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Enhancing Germination of Seeds of *Cassia abbreviata* and *senegalia nigrescens* using Pre-Sowing Seed Treatments In Botswana.

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Abstract

Cassia abbreviata and *Senegalia nigrescens* are indigenous trees found in North and Central regions of Botswana. However, inadequate knowledge of their silviculture and decline in population due to deforestation, expanding settlements, infrastructure and agriculture are major threats to most indigenous tree species in Botswana. Like many indigenous tree species, *Cassia abbreviata* and *Senegalia nigrescens*are slow-growing and are threatened by overexploitation for numerous uses. Because of the increased demand for medicinal uses, medicinal speciessuch *Cassia abbreviata* are rapidly disappearing in many habitats and threatened to extinction.This study assessed the response of pre-sowing treatment methods on quiescency and germination of seeds of *Cassia abbreviata*and *Senegalia nigrescens* tree species found in semiarid Savanna Ecozone of Botswana. Pre-sowing treatment included immersion in 98% undiluted sulfuric acid (H_2SO_4) for 15, 30, 45 and 60 minutes,submergence in boiling water for one, two, three minutes and submerged in warm water for 24 hours,mechanical scarification of the seed coat and control. ANOVA showed that there is a highly significant difference ($P < 0.0001$) among the treatments of*C.abbreviata*in seed germination and no significant difference among the treatments of *S. nigrescens*. The highest germination percentage of *C.abbreviata*seeds was 81%,which was found fromseeds immersed in H_2SO_4 for 30 minutes, while for *S. nigrescens* seeds,the highest germination percentage was 99%,which was found from seeds treated with mechanical scarification and those immersed in H_2SO_4 for 15 and 45 minutes.The objective of this study is to determine the effect of pretreatment methods on germination of *C. abbreviata* and *S. nigrescens* seeds. Based on these results, we recommend sulphuric acid and mechanical scarification as suitable seed pre-sowing treatments for enhancing the germination of *C. abbreviata* and *S. nigrescens*, respectively.

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Introduction

Forests and rangelands are the fundamental constituents of arid zone ecosystems which come up with sustentation for satisfactory conditions for agriculture and human livelihoods. They provide numerous goods and ecosystem services. In addition, they are essential habitat for many other organisms.[1] Globally, forests and tree resources have been declining over the past few decades due to deforestation, which also threatens othertree dependent organisms.[2] Deforestation has been credited to humanexercises, such as cutting down trees for construction or manufacturing timber and fuelwood, clearing land for cropping or grazing and human settlement.[3] Deforestation has been exacerbated by climate change, drought and forest fires.Consequently, there is crucial need to reclaim degraded land in arid environments using multipurpose indigenous trees and shrubs. These trees and shrubs are adaptable and have evolved to withstand harsh environmental conditions.

Cassia abbreviata Oliv.,commonly known as Monepenepe in Setswana[4] belongs to the family Fabaceae, sub-family Caesalpiniaceae. It is a deciduous shrub or a multiple-branched tree with a light open crown and grows up to 10m high.[5] *Cassia abbreviata* has a light brown to dark, fissured flaking bark. It has a rounded crown and compound leaves that are bright green in colorwhen new in early summer andbecome darker and less striking with maturity. Flowersare single, pea-like, yellow in colorand clustered at the end of branches.[6] *Cassia abbreviata* is found in dry thorn bushwoodlands from Somalia to South Africa, mostly at low to medium altitudes (220-1520m).[5] The wood may be used for fuelwood, furniture-making, and construction. *Cassia abbreviata* is also planted around homes and parks for ornamentalpurposes.[7] It is a good medicinal tree used to treat various infections.[8] The roots and bark of *Cassia abbreviata* is extensively utilized in Botswana as a general blood purifier and in the cure of period, womb, and abdominal pains. The plant has also been incriminated by anecdotal authentication as appetite booster, in decreasing HIV levels and in fighting STIs, such as syphilis and gonorrhea.[9] Current evidence reveals that the population of *C. abbreviata* has declined in its natural habitat due to removal of the bark for medicine.[9] The decline in population is intensified by drought, deforestation due to anthropogenic activities, fires, and climate change.

