_i N_i/S$

where, $T_i$ = number of days from the beginning of the experiment, $N_i$ = number of seeds germinated per day and S = total number of seeds germinated.

GI = $(G_1/1) + (G_2/2) +...+ (G_x/x)$

where, G = germination day 1, 2…, and x = the corresponding day of germination.

To test if there were significant differences among treatment means, the data were further subjected to one-way analyses of variance (ANOVA) using Analytical Software (2003), following arcsine transformation of all percentage data. Significant differences of means were tested using Tukey's Studentized Range (HSD) at the significance level of $P \leq 0.05$.

## RESULTS AND DISCUSSION

Table 1. Effect of seed pre-treatment on mean germination parameters of *V. rehmanniana*.

| Treatments | Cumulative mean germination (%) | GMT (days) | GI |
|---|---|---|---|
| Control | 3.00$^{d}$ | 1.50$^{c}$ | 0.01$^{c}$ |
| Mechanical scarification | 69.00$^{ab}$ | 4.53$^{abc}$ | 0.82$^{ab}$ |
| Boiling water | | | |
| 1 min | 24.00$^{c}$ | 6.88$^{a}$ | 0.22$^{c}$ |
| 3 min | 73.00$^{a}$ | 4.56$^{abc}$ | 0.87$^{a}$ |
| 5 min | 68.00$^{ab}$ | 4.54$^{abc}$ | 0.81$^{ab}$ |
| Hot water-24 hrs | 4.00$^{d}$ | 1.00$^{c}$ | 0.03$^{c}$ |
| Sulphuric acid (98%) | | | |
| 15 min | 4.00$^{d}$ | 1.63$^{bc}$ | 0.03$^{c}$ |
| 30 min | 24.00$^{c}$ | 6.42$^{a}$ | 0.21$^{c}$ |
| 45 min | 52.00$^{b}$ | 6.27$^{ab}$ | 0.62$^{b}$ |
| 60 min | 71.00$^{ab}$ | 4.32$^{abc}$ | 0.85$^{ab}$ |
| Significance | ** | ** | ** |
| HSD | 19.99 | 4.76 | 0.23 |
| CV (%) | 21.13 | 47.33 | 21.28 |

** Highly significant at $P < 0.01$. Means separated using Tukey's Studentized Range (HSD) Test at $P \leq 0.05$; means within columns followed by the same letters are not significantly different. GMT = germination mean time and GRI = germination index.

The mean length, width and breadth of seeds were 7.1 ± 0.08, 6.3 ± 0.08 and 3 ± 0.06 mm, respectively, with minimum and maximum ranges of 6.7-7.5, 5.8 - 6.6 and 2.7-3.2 mm, respectively. The mean thousand seed weight of the seeds was 93.5 ± 0.95 g, ranging between 88.3 and 97 g.

The results indicated that seed germination percentage, germination mean time and germination index were significantly ($F_{(9, 30)} = 51.4$, $P < 0.01$) affected by pre-sowing treatment methods (Table 1). Overall, the highest cumulative germination percentages at termination of the experiment were recorded in seeds subjected to boiling water for 3 minutes (73%), followed by seeds subjected to sulphuric acid for 60 minutes (71%), nicking (69%) and seeds subjected to boiling water for 5 minutes (68%) (Figure 1). No statistical differences in germination percentages were observed among the above treatments. Results also show no significant differences ($P > 0.05$) in cumulative germination percentages between seeds soaked in boiling water (1 minute) and those soaked in sulphuric acid (30 minutes). The lowest cumulative germination percentages were recorded in the control (3%) followed by hot water (24 hours) and sulphuric acid (15 minutes).

