Application and Use of Presowing Treatment Methods to Improve Germination of Vachellia karroo (Hayne) Banfi & Galasso

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ABSTRACT

A germination experiment of Vachellia karroo seeds was conducted at the Botswana University of Agriculture and Natural Resources, Department of Crop and Soil Sciences laboratory, from September to October 2018. Seeds were collected along the Segoditshane River in Gaborone to investigate the effect of different pre-sowing treatment methods on their 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 098.8%), Boiling water (30, 60, 180 and 300 seconds) and concentrated sulphuric acid (15, 30, 45 and 60 minutes) had four levels of exposure time. The highest significant (p < 0.01) cumulative germination percentages were recorded in seeds subjected to sulphuric acid for 45 and 60 minutes, mechanical scarification (shortest germination mean time of 2.0-2.3) and boiling water at 30 and 60 seconds (moderate germination mean time of 5-5.9) whereas, the control treatment had the least cumulative germination percentage of 2%. As expected, the same trend was revealed for germination index. The seeds possess seed coat imposed dormancy, which requires sowing treatments. The best treatments for releasing dormancy in V. karroo were sulphuric acid and mechanical scarification and because of the risks associated with the use of sulphuric acid, the researchers recommend mechanical scarification as the suitable treatment method in tree nurseries.

1. Introduction

Vachellia karroo (Hayne) Banfi & Galasso (Family: Fabaceae, sub-family: Mimosoideae), commonly known as "sweet thorn", is one of the most abundant and widespread Vachellia in southern Africa (Timberlake, 1980; Lagerwall, 2016). Formerly known as Acacia karroo, the species was recently transferred to the genus Vachellia (Kyangalilwa, 2013). Vachellia karroo is a multipurpose species native to Zimbabwe, Botswana, Swaziland, Lesotho, Namibia, South Africa, Mozambique, Zambia, Malawi and south of Angola (Timberlake,1980; Barnes et al.,1996). The species grows well in open woodland and wooded grassland (Van Wyk, 1990).

Vachellia karroo is a deciduous small shrub 3.5 m or tree up to 15 m tall, with a spreading, roundish crown and low branching stem (Timberlake, 1980; Van Wyk 1990; Palgrave 2002). The species is characterized by a dark and rough bark; striking, long, paired, white thorns; yellow, sweet smelling, ball-like flowers producing copious amounts of nectar (Setschego and Venter 2003). The pods are sickle-shaped and dehisce by splitting lengthwise in the middle (Setschego and Venter, 2003). Under favourable conditions, this species grows fast and is well adapted to arid and semi-arid environments constrained by drought, high temperatures, salinity and extreme light (Van Wyk, 1990; Palgrave, 2002; Ben Zetta et al., 2017).

It is one of the fastest-growing Vachellia species and produces high-density wood (800-890 kg m-3) (Kheloufi et al., 2017). The species provides different types of goods and services to the community. It produces palatable leaves, flowers and pods that provide excellent fodder for cattle, goats and game (Van Wyk, 1990; Setschego and Venter, 2003). The species produces edible gum, which is also used for pharmaceutical products (Van Wyk, 1990). It is an excellent source of pollen and nectar (Setschego and Venter 2003; Kheloufi et al., 2017), which make the species ideal for bee farmers (Venter and Venter, 2012). The bark contains 19% tannin (Venter and Venter, 2012) used for tanning leather (Setschego and Venter, 2003, Venter and Venter, 2012). Like many Vachellia species, the ability of V. karroo to develop dual symbiosis with Rhizobia and endomycorrhizal fungi contributes to fix nitrogen, provides shade, and stabilizes sand dunes and disturbed areas. It has a moderately dense crown that provides a suitable environment for sustained production of nutritious perennial grasses, such as Panicum maximum Jacq. and Cenchrus ciliaris L. (Barnes et al., 1996).
The successful regeneration of shrub and tree species under varied environmental conditions depends on their ability to produce sufficient seeds, exhibit high seed viability, seedling survival and growth (Khumbongmayum et al., 2005). The use of seeds is the cheapest method of propagating many tree species (Tigabu and Oden, 2001) worldwide. However, on numerous occasions viable seeds of some plant species fail to germinate when exposed to favourable conditions (sufficient water, good aeration and a suitable temperature) due to dormancy (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006; Ibiang et al., 2012; Chahtane et al., 2017). According to de Morais et al. (2014), dormancy is an inherited trait in seeds assigned to the palisade cell layer whose cell walls are thick and covered externally by a waxy cuticle. Dormancy evolved differently across shrub and tree species due to adaptation to their environment (Botsheleng et al., 2014). Numerous plants use dormancy as a strategy to survive (Donohue et al., 2010; Huang et al., 2010; Salazar et al., 2011) in the soil seed bank until conditions are suitable for germination (Graeber et al., 2012; Mark and Ooi, 2012).