Senegalia nigrescens (Oliv.) P.J.H. Hurter (previously known as *Acacia nigrencens* Oliv) and commonly referred to asknob thorn,[10] is a multipurpose leguminous tree belonging to the family Fabaceae. It is an average sized deciduous tree extendingup to 30m high. The treeshavestraight stemswith diameters of 75-90cm. The bark is very distinctive, dark brown to blackish, rough and deeply fissured.[11] The most outstanding feature of *S. nigrescens* is the scattered, irregular knobbed prickles that cover the trunk and large branches.[12] Leaves are alternate, bipinnately compound, with 2 - 4 pairs of pinnae each bearing 1 or 2 pairs of large, nearly circular, soft pale green or grey green leaflets.[13] Flowers are in creamy-white spikes up to 9cm long[12] and characterizedby a strong scent.[13] Flowersappear towards the end of the dry season (August -November) before leaves.[14] Fruits are oblong pods, initially green and become very dark to blackish in color with maturity.[12] It is a common savannah species that occurs from Tanzania southward to eastern Namibia, Botswana, and northeastern South Africa. It is found in woodland and bushland at low to medium altitudes (1200-1600m) on shallow soils, rocky hillsides, and alluvial soils in valleys.[11]

The wood of *S. nigrescens* is very durable, hard, heavy[12] and resistant to damage by termites.[13] It is used for firewood, furniture making, fence posts and centre posts for houses.[15] Flowers and foliage are browsed by giraffe,while the seed pods and foliage are browsed by a wide range of mammals, including elephants.[14] Elephants also eat the roots and the inner bark, by stripping of the bark, which has left many trees dead.[13] The damage to the bark by elephants increases during the late winter to early spring (July-October).[12] Flowers attracts many insects andare used by honeybees to produce good quality honey.[12] The two study speciesare propagated by seed or wildings, which are the most common and cheap method of raising many plant seedlings. Natural regeneration of indigenous species is not abundant in fragile arid environments due to poor seed germination and predation of seeds and seedlings by animals. In addition, arid environments are limited by low and unreliable rainfall, relatively with high temperatures,

thus, resulting in limited water resources. Poor germination poses as a serious challenge to the propagation of severalindigenous tree and shrub speciesfor planting in arid environments. Most indigenous trees and shrubs, especially leguminous species are marked by an impermeable seed coat that imposes physical exogenous dormancy.[17] The hardened seed coat hinderspermeation and gas interchange,resulting in poor and erratic germination, which limits the cultivation of indigenous tree and shrub species in both tree and homestead planting programmes. Overcoming hard seed coat-imposed dormancyis an important initial step in the use of endemic shrub and tree species in planting and restoration programmes.In a bid to realizemassive and uniform early germination, it is foremost to tackle problems associated with dormancy imposed by hard seed coats.Variousinvestigations have indicated that seed weight within a species or even an individual plant can differincredibly. This difference in seed weightnot outside a species may influence germination.[18]

Several scarification techniques,including nicking, acid, boiling/hot and cold water have been used to break seed dormancy in leguminous tree and shrub species of arid lands.[19] These methods can augment germination by reducing seed dormancy within a comparably short period of time.[2] Rapid, uniform and highseed germinationare critical in propagating planting stock for afforestation and restoration programmes.Therefore, the objective of this study was to determine the effect of pretreatment methods on germination of *C. abbreviata* and *S. nigrescens* seeds.

Materials and Methods

Study Site

The research study was run at the Herbarium of the Department of Range and Forest Resources in the Botswana University of Agriculture and Natural Resources (BUAN)in July and August 2019. The University is located at Sebele (23°34' S and 25°57' E, altitude of 994m) ,relatively 10 km from the centre of Gaborone, the Capital City of Botswana along the A1 North-South Highway.

Seed Source

Seeds of *C. abbreviata* were gathered from Botswana University of Agriculture and Natural Resources Agroforestry plots during July 2019. A long-hooked stick was used to shake the tree crown and get the mature and healthy fruits/pods. The mature dry pods were placed in paper bags and transported to the Herbarium. Seed were extracted from pods by crushing the pods by hand and then, cleaning them in water to remove the chaffer.

Seeds of *S.nigrescens* were collected between Mosetse and Nata along the A3 Road during August 2018. Pods were collected direct from crowns or by shaking with long hooked sticks and were put in paper bags and moved to BUAN where they were kept in a refrigerator set at about 5 °C till the time of use. The seeds were extracted by crushing with hands and then winnowed to separate the husk. Prior to sowing, seeds of the two species were put througha viability test using the floating method, to which the floated seeds were treated unviable and discarded.

