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Seed characteristics and the influence of scarification treatments on the germination of *Pterocarpus angolensis* in Botswana

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KEYWORDS

Scarification methods

Seed dormancy

Seed germination

Seed size

ABSTRACT

Pterocarpus angolensis, a vital timber tree species of the Miombo and other woodlands, is native to eastern and southern Africa. Gemination studies were carried out at the Botswana University of Agriculture and Natural Resources to ascertain the seeds' features and find the most effective scarification treatments that yield the fastest, highest, and most consistent germination of *P. angolensis*. Ten seed pretreatments were included in the completely randomized design of the experiments. These treatments included nicking, immersion in boiling water for one, three, and five minutes, concentrated sulphuric acid for fifteen, thirty, forty-five, and sixty minutes, and hot water left to cool overnight. Treated seeds were allowed to germinate at room temperature (25 °C) for 30 days. An analysis of variance (ANOVA) was performed on the raw data. The findings demonstrated that nicking and sulfuric acid treatments significantly increased seed germination (p=0.0001) compared to untreated seeds. The germination rate of the seeds emersed in boiling water for one, three, and five minutes was noticeably lower than that of untreated seeds. Because of their tough seed coat, *P. angolensis* seeds must be pretreated before sown. This study found that nicking and sulphuric acid treatment were the best techniques for seed germination of *P. Angolensis.*

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1 Introduction

Dryland forests found in arid and semiarid regions have deteriorated due to human activities such as deforestation, droughts, climate change, and numerous other issues. Various arid and semiarid woody species are constantly harvested at frightening rates for their wood, fuel, and other purposes (Bertuzzi et al. 1997). Numerous studies have indicated that the loss of biodiversity occurring globally is partly caused by deforestation and forest degradation (Angelsen and Kaimowitz 1999; Blakesley et al. 2002; FAO 2020). According to estimates, between 1990 and 2020, deforestation would have destroyed 420 million hectares of land (FAO 2020). Plant species extinctions are happening at an alarming rate due to ongoing deforestation. In arid and semiarid regions, arborists and other tree planters have prioritized planting exotic trees and shrub species for many years, giving indigenous species little attention.

Indigenous woody trees can be used successfully in afforestation and reforestation projects to repair or replenish the depleted forest resources in tropical regions. However, their involvement in planting programmes is extremely low because foresters and other tree planters do not understand their propagation and management protocols. The scarcity of high-quality seeds and seedlings is another significant barrier to planting indigenous tree species in Botswana and elsewhere. Though not at the planned rate, efforts to produce and manage indigenous woody species have increased globally in recent decades (Shono et al. 2007; Raman et al. 2009).

Pterocarpus angolensis DC (mukwa), a member of the legume family, can grow to a height of 20 meters (Venter and Venter 2012). Its spreading, flat-topped crown is supported by a straight, cylindrical stem (Palgrave 2002; Therrell et al. 2007; Venter and Venter 2012). *P. Angolensis* grows naturally in east and southern Africa (Caro et al. 2005; Chisha-Kasumu et al. 2007; Barstow and Timberlake 2018) and is widespread in dry evergreen and dry deciduous forests (Sawe et al. 2014). It thrives in grasslands and woodlands between 300 and 1,550 meters above sea level (Fichtler et al. 2004). It is distinguished by a circular, indehiscent pod that is 3 cm wide and has a membranous wing (van der Riet et al. 1998). A hard bristle covers the inner fruit with one or two reddish brown seeds inside (van der Riet et al. 1998). Mukwa produces quality timber used in the furniture and veneer industries (Storrs 1995; Pagrave 2002; Takawira-Nyenya 2005). Some parts of the mukwa tree are used as medicine by local people to treat different diseases (Mulofwa et al. 1994; Storrs 1995; Schwartz et al. 2002; Graz 2004). Traditionally, the sap is used to stop bleeding from the nose (Palgrave 2002), kill ringworms, and cure ulcers (Van der Reit et al. 1998). Furthermore, the sap treats black water fever, eye cataracts, malaria, and skin inflammation (Watt and Breyer-Brandwijk 1962; Palgrave 2002) as well as urinary schistosomiasis (Ndaba et al. 1994; Nyazema et al. 1994).

