INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 12, No. 2, p. 230-236, 2018
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Effects of different pre-Sowing treatments on germination of *Peltophorum africanum* Sond seeds from two provenances in Botswana

Witness Mojeremane*, Thembinkosi Mathowa, Demel Teketay

Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources, Private Bag, Gaborone, Botswana

Article published on February 28, 2018

Key words: Dormancy, *Peltophorum africanum*, Pre-treatment, Germination percentage

Abstract

A germination study of *Peltophorum africanum* seeds was conducted in the laboratory of the Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources from May to June 2017. Seeds were collected from Butale and Gaborone to assess effect of pre-sowing treatments on germination. The experiment followed a completely randomized design (CRD) with five treatments; control, boiling water, hot water, mechanical scarification and concentrated sulphuric acid. The boiling water treatment had three different levels of time exposure, while the sulphuric acid had four different levels of time exposure. Seed germination percentages, germination mean time and germination index were significantly ($P < 0.0001$) affected by pre-sowing treatment methods. The maximum germination percentages (82-87) of Butale seeds were recorded in the mechanical scarified seeds, seed immersed in hot water and all sulphuric acid soaking times. The maximum germination percentages (75-88) in the Gaborone seeds were recorded in mechanical scarification, immersion in hot water, boiling water (1min) and sulphuric acid. Mechanical scarification and hot water are methods recommended for use by tree growers and farmers to break dormancy *P. africanum* because the acid is expensive and need trained people.

***Corresponding Author:** Witness Mojeremane ✉ wmojerem@buan.ac

Introduction

Peltophorum africanum (Sond) known as weeping wattle or huilboom (Van Wyk and Van Wyk, 1997) belongs to the Mimosoideae subfamily of the Fabaceae family (Van Wyk and Gericke, 2000; Bizimenyera *et al.,* 2005). The *Peltophorum* genus is found throughout the tropics and *P. africanum* is the only species indigenous to southern Africa (Bizimenyera *et al.,* 2005; Bosch, 2006) distributed from the Democratic Republic of Congo to South Africa and Swaziland (Bosch, 2006). *P africanum* is cultivated as an ornamental tree in Kenya, Tanzania, Madagascar, Australia and the United States (Bosch, 2006). It is a deciduous or semi-deciduous shrub or tree reaching 9-15m high (Mazimba, 2014). It has crooked multi-stems (Storrs, 1995) and a wide canopy (Palgrave, 1983). It strives on sandy soils (Van Wyk and Van Wyk, 1997) at medium to low altitudes in wooded grasslands (Palgrave, 1983) in areas receiving annual rainfall of 300-900mm (Bosch, 2006). The wood is used in turning and carving (Van Wyk and Van Wyk, 1997). Young leaves and pods are eaten by livestock and wild animals (Storrs, 1995). Flowers provide nectar and pollen for honeybees (Venter and Venter, 1996; Setshogo and Venter, 2003). The bark, leaves and roots are used in traditional human medicine (Van Wyk and Gericke, 2000; Theo *et al.,* 2009; Chinsembu *et al.*, 2011; Maroyi, 2011) and in livestock (Bizimenyera *et al.,* 2005).

Indigenous leguminous trees are rarely planted in Botswana and other arid and semi-arid environments due to the hardness of their seed coats. Instead, they are deliberately maintained in homesteads, cropland and the veld in order to obtain their valuable products such shade, medicine, food, fuel wood and animal feed. Indigenous tree species face regular fires and indiscriminate cutting with little regards to include them in afforestation and reforestation programmes. The hard and impervious seed coats of most leguminous trees which strongly resists imbibition of water and gaseous exchange remains a hindrance to their inclusion in tree planting programmes. In order to obtain high and uniform germination in hard coated seeds, pre-sowing treatments are required (Teketay, 1996; Schultz and Rave, 1999; Yang *et al.,* 1999*).*

Many pre-sowing treatments that include sulphuric acid, cold, hot and boiling water and mechanical scarification have been used to break the dormancy of seeds with a hard and thick coat (Teketay, 1996; 1998; 2005; Rasebeka *et al.,* 2014; Fredrick *et al.,* 2016). Breaking dormancy in *P. africanum* would improve its regeneration potential and make it an ideal plant to use in afforestation and reforestation programmes. The present study sought to assess the impact of pre-sowing treatments on the germination of *Peltophorum africanum* seeds from two provenances in Botswana.

Materials and methods

Study site

The study was carried out in the Department of Crop Science and Production laboratory, Botswana University of Agriculture and Natural Resources (BUAN) from May to June 2017. BUAN is located at Sebele (23°34' S; 25°57' E, elevated 994m above sea level), 10km from the centre of Gaborone City, the Capital of Botswana along the A1 North-South highway.