Seed Characteristics

The number of seeds of the two study species in a pod were determined from five replications of 10 pods each. The seeds were, then, categorized as intact, aborted or dead/eaten. Once extracted from pods, seeds were submerged in cold water, and only those that descended and settled at the bottom of the container were chosen for the study. The floated seeds, which expressed non-viable seeds, were castaway. The sizes, i.e., length, width and breadth of seeds, of the two study species were determined by measuring five replications of 10 seeds each using a digital calliper. The weight of single seeds (seed mass) was determined by weighing five replicates of 10 seeds using a digitalized sensitive scale. Similarly, five replications of 100 seeds from each study species were weighed to determine the weight of thousand seeds.

Experimental Design and Treatments

In this study work, four experiments containing 10 treatments, including the control, were carried out. The four experiments were nicking, subjection to sulfuric acid, boiling and hot water. The treatments in the study were completely randomized in four replications of 25 seeds each.

Experiment 1 - Mechanical Scarification

The experiment comprised 100 seeds of each study species, with four replications of 25 seeds, were used. In all these seeds, oneto two millimeters of

the seed coat at the distal end, opposite the helium, was carefully cut using scissors so that the seeds could absorb water, which is necessary to initiate germination.

Experiment 2 - Exposure to Sulfuric Acid

The experiment encompassed four intervals of exposure to concerted sulfuric acid (98%), i.e., 15, 30, 45 and 60 minutes were used by applying the method outlined by (24). For each duration of exposure, the four replications of 25 seeds were placed into four 100ml heat-impervious non-caustic glass beakers, consisting of sulfuric acid by making sure that all the seeds were concealed by the acid. The seeds were incessantly stirred to confirm their consistentsubjection to the acid. After the specified intervals of exposure, the seeds were filtered out of the acid using an acid-impervious sieve while the acid was cleared off simultaneously into another beaker. The seeds were, then, entirely washed and rinsed to get rid of all the acid in a running tap water and distilled water, respectively.

Experiment 3 - Exposure to Boiling Water

The experiment incorporated three durations of subjection of seeds of the study species, i.e.,one, three and five minutes, to boiling water were used. For each time interval of subjection, four replications of 25 seeds were placed into four separate coffee filter papers and submerged into a cooking pot with boiling water for the designated period, after which they were removed and submerged in a small bucket containing cold distilled water to cool them down for a few minutes.

Experiment 4- Hot water (boiling water allowed to cool with seeds in 24 hours)

In this experiment, four replications of 25 seeds were enclosedin coffee filter papers and clipped to prevent them from falling before putting them in a beaker. Boiling waterwas poured into the beaker with seeds and left to cool for 24 hours at room temperature before placing seeds in petri dishes.

Control Experiment

Four replicates of 25 untreated seeds each were used as control for the two species.

In all the experiments, each replicate, 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 1–2 mm. The number of germinating seeds was recorded daily for 30 days. Germinated seeds were removed from petri dishes after counting and recording. Seeds that had not geminated after 30 days were tested for their viability using a cutting test.

Data collected on germinated seeds were used to calculate germination percentage (GP) as indicate in formula below.

GP= (Total number of seeds germinated) / (Total number of seeds sown)· 100, %

Data Analyses

The data collected was subjected to both descriptive statistics and One-Way 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.[20] Significant differences of means were tested using Tukey's Honestly Significant Difference (HSD) at the significance level of $P < 0.05$.

Results

Seed Characteristics

The mean numbers of seeds per pod were 7 ± 1.9 and 94 ± 0.8 in *S. nigrescens* and *C. abbreviata*, respectively. The mean numbers of intact, eaten, and aborted seeds per pod were 3 ± 1.6, 1 ± 0.4 and 4 ± 0.7, respectively, in *S. nigrescens*. In the case of *C. abbreviata*, the mean numbers of intact, eaten and aborted seeds were 62 ± 2.8, 0 and 32 ± 2.3, respectively (Table 1). The mean mass of single seeds of *S. nigrescens* and *C. abbreviata* was 0.19 ± 0 and 3.2 ± 0.03 grams, respectively. Similarly, the mean thousand seed weights were 172 ± 5 and 298 ± 6 grams for *S. nigrescens* and *C. abbreviata*, respectively.

