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Pre-sowing treatments for enhancing germination of *Guibortia coleosperma* and *Amblygonocarpus andongensis* seeds from Kazuma Forest Reserve, Botswana

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The effect of seed presowing treatment on *Guibortia coleosperma* and *Amblygonocarpus andongensis* was investigated. Seeds of both species were subjected to four experiments, containing 10 levels of presowing treatments: 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) and the control. The germination data were subjected to ANOVA followed by Tukey's HSD test to separate significantly different treatment means. The highest germination in *G. coleosperma* was recorded: mechanical scarified seeds, those soaked in sulphuric acid (15, 30, 45 and 60 min), hot water (and cooling down for 24 h) and the control seeds. For *A. andongensis*, seeds treated with mechanical scarification, exposure to sulphuric acid (15, 30, 45 and 60 min) and boiling water (1 min) had higher percent germination than the controls.

Key words: Germination mean time, germination percentage, pre-treatment, scarification.

INTRODUCTION

Forests and trees form an integral part of the ecosystem and offer numerous benefits to humans and the environment. They are critical habitats for biodiversity and also essential in the provision of a wide range of

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goods and ecosystem services important to human well-being (Brockerhoff et al., 2017). In addition, forests and trees are critical to mitigation and adaptation to climate change strategies in Africa (Fisher et al., 2010). The northern part of the Botswana is open woodlands characterized by *Baikiaea plurijuga* Harms and *Pterocarpus angolensis* DC, mostly located in forest reserves (Central Statistics Office, 2004). Tropical forest resources in general and those in Botswana are either disappearing or being degraded rapidly due to accelerated growth of human populations and wild animals, which result in the conversion of forested land to agriculture, and excessive exploitation of forests for fuelwood, construction material, and grazing by wild animals (Nduwayezu et al., 2015).

Guibourtia coleosperma (Benth) J. Léonard belongs to the family Fabaceae (Hyde et al., 2021). It is evergreen tree growing 6 to 20 m high (Palgrave, 2002) and 182 cm in diameter with a rounded dropping crown (Storrs, 1995). The bole is either basally swollen, slightly buttressed or fissured for the first 2 m (Storrs, 1995). The species is indigenous to Africa, occurring in Angola, southern Democratic Republic of Congo, Namibia, Botswana, Zambia, and Zimbabwe (Hyde et al., 2021) in open woodland and dry forest, almost exclusively on Kalahari sand (Storrs, 1995; Van Wyk and Van Wyk, 1997). The wood is used for canoes (Storrs, 1995), furniture, flooring, tool handles and railway sleepers (Storrs, 1995). Leaves are browsed by elephants and the bright red seed coats are eaten by starlings and parrots (Storrs, 1995) which help to disperse them (Storrs, 1995). The bark, leaves, and roots are used in traditional medicine (Storrs, 1995) to treat pneumonia and cure temporary madness (Storrs, 1995). The bark has been used for tanning or dyeing (Storrs, 1995). The seeds can be cooked or roasted for human consumption (Palgrave, 2002; Akinnifesi et al., 2008).

Amblygonocarpus andongensis (Welw. ex oliv.) Exell & Torre belongs to the family Mimosaceae (Leguminosae - Mimosoideae) (Lemmens, 2006). It is a large, spreading tree growing up to 91 cm diameter and 20 to 25 m tall (Storrs, 1995; Lemmens, 2006). *A. andongensis* is widely distributed in the savanna zone from northern Ghana east to Sudan, through Uganda and Tanzania, south to Botswana, Namibia, Zambia Zimbabwe, and Mozambique (Storrs, 1995; Lemmens, 2006). It grows on deep Kalahari sands or associated with mopane woodland (Palgrave, 2002). The wood is hard, heavy, and strong, very durable and termite resistant, and has been used for flooring, railway sleepers, wagons, and general construction (Storrs, 1995, Lemmens, 2006). The inner bark and roots are poisonous (Storrs, 1995), but medicinal uses have been recorded in them. Nwinyi et al. (2006) reported that *A. andongensis* is ethnomedicinally used in Northern Nigeria for the relief of pain. According to Ugwah et al. (2014) a decoction prepared from roots is used to treat food poisoning and against colic, cough and as a vermifuge. The stem bark has been used to treat diarrhoea (Burkill, 1995) and breast cancer (Kubmarawa et al., 2007).