Different pre-treatment methods have been used to break dormancy to stimulate prompt and uniform germination of seeds (Teketay, 2005; Sahoo *et al.*, 2007; Aref *et al.*, 2011; Botsheleng *et al.*, 2014; Rasebeka *et al.*, 2014; Frederick *et al.*, 2016). No single pre-treatment method has been reported to be effective across plant species (Amusa, 2011). Mechanical scarification has been shown to enhance germination in seeds of many species (Teketay, 1996, 1998, 2005; Likoswe *et al.*, 2008; Aref *et al.*, 2011; Olatunji *et al.*, 2013; Missanjo *et al.*, 2014). Present results show that mechanical scarification significantly ($P < 0.01$) improved germination compared with the control. These results demonstrated the effectiveness of mechanical scarification in overcoming physical dormancy, which is imposed by a hard seed coat that prevent the seed from taking up water, the first critical step in germination (Teketay, 2005; Chisha-Kasumu *et al.*, 2007). Chisha-Kasumu *et al.* (2007) recorded the highest rate of germination in mechanically scarified *Pterocarpus angolensis* DC. seeds within 5 days of sowing. Mechanical scarification cracks part of the hard seed coat which is a barrier to water uptake and gaseous exchange (Teketay, 2005; Azad *et al.*, 2011) and allow germination to proceed (Olatunji *et al.*, 2013). The results of the present experiment indicate that the seed dormancy found in *V.* rehmanniana is caused by physical rather than embryo dormancy.

Soaking seeds in boiling water (1, 3 and 5 minutes) was effective in improving germination compared with the control. However, seed germination percentages of seeds soaked in boiling water for 3 and 5 minutes were significantly higher ($P < 0.01$) than those soaked for 1 minute. Results indicated that soaking seeds in boiling water for a few minutes break their physical dormancy and allow their subsequent germination. The effectiveness of boiling water observed in this work could probably be attributed to the softening of the hard seed coat, which allowed entrance of water and air into the seed to induce

physiological changes (Teketay, 2005; Pahla *et al.*, 2014, Rasebeka *et al.*, 2014) that subsequently triggered germination (Rasebeka *et al.*, 2014). Present results are consistent with McDonald and Omoruyi (2003) who reported the highest seed germination (70%) in *Dialium guineense* Wild. seeds soaked in boiling water (100 °C) for 5 minutes. The fact that soaking seeds in boiling water for 1 minute was not as effective as the 3 and 5 minutes soaking durations could be attributed to the degree of the seed coat thickness. These results suggest that the seed coat thickness of V**.** rehmanniana requires soaking in boiling water for longer duration.

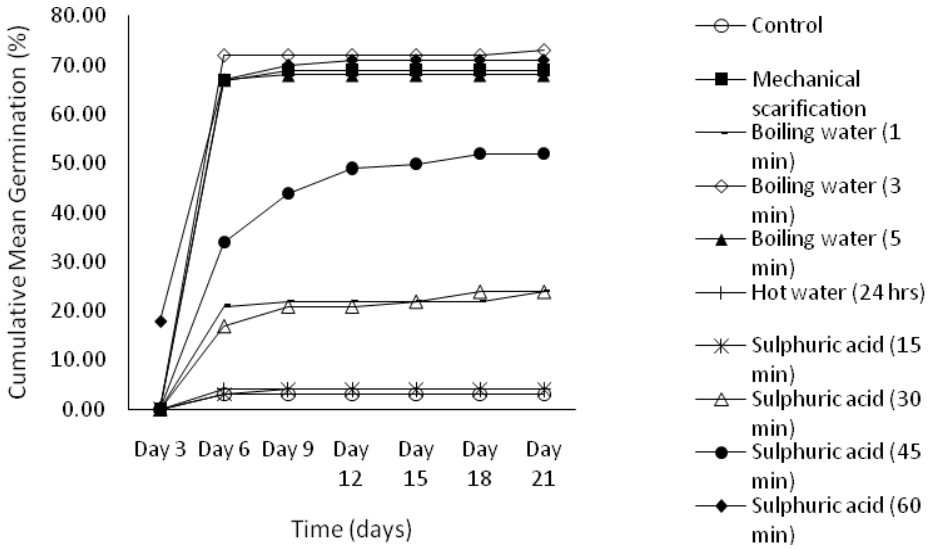


Figure 1. Cumulative mean germination (%) over time as influenced by presowing seed treatments.