Germination Percentages

The results indicated that percent germination was affected by pre-sowing treatments in the two species [*C.abbreviata*-One Way ANOVA: $F(9,39) = 54.91$, $P = 0.00001$ and *S. nigrescens* - One Way ANOVA: $F(9, 39) = 5.5$, $P=0.0002$)] (Table 2). For *C. abbreviata*, the highest mean percent germination was documented in seeds

exposed to sulfuric acid for 30 and 15 minutes (81 and 80%), respectively, followed by those subjected to the acid for 45 minutes (77%) and nicking (72%) (Table 2). Germination percentages of other treatments were not significantly higher than the control (66%), sulphuric acid 60 minutes (65%) and the hot water (53%). However, percent germination in seeds treated with boiling water (one, three and five minutes) were significantly lower than the control (Table 2). For *S. nigrescens*, there was no significant difference in percent germination among the control seeds and those treated with sulphuric acid (15, 30, 45 and 60 minutes), mechanical scarification, hot water (allowed to cool for 24 hours) and boiling water (one and threeminutes) (Table 2). Results show that percent germination of seeds exposedto boiling water for 5 minutes was significantly lower than the control.

Table 1: Seed characteristics (intact, eaten, and aborted) and total mean (± SEM) number of seeds pod^{-1} of *C. abbreviata* and *S. nigrescens*

Species	Seed characteristics							
	Intact		Eaten		Aborted		Total in pod	
	Number	Range	Number	Range	Number	Range	Number	Range
S. nigrescens	3 ± 1.6	0 – 5	1 ± 0.4	0 - 1	4 ± 0.7	3 - 5	7 ± 1.9	3 - 10
C. abbreviata	62 ± 2.8	42 - 81	0	-	32 ± 2.3	16 - 52	94 ± 0.8	85 - 100

Table 2: 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)

Treatment	*Cassia abbreviata*		*Senegalia nigrescens*	
	Germination (%)	Range	Germination (%)	Range
Control	66±5^{ab}	60 - 80	97 ± 2^{a}	92 - 100
Mechanical Scarification	72±3^{ab}	68 - 80	99 ± 1^{a}	96 - 100
Sulphuric Acid (15 minutes)	80±5^{a}	68 - 92	99 ± 1^{a}	96 – 100
Sulphuric Acid (30 minutes)	81±5^{a}	68 - 92	96 ± 1^{a}	96 - 100
Sulphuric Acid (45 minutes)	77±2^{ab}	72 - 80	99 ± 1^{a}	96 - 100
Sulphuric Acid (60 minutes)	65±7^{ab}	44 - 76	98 ± 2^{a}	92 - 100
Boiling Water (1 minute)	03±2^{c}	00 - 08	94 ± 3^{a}	88 - 100
Boiling Water (3 minutes)	01±1^{c}	00 - 04	61 ± 12^{ab}	32 - 80
Boiling Water (5 minutes)	00±0^{c}	00 - 00	21 ± 7^{b}	8 - 40
Hot Water (boiling water allowed to cool in 24 hours)	53±8^{b}	32 - 68	93 ± 6^{a}	72 - 100

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

Seed Germination Rates

The seeds of *C. abbreviata* treated with sulfuric acid (15, 30, 45 and 60 minutes) and hot water (allowing to cool in 24 hours) demonstrated the rapid and homogenous germination, reaching maximum percent germination within five days of sowing, succeeded by nicking (seven days) (Figure 1). While untreated seeds (control) reached maximum percent germination after 17 days. For *S. nigrescens*, seed treated with sulfuric acid (15, 30, 45 and 60 minutes) attained uniform, fastest and maximum germination just two days after sowing (Figure 2) followed by mechanical scarification (four days),

those treated in boiling water for one minutes and boiling water (allowed to cool for 24 hours) (14 days). The untreated seeds (control) took 21 days to reach maximum germination (Figure 2).