Germination of seeds is an important and cheapest means of propagating mass production of many woody plant species in Botswana. However, germination is one of the main difficulties in propagating many arid and semi-arid trees and shrubs. Germination in many woody species does not occur easily even under conditions favourable for germination. This cause has been attributed to hard seed coat which impedes the imbibition of water and gaseous exchange (Nasr et al., 2013; Smýkal et al., 2014) which exerts physical dormancy. To overcome seed dormancy, several presowing treatment methods have been developed in plant nurseries and laboratories. The methods include mechanical scarification (Seng and Cheong, 2020), sulphuric acid (Seng and Cheong, 2020; Peter et al., 2021), gibberellic acid (Seng and Cheong, 2020), hot water (Seng and Cheong, 2020; Peter et al., 2021), cold water and promalin solutions (Seng and Cheong, 2020; Peter et al., 2021). Each presowing treatment method has advantages and disadvantages, which depend on the plant species. For example, sulphuric is more effective in breaking dormancy in many tropical leguminous plants. However, it is expensive, dangerous, and abrasive to people and materials (Doran et al., 1983) and can only be used by trained personnel. It can pollute the environment if not properly disposed of (Mojeremane et al., 2020) and seeds can be damaged by over soaking (Nasr et al., 2013). Mechanical scarification is safer and more practical when scarifying few seeds (Mojeremane et al., 2020) but it is time consuming if large quantities of seeds are scarified (Mapongmetsem et al., 1999). Currently, there is little information on the germination requirement of seeds of indigenous woody plants in Botswana. Hence, the present study was undertaken to investigate the effect of presowing seed treatments of *A. andongensis* and *G. coleosperma* seeds by various methods to break the seed dormancy.

MATERIALS AND METHODS

Study site

The experiment was conducted in the laboratory at the Department of Range and Forest Resources, Botswana University of Agriculture and Natural Resources (BUAN). The university is located at Sebele, approximately 10 km from the Centre of Gaborone, the capital city of Botswana along the A1 North-South highway.

Seed collection

Mature pods of two study species were collected direct from mother trees and under trees at Kazuma Forest Reserve, northern

Botswana during 2018 and 2019 fruiting seasons. Pods were placed in paper bags and transported to the laboratory at the Department of Range and Forest Resources. At the laboratory, seeds were extracted by crushing the pods using hands, and a secateur (for *A*. *andongensis* seeds) followed by winnowing to separate the husk. Seeds were kept refrigerated at 5°C until the experiments were initiated.

Experiment and treatments

Prior to start of the experiments, seeds were immersed in distilled water, and only those that sank and settled at the bottom of glass beakers were used for the experiments. The study had four experiments containing 10 treatments, including the control. The four experiments were mechanical scarification, exposure to sulphuric acid, exposure to boiling water and hot water. The treatments in the experiments were completely randomized in four replications.

Sulphuric acid scarification

In this experiment, four levels of time exposure (soaking) of seeds in concentrated sulphuric acid (98%), that is, 15, 30, 45 and 60 min, were used by applying the method described by Teketay (1996). For each soaking time, four replicates of 25 seeds each 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 soaking time to ensure they were uniformly exposed to the acid. After the specified periods of soaking, the seeds were sieved out of the acid using an acid-resistant sieve after each specified period of soaking and the acid was drained off simultaneously into another beaker. Seeds were then thoroughly washed and rinsed to remove all the acid using tap water first and subsequently using distilled water, successively to remove the acid for safe handing.

Mechanical scarification

In this experiment, 100 seeds of each study species, with four replications of 25 seeds were used. In all seeds, 1 to 2 mm of the seed coat was removed using a pair of scissors so that the seeds could imbibe water, which is required to initiate germination.

Boiling water scarification

This experiment followed a method described by Ren and Tao (2004). Approximately 100 seeds having four replicates of 25 seeds each of the study species were wrapped in coffee filter papers which were folded and fastened with stapples to prevent seed loss. Seeds enclosed in coffee filter papers were then immersed into a cooking pot with boiling water for 1, 2, and 3 min. After each immersion time, seeds were removed from the boiling water and left to cool in a small bucket with tap water for about 5 min.

Hot water for 24 h

In this experiment, four replicates 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.

Control

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 continuously kept moist by adding distilled water whenever necessary until the end of the experiments. Seeds were considered to have germinated when the radicle penetrated the seed coat and reached 1 to 2 mm. The experiment was terminated after 30 days.

Data analysis

Data collected on germinated seeds were used to calculate germination percentage (GP), for each treatment using the equation:

$$\text{Germination percentage (GP)} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

The data collected were subjected to both descriptive statistics and one-way analysis of variance (ANOVA) using Statistix Software, Version 10 (Statistix 10, 1984-2003). Before the ANOVA, the germination percentage data were arcsine transformed to meet the requirement of normality (Zar, 2010). Significant differences of means were tested using Tukey's Honestly Significant Difference (HSD) at the significance level of $P < 0.05$.

