Germination studies on seeds of *Burkea africana* and *Erythrophleum africanum* from Kazuma Forest Reserve, Northern Botswana

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*Burkea africana* and *Erythrophleum africanum* are characterized by seed coat-imposed dormancy that prevents water entry and gaseous exchange, which are essential for the germination process. The objective of this study was to determine the best possible pre-sowing treatment method that maximizes seed germination of the two species. Seeds of both species were subjected to four experiments, containing 10 levels of presowing treatments: The control, mechanical scarification, soaking in concentrated sulphuric acid (for 15, 30, 45 and 60 min), immersion in boiling water (for 1, 3 and 5 min), and soaking in boiling water (and cooling down for 24 h). The germination data were subjected to ANOVA followed by Tukey's HSD Test to separate significantly different treatment means. The most promising results showed that seeds treated with mechanical, sulphuric acid and boiling water scarification had significantly higher mean percent germination than the controls for *B. africana*; whereas for *E. africanum*, mechanical scarification, exposure to sulphuric acid, boiling water (1 min) and immersion in boiling water (and cooling down for 24 h) had higher percent germination than the controls.

**Key words:** *Burkea africana, Erythrophleum africanum*, germination percentage, pre-sowing treatment, seed dormancy.

**INTRODUCTION**

Over the past few years, Botswana has put considerable efforts into forest conservation and afforestation programmes, such as the annual national tree planting day. This day dates back to 1985 when the then President Sir Ketumile Masire launched the first national tree planting day and has since been commemorated on the last Saturday of November each year (BOPA, 2013). At inception of the tree planting day, exotic tree species were planted in community woodlots and distributed for planting by individuals. Exotic species were promoted
because they establish easily, grow fast and are highly productive, especially on harsh sites where native tree species do not perform well (Dodet and Collet, 2012). They are highly productive because pests from their native habitats are absent (Nair, 2001). These characteristics contribute to their ability to invade local ecosystems (Dodet and Collet, 2012) and are a threat to native biodiversity (Bellard et al., 2016). Afforestation using exotic species has long been beneficial to the environment, and the aim of using exotic species was to repair damaged ecosystems (Richardson, 1998).

The use of indigenous tree species in afforestation and reforestation programmes is increasing worldwide (McNamara et al., 2006; Shono et al., 2007a; Raman et al., 2009). Similarly, Botswana has also been promoting their use in recent years (Rasebeka et al., 2014) because they cope well with prevailing harsh environmental conditions. However, the use of indigenous species in planting programmes is limited by the availability of quality planting materials (Elliott et al., 2002; Meli et al., 2014). There is need to identify indigenous tree species with readily available seed and propagation techniques that are suited to local environments (Shono et al., 2007b; Doust et al., 2008; Lamb, 2011; Meli et al., 2014).

_Burkea africana_ Hook. also known as _monato, mosheshe, Ohehe, nkalati_ in Botswana (Setshogo, 2002), burkea red syringa, Rhodesia ash, sand syringa, wild seringa and wild syringa (English) (Setshogo, 2002; Maroyi, 2010) belongs to the family Fabaceae (Caesalpinioideae) (Palmer and Pitman, 1972; Palgrave, 2002; Neya et al., 2004; Maroyi, 2010). The species is distributed throughout tropical Africa (Neya et al., 2004; Mair et al., 2018), from Senegal to Sudan and as far as South Africa (Maroyi, 2010). It has a flat-top and grows up to 61 cm in diameter and 20 m high (Fanshawe, 1972). The species grows naturally in open, wooded grassland and open woodland (Maroyi, 2010; Tanko et al., 2011) on sandy soil and lower slopes on rocky hills in the high rainfall areas, occasionally in miombo woodland (Mulofwa et al., 1994). The wood of _B. africana_ is hard, heavy and is used in constructional work such as bridges, sleepers, furniture, firewood, charcoal, fences and tool handles (Neya et al., 2004). The heartwood is very resistant to fungi (Neya et al., 2004). The bark, roots and leaves are used as medicine (Mulofwa et al., 1994; Mathisen et al., 2002). The bark has been used in medicine to treat colds, coughs, and constipation, gonorrhoea and syphilis (van Wyk and Gericke, 2007). _B. africana_ is planted as a roadside tree and ornamental (Maroyi, 2010). It is host to caterpillars of Saturnid moths (Cirina torda and Rohaniella pygmaea), which are eaten by local people. The flowers produce nectar collected by honeybees (Mulofwa et al., 1994). The bark and leaves are eaten by elephants and the tree yields a semi-translucent gel or green gum of high quality (Roodt, 1998).

