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Germination of *Senegalia mellifera* seeds in response to presowing treatments

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KEYWORDS

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Seed dormancy

Seed dimensions

Pre-sowing treatments

ABSTRACT

This study aimed to evaluate the size of *Senegalia mellifera* seeds and determine the most effective scarification techniques to improve germination. The experiment, conducted at Botswana University of Agriculture and Natural Resources, involved ten presowing treatments, including control, nicking, soaking in sulphuric acid for different durations, and boiling water for varying periods. A completely randomized design (CRD) was used for the experiment. Germination data was transformed using arcsine to meet normal distribution requirements and then analyzed using analysis of variance (ANOVA). The results showed that the presowing treatments had a statistically significant effect on germination ($P<0.00001$). Seeds treated with sulphuric acid, nicking, and those left untreated exhibited the highest germination rates, while seeds treated with boiling water showed the lowest germination percentages. These findings indicate that the seed coats of *S. mellifera* are permeable to water and air, and presowing treatments do not show any significant effect on the successful germination of *S. mellifera* seed.

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1 Introduction

Forests play a crucial role in the environment by providing a wide range of goods and services to both the environment and humans (Opoku et al. 2018; Turner-Skoff and Cavender 2019). Besides offering various environmental services, forests also supply economically important goods such as fruits, traditional medicine, animal feed, charcoal, firewood, and timber for building and household purposes (Cavendish 2000). They also serve as habitats for wildlife, protect water and soil, improve air quality, and store carbon. Anthropogenic activities, particularly deforestation, pose significant conservation issues in tropical regions, leading to a decrease in biodiversity and contributing to poverty in rural areas (Blakesley et al. 2002). This situation has been worsened by forest fires, occasional droughts, and climate change, as noted by Rampart et al. (2021). Deforestation not only endangers the flora and fauna that rely on forests and their resources but also threatens the livelihoods of indigenous people. Moreover, in arid and semi-arid regions, native woody species are extensively harvested without being replaced, despite the essential goods and services they provide.

The lack of attention given to incorporating native woody plants into planting projects and the excessive harvesting of these resources are putting their ongoing existence at risk. Planting initiatives struggle to include these indigenous woody plants due to a lack of knowledge on how to effectively propagate them in tropical areas. Additionally, the use of poor planting materials propagated in tree nurseries also contributes to their exclusion from planting programs (Elliot et al. 2002; McNamara et al. 2006). In recent years, there has been a growing trend towards replanting indigenous woody plants to restore native woodlands (Shono et al. 2007; Raman et al. 2009; Rasebeka et al. 2014; Meli et al. 2014).

Before acacias were renamed, *Senegalia mellifera* (Benth.) Seigler & Ebinger, was known as *Acacia mellifera* (Vahl) Benth. (Kyalangalilwa et al. 2013). *S. mellifera* belongs to the family Fabaceae (Timberlake 1980; Venter and Venter 2012). The tree is also known by several common or vernacular names, such as hook thorn, blackthorn, wait-a-bit, hook thorn, mongana (Timberlake 1980; Smit 2008), and many other names varying from place to place. The species is well-known for its tolerance and is common in arid and semi-arid regions of Africa and the Arabian peninsula (Hagos and Smit 2005; Heuze and Tran 2015). The species thrives in dry conditions and on a variety of soil types, including clayey and sandy Kalahari soils (Timberlake 1980; Tietema et al. 1992; Smit 2008). *S. mellifera* is a thorny shrub that grows up to 4 meters in height with multiple stems (Timberlake 1980) and rarely reaches 6 to 9 meters high (Venter and Venter 2012; Heuze and Tran 2015). The distinguishing feature of *S. mellifera* is a flat, round, or spreading crown with the potential to touch the ground (Venter and Venter 2012). Furthermore, the *S. mellifera* tree has a robust set of thorns with a sturdy hook at the nodes (Timberlake 1980; Smit 2008; Venter and Venter 2012).