RESULTS

Effect of seed treatment on germination

The germination percent of *G. coleosperma* seeds was significantly ($P< 0.0001$) affected by presowing treatments (Table 1). The results indicated that the highest germination percentage was recorded in mechanical scarified seeds (100%) which were not significantly different from the control (97%). In addition, the germination percentage of seeds treated with concentrated sulphuric acid for 15 and 60 min (80 and 94%) and hot water (allowed to cool for 24 h, 97%) were not significantly different from the control treatment. The germination percentages of *G. coleosperma* seeds treated with sulphuric acid for 30 and 45 min (50 and 68%) and boiling water for 1, 3 and 5 min (0 to 11%) were significantly lower compared to the control treatment (97%).

For *A. andongensis,* the results revealed that seed germination percentage was significantly ($P<0.001$) affected by presowing treatments (Table 1). The highest germination percentage (94-100%) was recorded from seeds scarified in concentrated sulphuric acid for 15, 30, 45 and 60 min which was significantly higher when compared with the control. Similarly, percent germination

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

Treatment	*G. coleosperma*		*A. andongensis*	
	Germination (%)	Range (%)	Germination (%)	Range (%)
Control	97±2^{ab}	92 - 100	5±1^{d}	04 - 08
Mechanical scarification	100±0^{a}	100 - 100	67±3^{b}	60 - 72
H_2SO_4 for 15 minutes	80±6^{bc}	64 - 88	94±2^{a}	92 - 100
H_2SO_4 for 30 minutes	50±14^{c}	32 - 92	99±1^{a}	96 - 100
H_2SO_4 for 45 minutes	68±8^{c}	44 - 84	100±0^{a}	100 - 100
H_2SO_4 for 60 minutes	94±2^{ab}	92 - 100	99±1^{a}	96 - 100
Boiling water for 1 minute	11±2^{d}	08 - 16	28±2^{c}	24 - 32
Boiling water for 3 minutes	00±0^{d}	00- 00	3±1^{d}	00 - 04
Boiling water for 5 minutes	00±0^{d}	00 - 00	6±3^{d}	00 - 12
Hot water (allowed to cool for 24 hours)	97±2^{ab}	92 - 100	15±4^{cd}	04 - 24

Means separated using Tukey's Honestly Significant Difference test at P ≤ 0.05. Means within columns followed by the same letter are not significantly different.

of seeds treated with mechanical scarification (67%) and boiling water for 1 min (28%) was significantly higher when compared with the control treatment. The germination percentages of seeds treated with boiling water for 3 and 5 min (3 and 6%) as well as well as hot water (allowed to cool for 24 h) were not significantly different to the control treatment.

Seed germination rate

The results showed that the control seeds of *G. coleosperma* and those treated with mechanical scarification, sulphuric acid for 15 and 60 min exhibited the fastest and uniform germination, reaching cumulative germination of 80 to 100% within 6 days after sowing, followed by those treated with concentrated sulphuric acid 45 and 30 minutes, reaching > 60 and >49% within eight days, respectively (Figure 1). The hot water treated seeds reached cumulative germination >90% within 16 days after sowing. Seed of *G. coleosperma* treated with boiling water for 1, 3 and 5 min exhibited not only the lowest, but also the slowest germination.

The results also showed that in the case of *A. andongensis*, seeds treated with sulphuric acid for 45 and 60 min exhibited the fastest and uniform germination, reaching 100 and 99% cumulative germination, respectively, within seven days (Figure 2). Seeds treated with sulphuric acid for 15 and 30 min reached cumulative germination of 94 and 99%, respectively within 20 days. On the other hand, the control, seeds treated with boiling water for 1, 3 and 5 min and hot water (allowed to cool for 24 h) exhibited, not only the lowest, but also the slowest germination, reaching maximum germination between 25 and 30 days.

DISCUSSION

Seed germination is the easiest and cheapest method of plant propagation (Thirupathi et al., 2012). Reducing germination time and increasing germination percentage are both important in propagating woody species for planting programmes. Prior studies demonstrated that seeds of leguminous woody plants are characterized by physical dormancy attributed to hard seed coats that are impermeable to water and oxygen (Nasr et al., 2013; Smýkal et al., 2014). In the present study, mechanical scarification (100%), soaking in hot water (allowed to cool for 24 h) and control treatment (97%), sulphuric acid for 60 min (94%) and 15 min (80%) showed the highest germination in *G. coleosperma* seeds which occurred within 6 to 8 days after sowing (DAS), except for hot water which occurred 16 DAS. *G. coleosperma* seeds soaked in concentrated sulphuric acid for 30 and 45 min showed 50 and 68% germination, respectively which occurred 8 DAS. The fact that the control treatment reached cumulative germination of 97% within 8 days clearly infers that *G. coleosperma* seeds are not characterized by physical dormancy imposed by the hard coat. Seeds soaked in boiling water for 1, 3 and 5 min showed the lowest germination (0-11%) which occurred 8 DAS probably because the boiling water ruptured the seed coat and caused damage to the embryo.