_Erythrophleum africanum_ (Welw. ex Benth.) Harms is known as _mmako, mobaku, ununza, mopombo and mokong ochi_ in Botswana (Setshogo, 2002) as well as African blackwood and ordeal tree in English and belongs to the family Fabaceae (Caesalpinioideae) (Burkill, 1995; Setshogo, 2002). It is a medium-sized to large tree growing up to 15 m high (Palmer and Pitman, 1972; Palgrave, 2002). It has a straight and cylindrical stem, up to 120 cm in diameter, and a dense and spreading crown (Kawanga, 2008). The bark is grey in colour and smooth in young trees and becoming red-brown, rough and fissured with age (Kawanga, 2008; Maroyi, 2019). The leaves are alternate, egg-shaped to oblong, finely velvety, particularly when young and on the under surface. The apex of the leaf is broadly tapering to rounded or notched and the base is broadly tapering with entire margins (Kawanga, 2008; Maroyi, 2019). Flowers are cream to yellow in colour, sweetly scented, occurring in dense spikes and often grouped together in large heads. The fruit is a pod, splitting along both sides simultaneously and each section curving backwards (Kawanga, 2008; Maroyi, 2019). It is indigenous to tropical Africa (Lock, 1989; Burkill, 1985; Germishuizen and Meyer, 2003; Smith and Allen, 2004; Hyde et al., 2020). The species grows naturally in hot and dry deciduous woodlands at 600 to 1400 m above sea level, and is absent from riparian woodlands and the dry savanna of the Sahel (Kawanga, 2008). It is indigenous to tropical Africa (Lock, 1989; Burkill, 1985; Germishuizen and Meyer, 2003; Smith and Allen, 2004; Hyde et al., 2020). The wood is used for furniture, heavy and light construction, posts, poles and tool handles. In addition, it is used for firewood and making high quality charcoal. The bark, roots and leaves are used in medicine. An infusion of the bark is drunk to treat stomach-ache or dysmenorrhoea. Steeped in water, the bark is applied externally and internally to cure cardiac diseases and epilepsy. The powdered root bark, mixed with urine, is applied to the skin to treat leprosy and a paste of root-bark is applied to the skin to cure scabies (Kawanga, 2008).

Germination of seed is important in propagating seedlings for mass planting of woody plant species. However, it can be a time-consuming process because seeds of some plants take a longer time to germinate, or may fail to do so under some culture regimes. According to Botumile et al. (2020), a high level of seed dormancy is a characteristic feature of many plants of dry regions, and

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it either completely prevents germination or allows very few seeds to germinate over a long period of time. Seed dormancy is an adaptive mechanism that blocks the germination of intact viable seeds under conditions when the chance of seedling survival and growth is low (Weibrecht et al., 2011; Smykal et al., 2014; Long et al., 2015). Seeds of many leguminous plants have hard coats, which make it difficult for the seeds to imbibe water and prevent gaseous exchange (Bolingue et al., 2010). In nature, hard seed coats are cracked or softened by fire (Mbalo and Witkowski, 1997; Walters et al., 2004), extreme temperatures, digestive acids in the stomachs of animals or the abrasion of blowing sand (Luna et al., 2009) that can promote germination.