S. mellifera is a valuable multipurpose tree species. Different parts of the tree, such as leaves, twigs, pods, shoots, and flowers, are eaten by domestic animals as feed (Palmer and Pitman 1972; Venter and Venter 2012; Abdalkreem et al. 2017). It provides nectar used by honeybees to produce high-quality honey (Bein et al. 1996; Smit 1999; Nonyane 2013). The termite-resistant wood of the tree is also used for making axe and pick handles (Venter and Venter 2012), constructing fences, and producing firewood and charcoal (Orwa et al. 2009; Nonyane 2013; Heuzé and Tran 2015). Local communities utilize the bark, leaves, and roots to treat a variety of illnesses (Hines and Eckman 1993; Mutai et al. 2004; Koch et al. 2005; Fatima and Mamoun 2013). According to Orwa et al. (2009), the tree has been used in agroforestry systems to mark farm boundaries and create live fences.

The purpose of a plant's seed is to establish new plants. However, the process of germination can only occur once because it is essentially a permanent one (Smýkal et al. 2014). Plant seeds have a very long dormancy period, and various mechanisms have evolved to minimize the period of germination and to overcome this dormancy (Foley 2001). Many woody plants, especially legumes, have tough outer seed coats that prevent the entry of water and oxygen to stimulate or initiate germination. Seeds with impermeable or physical dormancy are not able to germinate when conditions are conducive for non-dormant seeds to germinate (Vleeshouwers et al. 1995; Bewley 1997). Seed dormancy influences germination ecology and plant seed ecology (Forbis et al. 2002). Several researchers have used different pre-sowing techniques to crack impermeable seed coats to improve the germination of several plants (Ren and Tao 2004; Anand et al. 2012; Gilani et al. 2019; Botumile et al. 2020; Latiwa et al. 2023; Dasari et al. 2024; Longjam and Kotiya 2024).

The survival of plants depends not only on dormancy but also on seed qualities such as size and weight (Moles et al. 2005). Seed weight and size are critical factors for growth in the early stages of the plant life cycle, particularly in resource-limited areas (Saeed and Shaukat 2000; Moles et al. 2005). Seed size is also crucial for seed germination, dispersal, and the survival rates of plants (Tripathi and Khan 1990; Bonfil 1998; Moles and Westoby 2006). Limited information is available about the seed characteristics, dormancy, and germination requirements of various woody species, including *S. mellifera* in Botswana. This information is crucial for foresters to effectively propagate seedlings of native woody plants for afforestation and replanting initiatives. Therefore, the present study aimed to assess seed characteristics and determine the most effective scarification method for achieving speedy, successful, and consistent

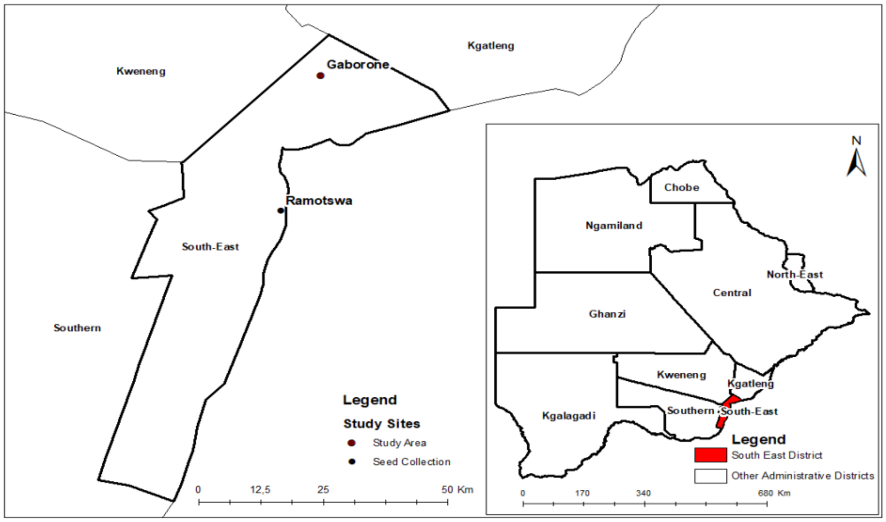

Figure 1 Map of Botswana showing Gaborone, where BUAN is situated, and Ramotswa, in the Southeast, where seeds were gathered (Cartographer T.I. Makoi)

Figure 2 *S. mellifera* pods and seeds (Photographer: F.O. Madisa)

germination in *S. mellifera*. The study's goals included measuring the size and weight of seeds, as well as testing various scarification methods on germination.