Low levels of *P. angolensis* natural regeneration have been reported in forest inventories conducted in Botswana (BUAN Consult and Botswana Tourism Organisation 2021; BUAN Consult 2021a, b) and Namibia (Cauwer et al. 2018). Due to timber exploitation (Sadiki et al. 2018), the species' population has declined (Barstow and Timberlake 2018). Mukwa is threatened by human activities (Shackleton 2002), wildfires, and elephants (Holdo 2007; Mmolotsi et al. 2012). The inability of seedlings to establish and mature into trees after seeds have germinated threatens the species (Shackleton 2002). In addition, elephants debark the tree to relieve their thirst, increasing its wildfire vulnerability. Elephant debarking exposes the cambium, which is why the mukwa trees are killed by wildfires. Even in a controlled laboratory setting, the species has been shown to have low seed germination rates (Boaler 1966; Van Daalen 1991; Von Breitenbach 1973). Munyanziza (1994) estimated that only 5% of seedlings that germinate survive past their first year.

Most forest trees are propagated sexually using seeds. However, sound seeds of many species fail to germinate even if placed under normal environmental conditions that are favourable for germination (Bewley 1997; Gilani et al. 2019). Such seeds are dormant, which is a common phenomenon in many tropical woody species. Dormant seeds can be described as viable seeds that fail to germinate (Finch-Savage and Leubner-Metzger 2006), establish, and grow into plants (García-Gusano et al. 2004) when sown or planted in an environment suitable for germination. Further, dormant seeds will only germinate when environmental conditions are conducive to the growth and survival of plants (Baskin and Baskin 1998; Olmez et al. 2009).

Pre-sowing treatments are used to break the impermeable seed coats that are common in many tropical leguminous woody plants to achieve rapid and homogenous germination. Nicking, chemical, cold, and hot water techniques are frequently used to disrupt the impermeable seed coat and accelerate germination (Kildisheva et al. 2013; Botumile et al. 2020). Furthermore, seed dormancy, a plastic characteristic of plants that influences germination and the recruitment of seedlings, is seed size (Souza and Fagundes 2014). It is regarded as a crucial evolutionary characteristic that affects how many plant species reproduce (Cordazzo 2002). It influences seedling establishment, growth, and survival, as well as germination time and percentage, emergence rate, dispersal, seed water relations, and vigour (Sanderson et al. 2002; Mölken et al. 2005; Yanlong et al. 2007; Silveira et al. 2012).

Indigenous woody species in arid and semiarid regions need to be included in planting initiatives aimed at restoring and rehabilitating native forests and woodlands to avoid their extinction. On the other hand, little is known about how to manage and reproduce native woody species. The main objective of the present study was to assess optimal presowing treatments that result in uniformly fast and high *P. angolensis* germination. The study's specific goals were to (a) measure the weight and size of the seeds, including their mass and weight of one thousand seeds, and (b) assess the effects of various seed treatment techniques, such as nicking, sulfuric acid, boiling, and hot water, on the overall germination percentage of *P. angolensis*.

2 Materials and Methods

2.1 Study Site

The study was conducted at the Botswana University of Agriculture and Natural Resources (BUAN) in Sebele, Gaborone, between November and December 2021. BUAN is located at latitude 24˚35' S and longitude 25˚56' E with an altitude of 994m. It is situated 10 km north of Gaborone, along the North-South highway. The climate of Sebele is semiarid, characterized by hot summers and mild winters.

2.2 Seed Collection

In September 2020, mature *P. angolensis* fruits were harvested from trees in Kazuma Forest Reserve in the Chobe District, northern Botswana. The fruits were gathered and brought to BUAN in paper bags. After the seeds were removed from the fruits, they were placed in clear plastic zip bags, sealed, and kept in a refrigerator at 4 °C until the experiments started.

2.3 Number of seeds within a fruit

Seeds in each fruit were counted using five replications, each with ten fruits. The seeds were then categorized into dead/eaten, aborted, and sound.

2.4 Seed weight and size

Five replications of ten seeds each were measured with a digital calliper to assess their length, width, and breadth. The weight of five replicates of ten seeds was measured using a digital balance and was used to calculate the weight of a single seed. To find the weight of 1,000 seeds, five replications of 100 seeds each were weighed.