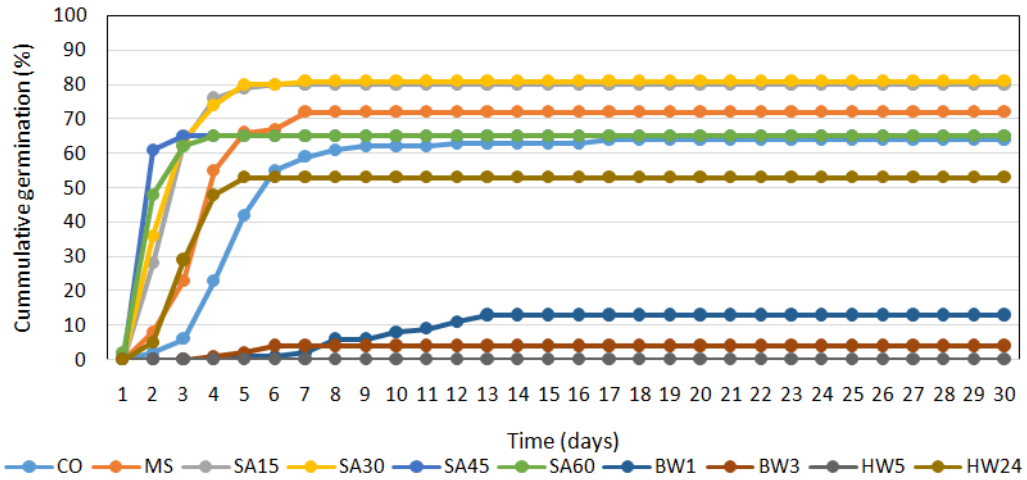

Fig. 1: Cumulative germination percentage of C. abbreviata recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 minute; BW3 = Boiling water for 3 minutes, HW5 = Boiling water for 5 minutes, HW24 = Boiling water allowed to cool in 24 hours, SA15 = Sulphuric acid for 15 minutes, SA30 = Sulphuric acid for 30 minutes, SA45 = Sulphuric acid for 45 minutes and SA60 = Sulphuric acid 60 minutes

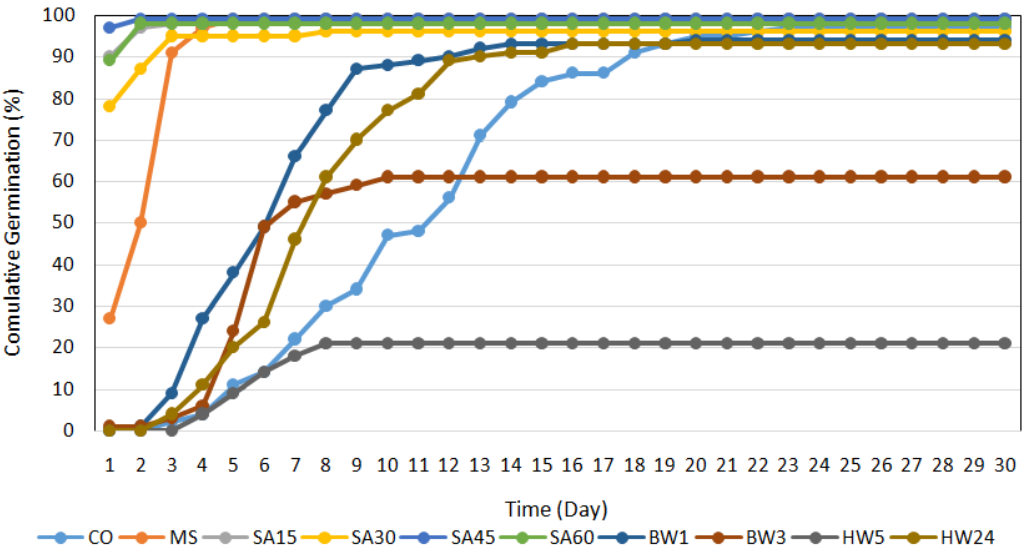

Fig. 2: Cumulative germination percentage of *Senegalia nigrescens* recorded for 30 days (CO = Control, MS = Manual scarification, BW1 = Boiling water for 1 minute; BW3 = Boiling water for 3 minutes, HW5 = Boiling water for 5 minutes, HW24 = Boiling water allowed to cool in 24 hours, SA15 = Sulphuric acid for 15 minutes, SA30 = Sulphuric acid for 30 minutes, SA45 = Sulphuric acid for 45 minutes and SA60 = Sulphuric acid 60 minutes

Discussion

Most tree and shrub species of arid and semi-arid regions fail to germinate when exposed to all states commendatory to germination. This phenomenon has been attributed to hard seed coats,which employ a physical exogenous dormancy.[21] The tough solid seed coats of numeroustree and shrub species have emerged to cope unsuitable conditions, such

as extreme heat from sunlight, spreading animals, grievous drought, and physical damage.[22] Seed dormancy imposed by hard seed coats normally applies to leguminous forest trees or shrubs.[23] Germination needsfracture of the seed coat and consequentimbibition of water by the seed.[21] Considerable pre-sowing techniques have been used to prevail over the hard seed coat-imposed dormancy, and to improve the absorption of the seed coat to waterto attain fast and uniform germination.[24] However, pre-sowing treatments used to break hard seed coats varyamong species.