Seeds of most Vachellia species exhibit poor germination or experience delayed germination due to dormancy that inhibit water imbibition and oxygen uptake by the developing embryo (Mucunguzi and Oryem-Origa, 1996; Grubb and Coomes, 1997; Baskin and Baskin, 2004; Walters et al., 2004; Aref et al., 2011). In nature, hard seed coats are cracked or softened by fire (Bradstock and Auld, 1995; Mbalo and Witkowski, 1997; Walters et al., 2004), extreme temperatures, digestive acids in the stomachs of animals or by the abrasion of blowing sand (Luna et al., 2009). In laboratories and tree nurseries, artificial methods are used to speed up germination. Seed dormancy influences the successful execution of afforestation and reforestation programs in arid and semi-arid environments. To break seed dormancy or enhance germination, Vachellia seeds often require pre-sowing treatment (Aref et al., 2011; Azad et al., 2011). Several artificial techniques have been used to break the hard seed coat and increase seed permeability. The techniques include physical (manual scarification, boiling water and cold water, dry heat and fire and chemical pre-sowing treatments (sulphuric acid, alcohols and organic solvents) (Hanna, 1984; Teketay, 1996; 1998; Uniyal et al., 2000; Okunomo and Bosah, 2007; Aref et al., 2011; Moussavi et al., 2011; Ghassali et al., 2012; Botsheleng et al., 2014; Fredrick et al., 2016; Mojere mane et al., 2017, 2018; Odirile et al., 2019). Vachellia karroo is one of the species characterised by physical dormancy (Baskin and Baskin, 2014). The aim of the present study was, therefore, to evaluate the influence of pre-sowing treatments on the germination of Vachellia karroo seeds.

2. Materials and Methods

2.1. Study site

The study was conducted at Botswana University of Agriculture and Natural Resources, located at Sebele (23°34’ S and 25°57’ E, altitude of 994 m) 10 km from the center of Gaborone, the Capital City of Botswana, along the A1 North-South highway. Seeds used were collected from 15 randomly selected trees along the Segoditshane River, Phase 2 and Gaborone in July 2018.

2.2. Experimental design

The experiment was set up in September 2018 in a laboratory, and room temperature of 25 ± 3°C was maintained throughout the experiment. The experiment was laid-out in a completely randomized design (CRD) with five main treatments (control, mechanical scarification, boiling water, hot water and concentrated sulphuric acid (98.8%). Boiling water had four different levels of time exposure (30, 60, 180 and 300 seconds) whereas, concentrated sulphuric acid had four different levels of time exposure (15, 30, 45 and 60 minutes). Each treatment had four replications of 25 seeds. Seeds were germinated in 90 mm petri dishes lined with cotton wool and closed with lids to reduce water loss by evapotranspiration (Schroder et al., 2013). The petri dishes were kept moist by spraying with distilled water.

2.3. Seed characteristics

The number of seeds in a pod was determined from five replications of 10 pods. Seeds were, then, categorized as intact, aborted or dead/eaten. Seed length, width and breadth were determined from five replicates of ten seeds using an electronic digital caliper (0-150 mm). The mass (weight) of single seeds was determined by weighing the using an electronic analytic balance (Model: PW 124) in four replications of 25 seeds. Ten replications of 100 seeds were weighed to determine the 1000 seed weight.