2.5 Seed germination treatments

Four experiments comprising ten treatment levels, including untreated seeds as the control, were conducted for the study. These treatments included nicking, immersion in boiling water for one, three, and five minutes, concentrated sulphuric acid for fifteen, thirty, forty-five, and sixty minutes, and hot water left to cool overnight. A complete randomized design was used to set up the experiments. Each treatment contained 25 seeds and was replicated four times. The seeds were submerged in cold water before the experiments began, and only the ones that settled at the bottom of the beaker were used. The seeds that floated were considered non-viable and were thrown away. For nicking, seed coats were scarified by removing a small part of the testa on the slightly rounded edge opposite the embryo's location using a nail cutter with care to minimize damage or embryo removal.

In the acid treatments, seeds were exposed to sulphuric acid for four different durations: 15, 30, 45, and 60 minutes using Teketay's (1996) approach. Seeds were placed inside four 100-ml non-corrosive, heat-resistant glass beakers. Then, sulphuric acid was added, ensuring it covered all the seeds. The seeds were stirred every five minutes throughout the designated soaking periods to expose them to the acid uniformly. The chemical was poured into a fresh beaker at the end of each acid-soaking time. Seeds were removed from the chemical using an acid-resistant filter. We thoroughly cleaned the seeds and rinsed them under a running tap and distilled water to remove any remaining acid.

Three distinct boiling water exposure times (one, three, and five minutes) were applied to the seeds in the boiling water treatments. The seeds were placed inside four distinct paper coffee filters and immersed in boiling water in a cooking kettle for each exposure period. Following removal from the heat source, the seeds were placed in a beaker with cold to cool them. The hot water treatment seeds were placed in paper coffee filters and put inside beakers. After that, boiling water was poured, and the seeds were allowed to cool for a full day. Four replicates of untreated seeds were used as the control for all the treatments.

Closed 90 mm petri dishes were used for the germination of the seeds. The petri dishes were lined with cotton wool and watered whenever necessary to prevent seeds from drying. Seeds were considered germinated when the radicle reached a length of 1-2 mm. Seeds were observed daily for 30 days to record those that had germinated. To prevent double counting, germinated and counted seeds were taken out of petri dishes daily.

2.6 Analysis of Data

The germination percentage (GP) was computed using data gathered on germinated seeds. The formula used to determine the final germination was $GP = (TG/TS) \times 100$, where TG stands for the total number of germinated seeds and TS for the total number of sown seeds. Using Statistix Software, Version 10 (Statistix 10, 1984-2003), the collected data were subjected to one-way analysis of variance (ANOVA) as well as descriptive statistics. The germination percentage data were arcsine transformed before the ANOVA to ensure they met the normalcy requirement (Zar 1996). Tukey's Honestly Significant Difference (HSD) Test was used to test significant differences in means at a significance level of $P < 0.05$.

Table 1 Number of seeds pod^{-1} and their status

Seed condition	Seed number	Range
Intact	0.65 ± 0.11	0 – 1
Dead/eaten	0.0 ± 0.0	0 – 1
Aborted	0.35 ± 0.11	0 – 1

The ‘‘±’’ shows the standard error of the mean.

Table 2 Seed characteristic of *P. angolensis*

Dimension of seeds	Size of seeds (mm)	Range (mm)
Length	11.44±0.25	10.76-12.37
Width	7.37±0.14	6.70-8.26
Breadth	4.63±0.81	4.38-5.32

The ‘‘±’’ shows the standard error of the mean.

3 Results

3.1 Seed characteristics

The average number of seeds in each fruit was 1. The mean number of intact and aborted seeds were 0.65 ± 0.11 and 0.35 ± 0.1 (Table 1). There were no signs of dead/eaten seeds, indicating they are well protected from damaging agents by the hard, bristly fruit cover.

According to Table 2, the average seed dimensions were 11.44 ± 0.25 mm for length, 7.37 ± 0.14 mm for width, and 4.63 ± 0.81 mm for breadth. The single seeds mass ranged between of 0.25 - 2.31 grammes with a mean of 0.66 ± 0.4 grammes. Likewise, the weight of the thousand seeds varied between 184 and 231 grammes, with an average of 205.5 ± 5.12 grammes.