2 Materials and Methods

2.1 The study area description

This study was conducted in 2021 at the Botswana University of Agriculture and Natural Resources (BUAN) in Botswana. The experimental site at BUAN is located at an elevation of 994 meters above sea level, with an altitude of 24° 33'S and a longitude of 25° 54'E. Gaborone has a subtropical, semi-arid climate characterized by warm and wet periods from November to March and dry periods from April to October. The coolest month is July (13.5°C), and the hottest month is January. Gaborone receives approximately 485mm of rainfall each year.

2.2 Collection of plant material

Mature fruits were collected from various *S. mellifera* trees in the Disaneng grazing land of Ramotswa, located in the Southeast District of Botswana (Figure 1). Disaneng has semi-arid conditions with consistently sunny summers. The savanna vegetation at Disaneng is primarily composed of shrubs. Dry fruits were manually harvested from randomly chosen trees. Paper bags were used to gather the fruits, which were then transported to BUAN. The seeds were extracted by splitting and opening the fruit, followed by removing the outer covering (Figure 2).

2.3 Seed properties

A digital vernier calliper was used to measure the length, width, and thickness of each seed from five sets of ten seeds. Five replicates of ten seeds were weighed on a digital precision scale to determine the weight of individual seeds. Similarly, the weight of 1000 seeds was determined by weighing five batches of 100 seeds each.

2.4 Effect of various seed treatments on germination of *S. mellifera*

In this study, ten treatments were conducted, including control or untreated seeds. The treatments included nicking, soaking in boiling water for one, three, and five minutes, using hot water (cooled for 24 hours), treatment with concentrated sulphuric acid (H_2SO_4) for 15, 30, 45, and 60 minutes, as well as untreated seeds. The study used a completely randomized block design (CRB), and each treatment, including the control, was repeated four times. Before starting the experiment, the viability of the seeds was tested by soaking them in water. Viable seeds settled at the bottom of the jar, while those that floated were considered not viable and were discarded. Each replicate contained 25 seeds sown inside 90 mm petri dishes lined with cotton wool, kept moist by adding distilled water as needed. Seeds were considered germinated when the radical had penetrated the seed coat. All germinated seeds were counted and then removed from the petri dish to avoid counting them the next day. The germination study was conducted for 30 days.

2.4.1 Nicking treatment

In this treatment, 100 seeds were divided into four groups of 25 seeds for each replication. Each seed underwent a 1-2mm seed coat incision on the curved edge opposite the embryo location to prevent damage or displacement of the embryo.

2.4.2 Sulphuric acid treatments

Seeds were treated with concentrated H_2SO_4 for 15, 30, 45, and 60 minutes using the method described by Teketay (1996b). Each soaking time and treatment involved twenty-five seeds placed in separate heat-resistant beakers. These beakers were filled with enough H_2SO_4 to soak the seeds and were stirred every five minutes to ensure uniform acid distribution. After each soaking time, an acid-resistant sieve was used to separate the seeds from the acid. The acid was then transferred to a different beaker. The seeds were cleaned and washed with running tap water to eliminate any acidic residues.

2.4.3 Boiling water treatment

In this treatment, seeds were subjected to three different boiling water periods: one, three, and five minutes. Each soaking period comprised of four replicates of 25 seeds enclosed inside coffee filter papers. The seeds were placed in a pot of boiling water on a stove and then removed after one, three, and five minutes. Finally, they were cooled in a small bowl of cold water.