2.4. Experimental procedures

For the 98% concentrated sulphuric acid, four replications of 25 seeds for each exposure time (15, 30, 45 and 60 minutes) were put into heat resistant non-corrosive glass beakers (Botsheleng et al., 2014). The acid was added slowly to a level covering all seeds and shaken occasionally. After each exposure time, the seeds were sieved-out using an acid resistant sieve and the acid drained off simultaneously into the beaker and, then, washed thoroughly in running tap and distilled water to remove all the acid for safe handling. With regard to boiling water, four replications of 25 seeds for each exposure time (30, 60, 180 and 300 seconds) were enclosed in coffee filter papers, which were clipped tightly to prevent them from falling before immersing in boiling water
Seed germination percentage, germination mean time and germination index were significantly (p < 0.01) affected by pre-sowing seed treatments (Table 1). Germination in nicking, hot water, boiling water (30 and 60 seconds) and sulphuric acid (45 and 60 minutes) treatments gave a significantly (p < 0.01) higher germination percentages than other treatments, including the control. There was no statistical difference in germination among the nicked, hot water, boiling water (30 and 60 seconds) and sulphuric acid (45 and 60 minutes) treated seeds. Several studies reported that seeds of *Vachellia* species are characterized by hard seed coats that act primarily as physical barrier to imbibition of water and entrance of oxygen (Holmes et al., 1987; Nasr et al., 2013; Ben Zetta et al., 2017) as well as the growth of the embryo (Nasr et al., 2013).

The results revealed that nicking improved the germination of *V. karroo* seeds compared with the control (Table 1). This result is consistent with those of studies conducted using other plant species (Teketay, 1996; Shiferaw et al., 2004; Travlos et al., 2007; Bamel et al., 2007; Botsheleng et al., 2014; Mojeremane et al., 2017; Odirile et al., 2019), Travlos et al. (2007) reported that nicking or scratching the seed coat promoted germination of *Tylosoem esculentum* (Burch.) A. Schreiber seeds. Shiferaw et al. (2004) reported that nicking or scratching *Prosopis juliflora* (Sw.) DC. seeds resulted in 100% germination. Odirile et al. (2019) nicked Vachellia erioloba (E.Mey.) P.J.H.Hurter seeds and reported 98% germination. Mojeremane et al. (2017) observed 69% germination in nicked *Vachellia rehmanniana* (Schinz) Kyal. & Boatwr. seeds compared with 3% in the control treatment. Nicking cracked the hard seed coat and allowed uptake of water and oxygen, which may have stimulated rapid and uniform germination (Teketay, 2005; Botsheleng et al., 2014), thereby, elongating the embryonic axis (Botsheleng et al., 2014). Although nicking improved seed germination, it may be a bottleneck when treating small sized seeds or a large number of seeds to produce many seedlings (Mapongmetsem et al., 1999; Baskin and Baskin, 2014). There is also a possibility of damaging the endosperm, cotyledons or embryo during nicking, which may result in low germination.
Soaking seeds in hot water for 24 hours enhanced germination in the present study. This result is consistent with results of germination experiments conducted on many tropical shrub and tree species (Sajeevukumar et al., 1995; Aref, 2000; Mwase and Mvula, 2011; Daz, 2014; Mojeremane et al., 2018; Odirile et al., 2019). Improved germination may be attributed to softening of the hard coats by hot water, which allowed seeds to imbibe water prior to sowing. Prior studies reported that pre-treating seeds in hot water is simple, cheap, reliable and suitable for a large number of seeds (Ortega-Baes et al., 2002, Himanen et al., 2012).

Soaking V. karroo seeds in boiling water for 30, 60, 180 and 300 seconds was effective in improving seed germination compared with the control (Table 1). However, seed germination decreased with increasing soaking time, which is consistent with results of other studies (Idu and Omonhinmin, 1999; Mojeremane et al., 2018). Idu and Omonhinmin (1999) scarified Dichrostachys cinerea L. Wight & Arn. seeds in boiling water for 10, 40, 60, 300 and 900 seconds and recorded the highest germination in seeds immersed in boiling water for 10 and 40 seconds. Mojeremane et al. (2018) soaked Peltophorum africanum Sond. In boiling water for 60, 180 and 300 seconds and recorded germination of 87, 32, and 24%, respectively. These results suggest that soaking seeds in boiling water for 180 and 300 seconds caused damage to the internal parts of some seeds, resulting in low germination. In contrast with our results, other studies conducted on other shrub and tree species observed no germination in seeds soaked in boiling water for 60, 180 and 300 seconds (Kahaka, 2017; Mojeremane et al., 2017). They attributed poor seed germination to the boiling water, which probably damaged the embryo.