3.2 Seed germination

The findings showed that the mean germination percentages of nicked seeds (89%) and those soaked in sulphuric acid (86-99%) were significantly higher than their untreated counterparts [One Way ANOVA: (F (9, 30) =45.78, P = 0.00001] (Table 3). The maximum germination percentage (99.0%) was recorded in seeds immersed in sulphuric acid for thirty minutes. This was followed by 45 (92%) and 15 (91%) minutes of sulphuric acid treatment, nicking (89%), and 60 minutes of sulphuric acid (86%) (Table 3). The percentage of germination seeds differed significantly between those treated with hot water left to cool overnight and the control or untreated seeds. The lowest mean germination percentages were seen in seeds that were boiled for one (32%), three (10%), and five (4%) minutes, respectively (Table 3).

4 Discussion

4.1 Seed characteristics

Seed size, expressed as weight, volume, length, breadth, and thickness, is essential to many facets of plant ecology (Pothasin et al. 2022). It is a significant characteristic that may affect a plant's

Table 3 Means and ranges of *P. angolensis* seed germination.

Treatment	Germination Percentage	Range
Control	$79.0±6.6^{b}$	60-88
Nicking	$89.0±2.5^{ab}$	84-96
Sulfuric acid (15 minutes)	$91.0±3.7^{ab}$	80-96
Sulfuric acid (30 minutes)	99.0±1.0a	96-100
Sulfuric acid (45 minutes)	$92.0±2.8^{ab}$	88-100
Sulfuric acid (60 minutes)	$86.0±5.0^{ab}$	72-96
Boiling water (1 minute)	$32.0±6.9^{c}$	12-44
Boiling water (3 minutes	$10.0±3.4^{cd}$	4-20
Boiling water (5 minutes)	$4.0±2.8^{d}$	0-12
Hot water (allowed to cool overnight)	$72.0±4.8^{b}$	64-84

The ‘‘±’’ shows the standard error of the mean. Means within columns that are separated by the same letters do not differ significantly.

ability to germinate, emerge, grow, and perform in the field (Singh et al. 2021). Larger seeds store more food reserves in the embryo and endosperm than their smaller counterparts. This phenomenon influences the continued existence and development of seedlings growing in the wild. Large seeds contain nutrient-rich food reserves, which offer advantages for establishing seedlings, especially on nutrient-poor soils (Vaughton and Ramsey 2001). Plants from large seeds are expected to have bigger and heavier leaves (Elliott et al. 2007) than those from small seeds. Plants from large seeds have increased survival and establishment rates compared to those from small seeds (Stock et al. 1990). However, to make more offspring, smaller seeds can be produced in more significant quantities per canopy area than large seeds, and some seeds have a higher chance of dispersing into an appropriate habitat (Westoby et al. 1996).

These results indicated that *P. angolensis* produced a single seed per pod. This is consistent with Vermeulem's (1990) observation that the species seldom yields more than one seed per pod, though this may vary depending on the geographic location. About 65% of the seeds that were recorded in this study were intact and showed no signs of damage, whereas 35% showed indications of abortion. According to Hines and Eckman (1993) and Takawira-Nyenya (2005) only 50% of *P. angolensis* fruits have seeds, and the remaining fruits are considered barren. The dimensions of the seeds were similar to those recorded for *Senegalia erubescens* (Welw. Ex Oliv.) Kyal. & Boatwr (Kahaka et al. 2018), *Vachellia robusta* (Burch.) Kyal. & Boatwr (Botumile et al. 2020), and *V.erioloba* (E.Mey.) P.J.H. Hunter (Odirile et al. 2019). Compared to *S. galpinii* (Botumile et al. 2020) and *V. rehmanniana* Schinz (Mojeremane et al. 2017), *P. angolensis* seeds were shorter, less comprehensive, and broader. Compared to *Dichrostachys cineria* (L.) Wight & Arn and *V. nilotica* (L.) Delile, the seeds were longer, wider, and broader (Kahaka et al. 2018).

In comparison to *S. galpinii*, *V. robusta* (Botumile et al. 2020), *D. cinerea*, *S. erubescens*, and *V. nilotica* (Kahaka et al. 2018), *P. angolensis* had a higher single seed mass. *P. angolensis* had a thousand seed weight that was higher than that of *V. rehmanniana* (Mojeremane et al. 2017), *D. cinerea*, *S. erubescens*, and *V. nilotica* (Kahaka et al. 2018), but similar to that of *V. robusta* and lighter than *S. galpini* (Botumile et al. 2020).