2.4.4 Hot water treatment

For this treatment, four coffee filters were filled with 25 seeds each and placed in a 250 ml beaker. After that, boiling water was taken off the heat and poured into the beaker, and the seeds were left for 24 hours to cool down.

2.4.5 Control

In this study, each experiment used a control group consisting of four replicates of 25 untreated seeds.

2.5 Statistical analyses

The percent germination of the seeds was calculated by directly counting the germinated seeds and using the following formula.

$$\text{Seed germination (\%)} = \frac{\text{Number of seedlings emerged}}{\text{Total number of seeds sown}} \times 100$$

The data was analyzed using Statistix Software, Version 10 (Statistix 10, 1984-2003) for one-way ANOVA and descriptive statistical analysis. The percentage germination data was transformed with arcsine to meet normality requirements before being subjected to analysis of variance (ANOVA) (Zar 1996). The Tukey's Honestly Significant Difference (HSD) Test was utilized to identify significant differences in means at a significance level of $P < 0.05$.

3 Results

3.1 Characteristics of seeds

Each pod contained between 2 and 3 seeds, with 1 to 2 of these being sound seeds. No insect-damaged or aborted seeds were found in the collected samples. The observed measurements for the length, width, and thickness were 10.32 ± 0.27 mm, 8.52 ± 0.06 mm, and 1.76 ± 0.08 mm, respectively (Table 1). The results in Table 1 show that the weight of individual seeds and one thousand seeds were 0.14 ± 0.01 g and 111.66 ± 1.11 g, respectively.

3.2 Effect of seed treatment on germination of *S. mellifera*

The influence of pre-sowing treatments on the germination of *S. melifera* was found to be extremely significant ($P < 0.00001$).

Table 1 Different characteristics of the *Senegalia mellifera* seed samples

Seed length (mm)	Seed width (mm)	Seed breast (mm)	Single seed weight (g)	Thousand seed Wight (g)
10.32 ± 0.27	8.52 ± 0.06	1.76 ± 0.08	0.14±0.01	111.66±1.11

The "±" standard error of mean

Table 2 Effect of various pre-sowing treatments on the cumulative germination of *S. mellifera* seeds

Treatment	Germination percentage	
	Mean germination	Range
Control	79.2± 4.2[a]	69.7 – 90.0
Nicking	80.8 ± 5.4[a]	69.7 – 90.0
Sulphuric acid (15 min)	78.5 ± 0.0[a]	78.5 – 78.5
Sulphuric acid (30 min)	80.1 ± 3.5[a]	73.6 – 90.0
Sulphuric acid (45 min)	87.1 ± 2.9[a]	78.5 –90.0
Sulphuric acid (60 min)	80.1± 3.5[a]	73.6 – 90.0
Boiling Water (1 min)	27.7 ± 3.0[c]	20.3 –34.5
Boiling Water (3 min)	8.0 ± 4.9[d]	0 – 20
Boiling Water (5 min)	2.9 ± 2.9[d]	0 – 11
Hot water (allowed to cool for 24 hrs)	54.3 ± 0.70[b]	53.1–55.6

Values are means ± standard error; Means were separated using Tukey Honestly Significant Differences (HSD) Test at $p \leq 0.05$; Means within columns followed by the same letters are not significantly different.

When compared to untreated seeds, the germination percentage for seeds that were boiled for one, three, and five minutes, as well as those soaked in hot water (and then allowed to cool for 24 hours), was significantly lower (Table 2). Moreover, there were no noteworthy variances in germination percentage between seeds treated with sulphuric acid (for 15, 30, 45, and 60 minutes), nicking, and the control. The seeds that exhibited the highest germination percentage were those treated with sulphuric acid for 45 minutes (87.1%), followed by nicking (80.8%), sulfuric acid treatment for 30 and 60 minutes (80.1%), and the control (79.2%). Conversely, the seeds subjected to the five-minute boiling treatment displayed the lowest germination percentage (2.9%) (Table 2).