Hard seed coat-imposed dormancy of leguminous species hinders their successful artificial regeneration (Teketay, 1996a, b; Mojерemane et al., 2017, 2018; Odirile et al., 2019; Setlhabetsi et al., 2019). Several pre-sowing treatments have been used to enhance germination of seeds characterised by hard coats. These include mechanical, acid, cold, hot and boiling water scarification (Teketay, 1996b, 1998, 2005; Alamgir and Hossain, 2005; Amri, 2010; Azad et al., 2011; Rasebeka et al., 2014; Fredrick et al., 2017; Kahaka et al., 2018; Opoku et al., 2018; Botumile et al., 2020), among others. These techniques can improve germination by overcoming seed dormancy within a relatively short period of time (Tadros et al., 2011; Mojерemane et al., 2017, 2018; Odirile et al., 2019; Setlhabetsi et al., 2019).

*B. africana* and *E. africanum* are among the excellent candidate species for introducing in planting programmes in dry regions, because of their multiple uses and adaptation to the local environment. The hard seed coat is seen as a hindrance to uniform and rapid germination of tree and shrubs species, hence, there is a need for pre-sowing seed treatments to enhance germination. Therefore, the objective of this study was to determine some of the best possible pre-sowing treatment methods that maximize the germination of *B. africana* and *E. africanum* seeds.

**MATERIALS AND METHODS**

**Study site**

The experiment was conducted in the laboratory at the Botswana University of Agriculture and Natural Resources (BUAN) from January to February, 2019. The university is located at Sebele (23°34’ S and 25°57’ E, altitude 994 m), approximately 10 km from the Centre of Gaborone, the capital city of Botswana along the A1 North-South highway.

**Seed source**

Seeds were collected from Kazuma Forest Reserve (18° 4259’ S and 25.4970 E, altitude 997 m) in the Chobe district during August 2018. Mature and healthy fruits/pods were collected from the tree crown by shaking with a long-hooked stick. The mature dry pods were placed in paper bags and transported to the Department of Range and Forest Resources Laboratory, Botswana University of Agriculture and Natural Resources. Seeds were extracted by crushing the pods by hand, followed by winnowing to separate the husk. Seeds were kept refrigerated at 5°C for four months awaiting commencement of experiments. Prior to sowing, seeds were tested for viability using the floating method, in which the floated seeds were considered unviable and discarded.

**Experimental design and treatments**

In this study, four experiments, containing 10 levels of treatments, including the control, were carried out. The four experiments were mechanical scarification, exposure to sulphuric acid, exposure to boiling water and exposure to hot water for 24 h. The experiments were laid down in completely randomized design having four replications.

**Experiment 1: Mechanical scarification**

In this experiment, 100 seeds of each studied species, with four replications of 25 seeds, were used. In all these seeds, a pair of scissors was used to cut way 1 to 2 mm of the seed coat on a convex edge opposite where the embryo is located and avoiding removal of endosperm as much as possible.

**Experiment 2: Exposure to sulphuric acid**

In this experiment, four periods of exposure of seeds of the studied species using sulphuric concentrated sulphuric acid (98%), that is, 15, 30, 45 and 60 min, were used by employing the method described by Teketay (1996a). For each period of exposure, the four replications of 25 seeds were put into four 100-ml, heat-resistant, non-corrosive glass beakers containing sulphuric acid by making sure that all the seeds were covered by the acid. Seeds were hand stirred every 5 min during the specific treatment time to ensure their uniform exposure to the acid. After the specified periods of exposure, the seeds were sieved out of the acid using an acid-resistant sieve, while the acid was drained off simultaneously into another beaker. Seeds were then, thoroughly washed and rinsed to remove acid using running water tap first and subsequently using distilled water, successively.

**Experiment 3: Exposure to boiling water**

In this experiment, three periods of exposure of seeds of the studied species, that is, 1, 3 and 5 min, to boiling water were used. For each period of exposure, four replications of 25 seeds were put into four separate coffee filter papers and immersed into a cooking pot with boiling water for the specified period, after which they were removed and immersed in a small bucket containing room temperature distilled water to cool them down for a few minutes.

**Experiment 4: Exposure to boiled water for 24 h**

In this experiment, four replications of 25 seeds were put into four separate coffee filters and placed into a 250 ml beaker. Boiling water was, then, poured into the beaker and left to cool with the seeds inside for 24 h.