In the present study presowing treatments were effective in breaking dormancy and enhance germination of *A. andongensis* compared to the control treatment. The seed soaked in concentrated sulphuric acid (15, 30, 45 and 60 min) showed the highest germination (94-100%) which occurred between 7 and 20 DAS. The increased germination observed in the concentrated sulphuric acid treatments is evidenced that the acid

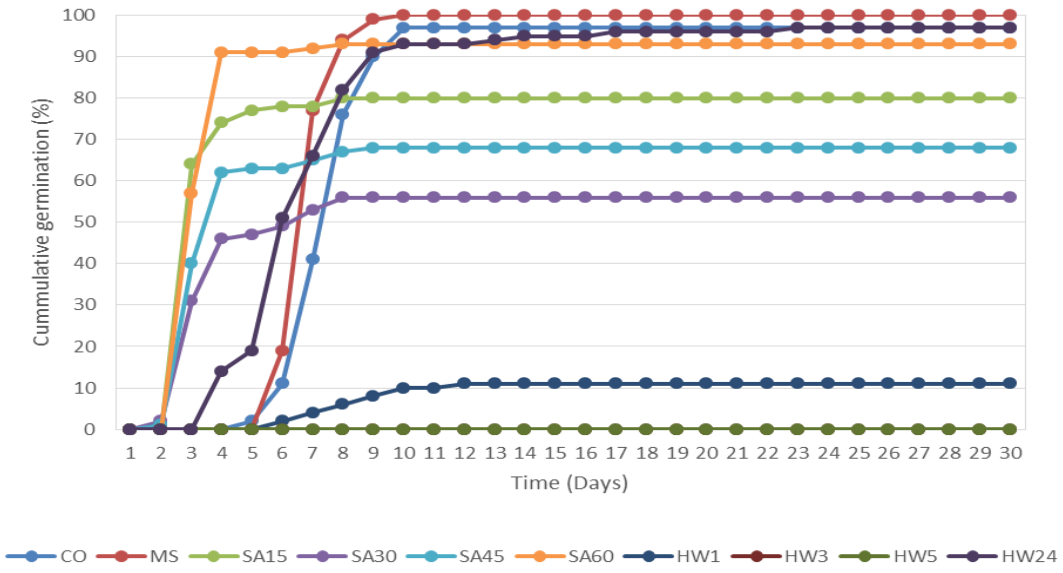

Figure 1. Cumulative germination percentage of *G. coleosperma* recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 min; BW3 = Boiling water for 3 min, HW5 = Boiling water for 5 min, HW24 = Boiling water (allowed to cool for 24 h), SA15 = Sulphuric acid for 15 min, SA30 = Sulphuric acid for 30 min, SA45 = Sulphuric acid for 45 min and SA60 = Sulphuric acid 60 min.