According to[25] seed size affects the germination, emergence, plant growth and performance of plants in the field. It is commonly acknowledged that considerably heavy seeds germinate superior to lighter seeds.[26] The outcome of this experiment is congruous with this general trend, which proposes that germination features depend relatively on the resources allotted to the seed by the mother plant. Larger seeds have significant levels of starch and other foods, and this might be one factor which determines the germination of seed and the growth of seedlings.[18] Our results showed that there was no significant difference in percent germination between *C.abbreviata* seeds treated with sulphuric acid (15, 30, 45 and 60 minutes), mechanical scarification, and the control. However, these treatments expressed the fastest and uniform germination compared with the control. The fact that these treatments gave earlier,and homogenousgermination suggest that the more rapidly the seed coat is ruptured, the faster the rate of germination.[19] Fast and uniform germination reported in these treatments could be accredited to the uptake of water and gaseous exchange due to softening andrupturing of the seed coat.[2] According to,[19] hard seed coatsprevent the entrance of water and exchange of gases,which cause seed dormancy in many species. Our results are consistent with[20] results on percent germination in Philenoptera violacea(Klotzsch) Schrire that showed no significant improvement by sulphuric, hot water and mechanical scarification compared with the control. Results also showed that seeds treated in boing water (one, three and five minutes) attainedgermination of 0-3%, which was significantly lower than 66% recorded for the control (Table1). This suggest that boiling water is not a suitable pre-treatment technique for *C.abbreviata*. In contrast,[15] reported 10% germination is untreated seeds of *C.abbreviata*. Lower percent germination in seeds treated with boiling water has been reported in other species.[2] For *S. nigrescens*, the findingsindicated that seeds treated with sulfuric acid (15, 30, 45 and 60), mechanical scarification, boiling water (one and three minutes) and hot water (boiling water allowed to cool in 24 hours) improved germination. However, percent germination in these treatments was not significantly higher than the control. Maximum germination in the above treatments was attained between 2-14 days compared to the 21 days recorded in the untreated seeds.This result agrees with[15] who reported that 85% *S. nigrescens* seeds treated with hot water germinated 10 days after sowing. The 97% cumulative germination recorded in the control treatment suggest that *S. nigrescens* is not characterised by hard seed coat-imposed dormancy. Therefore, some of these treatments may only be used to speed up germination.

Conclusions

Results from the present study showed that the germination of seeds of *C.abbreviata* and *S. nigrescens*is not impended by the seed coat. However, it is worth noting that seeds subjected tosulfuric acid, mechanical scarification and hot water reached maximum germination in a short time in contrastwith the control. Although, the germination of the two study species is not constrained by seed coat dormancy, seeds can be soaked in hot water and allowed to cool to speed-up uniform germination.

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Conflict of Interest

This is an official agreement that all listed authors have contributed to the data collection and write-up of this manuscript. They have also all agreed that it be published with the CurrentAgriculture Research Journal and do not have any conflict of interest.