Sulphuric acid (30, 45 and 60 minutes) enhanced seed germination in this study compared with the control (Table 1). This result is consistent with results of experiments conducted on seeds of other plant species, which demonstrated that sulphuric acid improved seed germination (Herron and Clemens, 2001; McDonald and Omoruyi, 2003; Cirak et al., 2004; Keshkar et al., 2008; Likoswe et al., 2008; Sosnoskie and Cardina, 2009; Aref et al., 2011; Mojeremane et al., 2018; Odirile et al., 2019). Sulphuric acid wears out hard seed coats (Ali et al., 2011; Nasr et al., 2013) and allows imbibition, which triggers germination (Aliero, 2004; Amusa, 2011). In contrast, soaking seeds in sulphuric acid for 15 minutes did not improve germination compared with control. Mojeremane et al. (2017) reported similar results in Vachellia rehmanniana (Schinz) Kyal. & Boatwr seeds. This may be attributed to the hard coat of V. karroo seeds that requires soaking periods of more than 15 minutes in concentrated sulphuric acid to disrupt the seed coat. Although sulphuric acid (30, 45 and 60 minutes) enhanced the germination of V. karroo seeds, the acid is expensive and hazardous to workers and the environment (Danthu et al., 1992; Nasr et al., 2013). The use of sulphuric acid also requires special equipment, personal protective gear and proper disposal after use (Luna et al., 2009). Furthermore, seeds can also be damaged by over-soaking in the acid (Nasr et al., 2013).

3.3 Germination mean time and germination index

Germination mean time and germination index were significantly (p < 0.01) affected by pre-sowing treatments (Table 1). Nicked seeds germinated within two days whereas, seed treated with sulphuric acid (45 and 60 minutes) germinated within 2.3 days, which was significantly lower than the rest. As would be expected, germination index was significantly higher for treatments with higher germination percentages.

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Table 1. Effect of pre-sowing seed treatments on germination parameters of V. karroo

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Germination parameters</th>
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<tr>
<td></td>
<td>Germination (%)</td>
</tr>
<tr>
<td>Control</td>
<td>0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nicking</td>
<td>87.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hot water (24 hours)</td>
<td>85.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boiling water (30 seconds)</td>
<td>85.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boiling water (60 seconds)</td>
<td>85.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boiling water (180 seconds)</td>
<td>60.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Boiling water (300 seconds)</td>
<td>34.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphuric acid (15 minutes)</td>
<td>11.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphuric acid (30 minutes)</td>
<td>51.1&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphuric acid (45 minutes)</td>
<td>89.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sulphuric acid (60 minutes)</td>
<td>88.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

** Highly significant at p < 0.01. Means separated using Tukey’s Honestly Significant Difference (HSD) Test at p ≤ 0.05. Means within columns followed by the same letters across treatments are not significantly different. GMT = germination mean time, GI = germination index and CV = coefficient of variation in percentage.
Sulphuric acid (45 and 60 minutes) and nicking exhibited more than 70% mean daily germination rate within 2 days whereas, the rest were below 40% (Figure 1). No significant increases in the number of germinated seeds were recorded between 10 to 13 days across treatments.

![Figure 1. Mean daily germination rate of V. karroo recorded over 20 days.](image)

4. Conclusion

Sulphuric acid (30, 45 and 60 minutes), hot water soaking for 24 hours, nicking and boiling water (30, 60 and 180 seconds) improved the germination of V. Karroo seeds. However, among these treatments, sulphuric acid is not a suitable pre-sowing seed treatment method in tree nurseries because it requires special equipment, personal protective gear and proper disposal after use.

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