4 Discussion

4.1 Seed Properties

The size of a plant's seeds can influence its ability to regenerate and maintain health (Chacon et al. 1998). Larger seeds have been found to have a positive impact on germination and plant survival (Zimmermann and Weis 1983; Chacon et al. 1998; Moles and Westoby 2006). The current study did not observe any damaged or aborted seeds, indicating that these seeds are unlikely to be affected by living or non-living factors. These findings are consistent with a study by Kahaka et al. (2018), which reported no damage or seed abortion in other legume plant species native to Botswana. Table 1 presents the findings on the seed dimensions (length, width, and breadth) of *S. mellifera*. These dimensions are similar to those of other species native to Botswana, particularly *Senegalia erubescens* (Kahaka et al. 2018) and *Senegalia galpini* (Botumile et al. 2020). The length and width of *S. mellifera* seeds are comparable to those of *Vachellia robusta* (Botumile et al. 2020). The weight of a single seed and 1,000 seeds is similar to that of *S. erubescens* (Kahaka et al. 2018) and smaller than that of *Senegalia galpinii* (Botumile et al. 2020). The mass of 1,000 seeds is higher compared to *Vachelia rehmanniana* (Mojeremane et al. 2017), *Dichrostachys cineria* (Kahaka et al. 2018), and *Vachelia erioloba* (Odirile et al. 2019). Furthermore, in comparison to small seeds, various studies have indicated that large seeds produce seedlings that are more resistant to drought and have increased survival and/or growth in nutrient-deficient environments (Stock et al. 1990; Milberg et al. 1998; Lloret et al. 1999; Seiwa et al. 2002).

4.2 Effect of seed treatment on *S. mellifera* germination

Leguminous woody plants have impermeable seed coats, which prevent the absorption of water and oxygen, leading to seed dormancy (Considine and Considine 2016). These plants are naturally found in dry environments and have impermeable seed coats to survive tough conditions such as intense heat, animal damage, extreme drought, and physical harm. The low germination rate caused by these impermeable seed coats hinders the use of local species in planting initiatives in dry environments (Smith et al. 2003; Mojeremane et al. 2021).

Based on the data presented in Table 2, *S. mellifera* does not have impermeable seed coats that would prevent water absorption or gas exchange. The results indicate no significant difference in seed germination between seeds treated with sulphuric acid, nicking, and untreated seeds. Untreated seeds showed a germination rate of 79.2%, which is not significantly different from the germination rates of seeds subjected to sulphuric acid and nicking treatments. This suggests that the species lacks the typical hard, impermeable seed coat found in other leguminous woody plants. These findings align with those of Tietema et al. (1992), who reported a 65%

germination rate for untreated *S. mellifera* seeds five days after sowing. Previous reports have indicated that untreated *S. mellifera* seeds can achieve higher germination rates without any prior treatment (Roodt 2008; Venter and Venter 2012; Nonyane 2013) and that soaking the seeds in warm water overnight can expedite their germination process.

Boiling seeds for one, three, or five minutes resulted in the lowest germination rates, ranging from 2.9% to 27.7%. The seeds' sensitivity to high temperatures may explain the low germination rates observed in these treatments (Kahaka et al. 2018). These recent study results are consistent with previous research on various types of trees in Botswana (Kahaka et al. 2018; Odirile et al. 2019; Botumile et al. 2020; Mojeremane et al. 2021; Latiwa et al. 2023). The germination rate of seeds soaked in hot water (allowed to cool for 24 hours) was also found to be lower compared to that of untreated seeds. This finding is supported by studies conducted in Botswana (Mojeremane et al. 2017; Mojeremane et al. 2020) and elsewhere (Diallo et al. 1996; Teketay 1996a, 1996b; Teketay 1998; Fredrick et al. 2017).

Conclusions

The laboratory germination tests revealed that *S. mellifera* seeds are not impacted by the impermeability of the seed coat, which typically hinders water intake and gaseous exchange necessary for germination. Untreated seeds displayed comparable germination rates to those treated with nicking and sulphuric acid. Consequently, these seeds do not necessitate any prior treatment before planting.

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Declarations

The authors have no conflicting interests.

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