4.2 Seed germination

Numerous leguminous woody plants in dryland environments produce seeds resistant to embryo growth due to physical dormancy or impermeable, thick seed coats (Amoakoh et al. 2017). The primary obstacle in establishing native forests using leguminous woody species is thought to be impermeable, and the presence of thick seed coats that hinders natural seed germination (Opoku et al. 2018). Therefore, before sowing, seeds need pre-sowing treatment to break the hard seed coat and promote quick, uniform, and high germination (Mojeremane et al. 2017). The propagation of seedlings of many indigenous leguminous woody species in tree nurseries is negatively affected by impermeable and hard seed coats, which prevent inhibition. Consequently, various authors have proposed seed dormancy-breaking techniques to improve the germination process and raise the germination rate (Rees 1996; Teketay 1996; Nadjafi et al. 2006; Tiwari et al. 2018; Maiguru et al. 2020; Mojeremane et al. 2020; Mmulotsi et al. 2020). For example, Tiwari et al. (2018) discovered that scarification using sandpaper was the most successful method for breaking dormancy in *Abelmoschus moschatus* Medik., *Bixa orellana* L., *Cassia angustifolia* Vahl., and *Psoralea corylifolia* L. According to Mmulotsi et al. (2020), nicking, boiling and hot water, and scarification with sulphuric acid all improve the germination of *Vachellia karroo* (Hayne) Banfi & Galasso seeds. *Burkea africana* Hook seeds treated with mechanical scarification, sulphuric acid, and boiling water treatment showed increased germination compared to untreated seeds (Mojeremane et al. 2020).

According to the result of the current study, the germination percentage of nicked seeds and their counterparts immersed in sulphuric acid was statistically higher than that of the control.

The findings align with previous studies carried out on different woody species in Botswana (Mmulotsi et al. 2020; Mojeremane et al. 2020) and other locations (Salim Azad et al. 2010; Gilani et al. 2019; Magray et al. 2023). In addition, similar results have been obtained from research on the seeds of different woody species that were nicked (Danthu et al. 1995, Diallo et al. 1996) and treated with sulphuric acid (Baes et al. 2002; Righini et al. 2004; Finch-Savage and Leubner-Metzger 2006; Naim 2015; Fredrick et al. 2017; Krishan et al. 2022; De Jesús-Velázquez et al. 2023; Mahajan et al. 2023). According to studies by Krishan et al. (2022) and Magray et al. (2023), nicking and sulphuric acid helped to overcome dormancy and enhance germination in *Phytolacca acinosa* Roxb. After mechanical scarification, Mahajan et al. (2023) reported that over 85% of the seeds of *Gloriosa superba L.* germinated. According to research by De Jesús-Velázquez et al. (2023), mechanical scarification proved to be the most successful technique for preparing the seeds of eight native woody species for replanting in tropical deciduous forests, as it yielded 100% germination. Research on seeds of *Albizia versicolor* Welw. ex Oliv and *Faidherbia albida* (Delile) A. Chev revealed that mechanically and sulphuric acid-treated seeds yielded higher germination than the control seeds (Mojeremane et al. 2021). Since sulphuric acid and nicking treatments significantly increase germination by breaking the seed coat and allowing the uptake of water and oxygen, it can be concluded that the impermeable seed testa prevents *P. angolenisis* from germinating.

Compared to untreated seeds, the germination of seeds immersed in boiling water for one, three, and five minutes was noticeably lower. This could be explained by the high heat that the boiling water transmitted through the seed coat to the interior parts, which most likely killed the embryo (Kahaka et al. 2018; Rampart et al. 2021b). The study's findings are in line with earlier investigations on other native tree species in Botswana (Kahaka et al. 2018; Mojeremane et al. 2018; Odirile et al. 2019; Botumile et al. 2020; Rampart et al. 2021a, 2021b), which revealed that the seeds soaked in boiling water had the lowest rate of germination.

Conclusions

The results showed that nicking and sulphuric acid treatment increased the germination percentages of seeds compared to untreated seeds. This implies that *P. angolensis* seeds possess the physical dormancy or thick, impermeable seed coat found in many other leguminous woody plants in the Miombo and other woodlands. The hard seed coat inhibits water absorption and gas exchange, which is essential for germination. Consequently, *P. angolensis* seeds must be treated before being sown.

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Statement of Conflicting Interests

The authors have no conflicting interests.

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