Four replications of 25 untreated seeds were used as control for all the experiments. In all the experiments and the control, each replication, containing the 25 seeds, was placed in 8-mm closed Petri dishes lined with cotton wool. The cotton wool was
controlled by 100% within seven days, respectively.}

**Table 1.** Means and ranges of the cumulative germination of seeds of the study species subjected to different pre-sowing seed treatments (± standard error of the means).

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>Burkea africana</em></th>
<th>Erythrophleum africanum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Germination (%)</td>
<td>Range</td>
</tr>
<tr>
<td>Control</td>
<td>5 ± 1°</td>
<td>04 - 08</td>
</tr>
<tr>
<td>Mechanical Scarification</td>
<td>80 ± 24abc</td>
<td>72 - 92</td>
</tr>
<tr>
<td>Sulphuric Acid (15 min)</td>
<td>74 ± 8ab</td>
<td>52 - 84</td>
</tr>
<tr>
<td>Sulphuric Acid (30 min)</td>
<td>73 ± 5ab</td>
<td>48 - 92</td>
</tr>
<tr>
<td>Sulphuric Acid (45 min)</td>
<td>93 ± 4a</td>
<td>84 - 100</td>
</tr>
<tr>
<td>Sulphuric Acid (60 min)</td>
<td>92 ± 2a</td>
<td>88 - 96</td>
</tr>
<tr>
<td>Boiling Water (1 min)</td>
<td>50 ± 6cd</td>
<td>36 - 64</td>
</tr>
<tr>
<td>Boiling Water (3 min)</td>
<td>62 ± 7bcd</td>
<td>52 - 84</td>
</tr>
<tr>
<td>Boiling Water (5 min)</td>
<td>66 ± 7bcd</td>
<td>48 - 80</td>
</tr>
<tr>
<td>Boiling Water (allowed to cool 24 h)</td>
<td>37 ± 7de</td>
<td>28 - 56</td>
</tr>
</tbody>
</table>

Means separated using Tukey’s Honestly Significant Difference (HSD) Test at P ≤ 0.05. Means within columns followed by the same letters for each species are not significantly different.

**RESULTS**

**Germination of seeds**

The results indicated that seeds treated with mechanical scarification, sulphuric acid and boiling water had significantly higher mean germination percentages than the control in *B. africana* ([One Way ANOVA: (F (9, 39) = 15.86, P = 0.00001)]. For *E. africanum* mechanical scarification, sulphuric acid, boiling water (1 min) and hot water (boiling water allowed to cool for 24 h) had significantly higher mean germination percentages than the control ([One Way ANOVA: (F (9, 39) = 22.19, P = 0.00001)] (Table 1). The ANOVA also indicated that there were significant differences among the different treatment times and conditions further clarified by the HSD significant differences within and among the treatment means (Table 1) as explained more fully in the following paragraph.

The highest mean germination percentages (93 and 92%) for *B. africana* were found in sulphuric acid (45 and 60 min) treatments, followed by those exposed to mechanical scarification (80%), sulphuric acid for 15 (74%) and 30 (73%) minutes as well as boiling water for 5 (66%), 3 (62%) and 1 (50%) min, respectively. Results of seeds immersed in hot water for 24 hours showed no significant effect on the germination of seeds compared with the control (Table 1). For *E. africanum*, the sulphuric acid (30, 45 and 60 min) treatments had the highest mean germination (95-98%), followed by mechanical scarification (90%), those treated in boiling water (1 min) (72%), hot water (boiling water allowed to cool in 24 h) (65%) and sulphuric acid (15 min) (58%). Boiling water treatments (3 and 5 min) had no significant effect on the germination of seeds (Table 1).

**Seed germination rate**

The results showed that seeds of *B. africana* that were treated with sulphuric acid (for 45 and 60 min) exhibited the fastest and uniform germination, reaching > 90% cumulative germination within five days after sowing, followed by mechanical scarification, reaching > 78% within 8 days and those treated with sulphuric acid (for 30 and 15 min), reaching > 71% and > 65% within seven days, respectively (Figure 1). On the other hand, untreated seeds (control) and seeds treated with hot water exhibited, not only the lowest germination percentage, but also the slowest germination.