Hot water treatment is frequently used to overcome dormancy in seeds with a hard coat. When using this method, seeds are soaked at 40-100 °C for a period of time, depending on the species and seed coat thickness or until the boiling water cools to ambient (Tadros et al., 2011). There was no significant difference in germination percentages between seeds soaked in hot water for 24 hrs and the control. These results are in agreement with studies conducted elsewhere using seeds of different tree species (Teketay, 1996, 1998; Botsheleng et al., 2014; Fredrick et al., 2016). The effectiveness of hot water in breaking seed dormancy varies from species to species (Tigabu and Oden, 2001; Teketay, 2005). For example, Albrecht (1993) reported that soaking seeds for 24 hrs in water boiled to 100 °C was effective in breaking the seed coat dormancy of *Adansonia digitata* L., *Calliandra calothyrsus* Meissner and *Sesbania sesban* (L.) Merr. Tadros et al. (2011) observed that soaking Leucaena *leucocephala* (Lam.) de Wit seeds in 70 °C water for 20 minutes was effective in breaking seed

dormancy and enhancing seed germination. The results indicate that V. rehmanniana has a very tough seed coat, which is in agreement with Aref (2000) who reported hard coats in other *Vachellia* species. The hard seed coat is one of the several strategies that the *Vachellia* species use to survive in the spatially and temporally variable environment (Aref, 2000). It is possible that the hot water in the present study cooled off before the hard seed coat was soft enough to allow entry of water and air.

Sulphuric acid (30, 45, and 60 minutes) improved seed germination compared with the control. This is consistent with results from other studies that reported prompt and uniform germination in hard water impermeable seed coated seeds soaked in sulphuric acid (Muhammad and Amusa, 2003; McDonald and Omoruyi, 2003; Keshtkar *et al.*, 2008; Likoswe *et al.*, 2008; Aref *et al.*, 2011). Seed germination in the present study increased with soaking period in sulphuric acid. Muhammad and Amusa (2003) recorded the highest germination in *Tamarindus indica* L. seeds soaked in sulphuric acid (50%) for 60 minute. Sulphuric acid disrupts the seed coat and expose the lumens of the macrosclereids cells, permitting imbibition of water, which triggers seed germination (Aliero, 2004; Amusa, 2011). The germination of seeds soaked in sulphuric acid for 15 minutes did not significantly differ from the control indicating that *V.* rehmanniana seeds are characterized by hard seed coat, which requires longer soaking periods in sulphuric acid to reduce its thickness.

The lowest mean germination durations (days) were recorded in seeds soaked in hot water for 24 hrs (1) followed by control (1.5) and seeds soaked in sulphuric acid for 15 min (1.63). These together with high coefficient of variation (47.33) revealed indicates that the three treatments had difficulties breaking the hard seed coat exhibited by *V.* rehmanniana recording no germination in almost all the replications. However, the rest of the treatments were evenly spread between 4.32 and 6.88 days with no statically differences. The few seeds which germinated as evidenced by the highest germination percentage (73%) after 21 days across the treatments germinated within a reasonable period. As would be expected, seeds subjected to boiling water for 3 and 5 minutes, sulphuric acid for 45 and 60 minutes and mechanical scarification had the highest germination index ranging from 0.6 to 0.9. As a ratio, this shows that treatments were superior to others.

## CONCLUSION

Mechanical scarification, boiling water (3 and 5 minutes) and concentrated sulphuric acid (45 and 60 minutes) proved to be effective in improving seed germination in V. rehmanniana in the present study. However, mechanical scarification and boiling water methods are recommended for use in nurseries and by farmers because sulphuric acid is expensive and needs to be handled by trained individuals. Authors also recommend increased exposure time over 5 and 60 minutes for boiling water and sulphuric acid respectively.

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