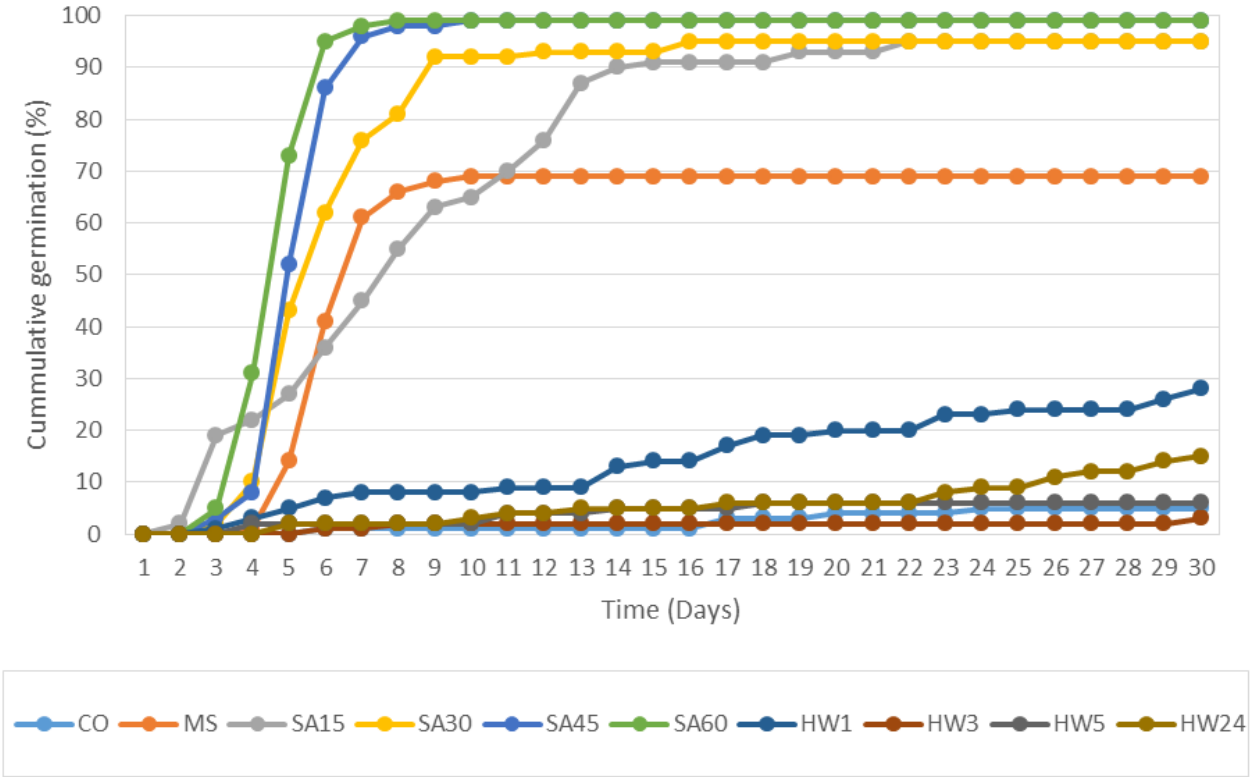

Figure 2. Cumulative germination percentage of *A. andongensis* recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 min; BW3 = Boiling water for 3 min, HW5 = Boiling water for 5 min, HW24 = Boiling water (allowed to cool in 24 h), SA15 = Sulphuric acid for 15 min, SA30 = Sulphuric acid for 30 min, SA45 = Sulphuric acid for 45 min and SA60 = Sulphuric acid 60 min.

softened the hard seed coats and triggered the imbibition process and gaseous exchange (Okunlola et al., 2011). The fact that concentrated sulphuric acid enhanced the germination of *A. andongensis* infers that the seed coat is a barrier to the germination of the species. This result is consistent with studies conducted on other plant species elsewhere which showed that concentrated sulphuric acid increased germination percentage (Filho et al., 2019; Al-Namazi et al., 2020).

Mechanical scarification by nicking seeds with a pair of scissors significantly increased germination percentage of *A. andongensis* compared to the control. Nicking seeds created scars on the surface of the hard seed coat which could have facilitated water imbibition and gaseous exchange thereby triggering germination. Seed coats of many leguminous plants act as physical barriers that limit water uptake, gas exchange and/or embryo expansion (Huang et al., 2017). This result agrees with results of seed germination studies conducted on many hard-coated legumes found in arid and semi-arid zones (Okunlola et al., 2011; Maldonado-Arciniegas et al., 2018) which affirmed the benefit of mechanical scarification in improving the permeability of the seed coat and improving seed germination. Soaking seeds in boiling water for 1 min increased germination only moderately (28%) which occurred 30 DAS. Soaking seeds in boiling water for 3 and 5 min was not effective in improving germination of *A. andongensis.* The low germination observed in the boiling hot water treatments could be attributed to the high level of heat transmitted from the boiling water through the seed coat to the internal parts of which may have killed the embryo. Although hot water has been reported to improve germination by softening the hard seed coat to allow water absorption and gaseous exchange (Haider et al., 2016; São José et al., 2019), this was not the case with *A. andongensis* seeds. This could probably imply that the hot water cooled down before uplifting water and oxygen permeability of the testa.

Conclusions

The study revealed that the seed coat is not a barrier to the germination of *G. coleosperma* because the control seeds attained are cumulative of 97% within six days of sowing. For *A. Amblygonocarpus*, mechanical scarification, and exposure to sulphuric acid significantly improved percent germination of seeds. Therefore, farmers, extension agents and researchers planning to raise seedlings of *G. coleosperma* can sow the seeds without treatment. *A. amblygonocarpus* require scarification treatments using mechanical scarification and sulphuric acid before sowing to render the seed coats permeable to water and trigger embryo growth. Mechanical scarification is recommended for nurseries since it is safer and requires less skill to administer while sulphuric acid treatment which is expensive and hazardous can be used in research laboratories.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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