The results also showed that in the case of
E. africanum, seeds treated with sulphuric acid (for 30, 45 and 60 min) and mechanical scarification exhibited the fastest and uniform seed germination, reaching > 95% and > 80% cumulative germination within five days, respectively (Figure 2). On the other hand, seeds treated with boiling water (5 min) and the control exhibited not only the lowest, but also the slowest germination.

DISCUSSION

Different techniques of breaking seed dormancy, in order to improve germination rate and speed up the germination process, have been suggested by other authors (Airi et al., 2009; Azad et al., 2010). Results of this study indicated that B. africana and E. africanum are characterized by physical seed dormancy imposed on the seeds by a water-impermeable seed coat. Mechanical scarification proved to be one of the most effective methods to break dormancy for both the two study species compared with the controls (Table 1). Removing 1-2 mm of the seed coat allows the seed to imbibe water, hence promoted radicle emergence. Once the seed imbibed water, the cumulative germination was improved significantly, and became more rapid and uniform. This result is consistent with work carried out on other leguminous plant species (Teketay, 1996a, 1998; Tigabu and Odén, 2001; Sy et al., 2001; Alamgir and Hossain, 2005; Rodrigues-Junior et al., 2014; Naim et al., 2015; Boateng, 2017; Fredrick et al., 2017; Mojeremane et al., 2017, 2018; Odirile et al., 2019; Botumile et al., 2020). Teketay (1996a) reported that mechanical scarification enhanced seed germination for most leguminous species. Tigabu and Odén (2001) recorded 100% germination in Albizia gummiifera seeds and 80% in Albizia grandibracteata compared with <10% germination of the untreated seeds. Mackay et al. (1995) also recorded 100% germination for mechanically scarified Lupinus havardii seeds. Botumile et al. (2020) obtained 96% germination for Vachellia robusta (Burch.) Kyalangiliwa and Boatwright and 88% for Senegalia galpinii (Burtt Davy) Seigler and Ebinger seeds. Mechanical scarification is a safer and more practical technique for scarifying few seeds. The technique is simple and effective in promoting rapid and uniform germination (Odirile et al., 2019). However, it requires a lot of time, especially if scarifying many seeds (Mapongmetsem et al., 1999; Himanen et al., 2012; Baskin and Baskin, 2014; Müller et al., 2017). According to Mmolutsi et al. (2020), it is also possible to damage the endosperm, cotyledons or embryo during nicking, which could result in low germination.
Sulphuric acid enhanced germination in *B. africana* and *E. africanum* compared with the controls (Table 1). Sulphuric acid is one of the most effective pre-sowing treatments for seeds with very hard coats. The acid wears out the thick seed coat and allows water to enter the seeds and trigger germination, which is more rapid and uniform. The results of the sulphuric acid treatments on the two study species are supported by similar studies conducted on other leguminous species elsewhere (Teketay, 1996a, 1998; Sy et al., 2001; Rincón-Rosales et al., 2003; Cirak et al., 2004; Phartyal et al., 2005; Aref et al., 2011; Nasr et al., 2013; Fredrick et al., 2017; Mojere Manene et al., 2017; Odirile et al., 2019). Although the sulphuric acid treatments are more effective methods for many tropical leguminous trees, the sulphuric acid used is expensive and a very dangerous and abrasive chemical to people and materials (Doran et al., 1983) as well as a potential pollutant of the environment unless properly disposed of. The acid needs to be handled with great care observing safety rules (Schmidt, 2007). Safety glasses, gloves and other protective clothing must be worn, and if possible, a fume cabinet used because inhaling the fumes is very harmful (Luna et al., 2009). There is also a possibility of damaging seeds by over soaking (Nasr et al., 2013). Disposing the waste acid safely can be serious challenge in some areas.