References

1. Bastin JF, Finegold Y, Garcia C, Mollicone D, Rezende M, Routh D, Zohner CM and Crowther TW. 2019. The global tree restoration potential. *Science,* 365(6448):76-79.
2. Botumile A, Teketay D, Mojeremane W, Mathowa T. 2020. Overcoming seed dormancy of Senegalia galpinii and Vachellia robusta through scarification pre-sowing treatments. *Agriculture and Forestry*, 66(1): 157-173.
3. Davalos LM, Sanchez KM and Armenteras D. 2016. Deforestation and coca cultivation rooted in twentieth-century development projects. *Bioscience*, 66(11):974-982.
4. Setshogo MP and Mbereki CM. 2011. Floristic diversity and uses of medicinal plants sold by street vendors in Gaborone, Botswana. *African Journal of Plant Science and Biotechnology,* 5: 69-74.
5. Mulofwa J, Simute S and Tengas B. 1994. Agroforestry manual for extension workers in the Southern Province, Zambia. Technical Handbook No.4. Regional Soil Conservation Unit/SIDA. Nairobi, Kenya. 91pp.
6. Jacana M and Twisisa S. 1997. Sappi tree spotting: Tree identification made easy, Jacana Education, Johannesburg, 302pp.
7. Dharani M, Kurunga G, Yenesew A, Mbora A, Mwaura L, Dawson I and Jamnadass R. 2010. Common antimalarial trees and shrubs of East Africa: A description of species and a guide to cultivation and conservation through use. *World Agroforestry Centre,* Nairobi, Kenya. 100 pp.
8. Keter LK and Mutiso PC. 2012. Ethnobotanical studies of medicinal plants used by traditional health practitioners in the management of diabetes in Lower Eastern Province, Kenya. *Journal of Ethnopharmacology,* 139(6):74-80.
9. Mojeremane W, Legwaila GM, Mogotsi K.K, Tshwenyane SO. 2005. Monepenepe (Cassia abbriviata): A Medicinal Plant in Botswana. *International Journal of Botany,* (2):108-110.
10. Kyalangalilwa B, Boatwright JS, Daru BH, Maurin O and van der Bank M. 2013. Phylogenetic position and revised classification of Acacia (Fabaceae; Mimosoideae) in Africa, including new combinations in Vachellia and Senegalia. *Botanical Journal of the Linnean Society,* 172: 500-523.
11. Lemmens RHMJ.2006. Acacia nigrescens Oliv. In: Louppe, D., Oteng-Amoako, A.A. and Brink, M (eds.). PROTA (Plant Resources of Tropical Africa), Wageningen, Netherlands. Accessed 1 May 2020.
12. Roodt V. 2008. Trees and Shrubs of the Okavango Delta: Medicinal uses and nutritional value. Shell Oil Botswana, Gaborone, Botswana. 221 pp.
13. Timberlake J. 1980. Handbook of Botswana Acacias. Ministry of Agriculture, Gaborone, Botswana. 120 pp.
14. Fleming PA, Hofmeyr SD, Nicolson SW. 2007. Role of insects in the pollination of Acacia nigrescens (Fabaceae). *South African Journal of Botany,* 73(1): 49-55.
15. Tietema T, Merkesdal E and Schroten J. 1992. Seed germination of trees in Botswana. African Centre for Technology Studies Press, Nairobi, Kenya. 106 pp.
16. Mojeremane W, Mathowa T, Teketay D, Stimela T, Kopong I and Rampart M. 2017. Pre-sowing seed treatment method to overcome dormancy in seeds of Vachellia rehmanniana. *Agriculture and Forestry,* 63(2):171-181.
17. Pound LM, Ainsley PJ and Facelli JM. 2015. Dormancy-breaking and germination requirements for seeds of Acacia papyrocarpa, Acacia oswaldii and Senna artemisioides, three Australian arid-zone Fabaceae species. *Australian Journal of Botany,* 62(7): 546-557.
18. Rampart M. 2019. Seed germination variation among crop years from a Pinus sylvestris clonal seed orchard. *Asian Journal of Research in Agriculture and Forestry,* 3(1):

2581-7418.

19. Fredrick C, Muthuri C, Ngamau K and Sinclair F. 2017. Provenance and pre-treatment effect on seed germination of six provenances of Faidherbia albida (Delile) A. Chev. *Agroforestry Systems,* 91(6): 1007-1017.
20. Setlhabetsi OT, Mojeremane W, Mathowa T and Teketay D. 2019. Breaking seed dormancy in Philenoptera violacea (Koltzsch) Schire using different pre-sowing treatment methods. *Journal of Agriculture and Environmental Sciences*, 8(1): 104-110.
21. Baskin CC. and Baskin JM. 1998. Seed ecology, biogeography, and evolution of dormancy and germination. *Academic Press,* San Diego.
22. Tadros M, Samarah N and Alqudah AM. 2012. Effect of different pre-sowing seed treatments on the germination of Leucaena leucocephala and Acacia farnesiana. *New Forests,* 42: 397-407.
23. Mojeremane W, Mathowa T and Teketay D. 2018. Effects of different pre-sowing treatments on germination of Peltophorum africanum. seeds from two provenances in Botswana. *Journal of Biodiversity and Environmental Sciences,* 12(2):230-236.
24. Steiner F, Zuffo MA, Busch A, Sousa TDO and Zoz T. 2019. Does seed size affect the germination rate and seedling growth of peanut under salinity and water stress? *Agricultural Research in the Tropics,* 49: 1-9.
25. Blate GM, Peart, DR and Leighton M. 1998. Post-dispersal predation on isolated seeds: a comparative study of 40 tree species in a Southeast Asian rainforest. Oikos, 82(3):522-538.