Hot water (boiling water allowed to cool for 24 h) increased the germination of *E. africanum* seeds compared with that of the control (Table 1). Soaking of seeds of *E. africanum* in hot water might have softened the seed coats and allowed for the imbibition of water. In contrast, *B. africana* seeds soaked in hot water (boiling water allowed to cool for 24 h) did not differ significant from the control. These contrasting results have been reported in other studies elsewhere (Albrecht, 1993; Teketay, 1996a, 1998; Sharma et al., 2008; Mwase and Mvula, 2011; Botsheleng et al., 2014; Fredrick et al., 2017; Mojere Manene et al., 2017; Botumile et al., 2020). Studies have shown that the effectiveness of hot water in improving seed germination vary with species (Tigabu and Oden, 2001; Teketay, 2005). For seeds treated in hot water at 100°C, Sharma et al. (2008) reported germination of 94 to 100% in *Albizia lebbeck* (L.) Benth., *Albizia procera* (Roxb.) Benth., *Peltogyne pterocarpum* (DC.) Backer ex Heyne, *Acacia auriculiformis* A. Cunn. ex Benth. and *Leucaena leucocephala* (Lam.) de Wit. Albrecht (1993) reported that treating seeds for 24 h in hot water at 100°C enhanced percent germination of *Adansonia digitata* L., *Calliandra calothyrsus* Meissner and *Sesbania sesban* (L.) Merr. Botumile et al. (2020) also reported that hot water improved percent germination in *Vachellia karroo* (Hayne) Banfi & Galasso.
compared with the control. According to Mwase and Mvula (2011), hot water softens hard seed coats, leaches out chemical inhibitors and allows imbibition and gaseous exchange. Mojeremane et al. (2017) found that hot water was not effective in improving percent germination of *Swartzia madagascariensis* just like those of *B. africana* in the present study. According to Teketay (1996a) the degree of the seed coat hardness among different species is the cause of different responses to various treatments. The poor performance of *B. africana* in the hot water treatment could be due to the thickness of the seed coat, which failed to break before the water cooled down. The fact that boiling water treatments (experiment 3 in this study) improved germination is evidence that the species is characterised by hard coat-imposed dormancy.

Boiling water (for 1, 3 and 5 min) was effective in increasing percent germination in *B. africana* compared with controls (Table 1). Results indicated that percent germination in this species increased with exposure time, suggesting physical dormancy imposed by the hard seed coat. In the case of *E. africanum*, percent germination was increased by treating seeds in boiling water (1 min) compared with the control. There were no significant differences in percent germination among the boiling water (3 and 5 min) treatments and the control (Table 1). Results show that percent germination decreased with increase in exposure time to boiling water. This result is consistent with Botumile et al. (2020) who reported a decrease in percent germination with increasing exposure time up to 5 min with boiling water for *Senegalia galpinii* and *Vachellia robusta*. Similar results were also reported for *Vachellia karroo* (Mmolutsi et al., 2020). The decline in percent germination with increase in boiling time could be due to the sensitivity of seeds to the heat, which might have damaged the embryo.

**Conclusion**

Dormancy in the legume species is mainly caused by their hard seed coat covering which prevents water uptake and gaseous exchange. Therefore, the hard seed coat needs to be subjected to pre-sowing treatments before seeds can germinate. The study has shown that the hard seed coat in *B. africana* can be overcome by mechanical scarification, exposure to sulphuric acid and boiling water. Seed germination in *E. africanum* was significantly improved by mechanical scarification, exposure to sulphuric acid, boiling water (1 min) and hot water (boiling water allowed to cool for 24 h). The results also indicated that sulphuric acid and mechanical scarification treatments resulted in the highest, fastest and uniform germination percentages relative to the control and boiling water treatments. Therefore, extension agents and researchers that have plans to raise seedlings of *B. africana* should consider scarification treatments using mechanical scarification, sulphuric acid and boiling water before sowing. For *E. africanum*, they should subject seeds to mechanical scarification, sulphuric acid and boiling water (1 min) and hot water. Mechanical scarification and boiling water treatments are recommended for farmers and nurseries since they are safer and require less skill to administer; while sulphuric acid treatments can be used in research laboratories. When using mechanical scarification, care should be taken to ensure that the scarification treatment does not bruise the endosperm or the embryo since it could lead to fungal attack and death of the seed.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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