Seed collection and processing

Mature pods of *P. africanum* were collected from different healthy mother trees at Butale village and around Gaborone City between July and September 2016. Butale Village is situated in the North East District, approximately 500km from Gaborone. Pods were placed in paper bags and transported to the laboratory (BUAN) for processing. Seeds were extracted from pods and mixed according to provenance. They were then screened to remove the damaged ones. Seeds were kept in tightly sealed bottles and stored at 5°C in a refrigerator until the experiment commenced. Before pre-treatment experiments, seeds were sorted as viable and non-viable seed by immersing them in distilled water at room temperature. The floating seeds were classified as non-viable and discarded; and viable seeds (sub-merged seeds) were used for the experiments.

Seed characteristics

Seed characteristics were evaluated by measuring their length, width and breath using an electronic digital caliper (0-150mm).

The 1000 seed weight was assessed by weighing seeds in an electronic analytical balance (Model: PW 124). Five replicates of 10 seeds and ten replicates of 100 seeds were used to determine the mean dimensions and thousand seeds respectively. The 1000 seed weight was then, computed from the mean weight of 100 seeds. Seed characteristics are shown in Table 1.

Table 1. Mean seed size and 1000 seed weight of Butale and Gaborone. The "±" indicate the standard error of mean.

Locality	Seed size (mm)			1000 seed weight (g)
	Length	Width	Breath	Weight
Butale	10.7±0.2	6.3±0.2	1.9±0.1	84.2±0.5
Gaborone	8.5±0.2	6.1±0.1	2.0±0.1	78.8±0.8

Experimental design and germination experiment

The experiment was laid out using a completely randomized design (CRD). There were five main treatments that included; mechanical scarification, boiling water, hot water, concentrated sulphuric acid (98.8%) and the control. Each treatment had four repetitions. The boiling water treatment had three different levels of exposure time (1, 3 and 5 minutes) and the concentrated sulphuric acid treatment had four different levels of exposure time (15, 30, 45 and 60 minutes). Each repetition had 25 seeds germinated in petri dishes lined with cotton wool and moistened with distilled water. Observations were done daily and petri-dishes watered when necessary. Seeds were considered germinated when the radicle protruded from the seed coat.

Experimental treatments

Control

Seed germinated in petri dishes without pre-treatment applied were used as the control.

Boiling water

Each replicate of seeds was enclosed in a coffee filter bag which was then folded and fastened with staples to prevent seed loss. The seeds were then immersed in boiling water for 1, 3, and 5min. After immersion, they were removed from the boiling water, soaked in cold water and left to cool for about 5min.

Hot water

Hot water treatment was done by boiling the water to 100°C and then pouring it in a 100ml heat resistant glass beaker containing 100 seeds. Soaked seeds were left in the water to cool gradually for 24 hours at room temperature before sowing.

Mechanical scarification

Mechanical scarification was done by nicking the seed coat with a nail cutter at the opposite side of the caruncle helium until the cotyledon was exposed.

Sulphuric acid treatment

Acid scarification was done by soaking seeds in concentrated sulphuric acid for 15, 30, 45 and 60 minutes followed by thorough washing in running tap water until considered safe to handle.

Statistical analysis

Seeds were checked for germination daily for 21 days. Daily germinations were summed up to obtain cumulative germination percentage. Germination mean time (GMT) and germination index (GI) were calculated as follows (Botsheleng *et al.*, 2014).

GMT (days) = $\Sigma T_i N_i/S$, where, T_i = number of days from the beginning of the experiment, N_i = number of seeds germinated per day and S = total number of seeds germinated. (1)

GI = $(G_1/1) + (G_2/2) + ... + (G_x/x)$, where, G = germination day 1, 2..., and x = the corresponding day of germination. (2)

Data was subjected to an analysis of variance (ANOVA) with Analytical Software (2013) after arcsine transformation of all percent germination data (Zar, 2010). A significant level of 0.05 was used for all statistical tests and mean comparisons done using Tukey's HSD test.

Results

The germination percentages, GMT and GI of seeds from the two collection sites were significantly (P<0.0001) affected by seed pre-treatment methods (Table 2).

Mechanically scarified seeds, seeds immersed in hot water for 24 hours and all sulphuric acid soaking times of Butale seeds produced maximum GP (82-87%) compared to the control. There was no germination recorded in all boiling water soaking times (Table 2). The GMT for Butale varied significantly (P<0.0001) among the treatments. The maximum GMT was observed in the control and seeds soaked in sulphuric acid for 15 minutes, but they did not differ from mechanically scarified seeds and their counterparts soaked in sulphuric acid for 30 and 45 minutes. The GI did not differ among the scarified seeds, seeds immersed in hot water, and all sulphuric acid soaking times which were significantly (P< 0.0001) higher that the control. The GI for boiling water soaking times followed trends observed in the GP and GMT (Table 2).

Germination percentages for Gaborone seeds ranged from 3 to 87 (Table 2). The GP of mechanical scarified seeds, seeds immersed in hot water for 24 hours, boiling water for a minute and sulphuric acid soaking times of 30, 45, 60 minutes were significantly (P<0.0001) higher than the control, soaking in sulphuric acid for 15 minutes and boiling water soaking times of 3 and 5 minutes. However, the GPs recorded in seeds soaked in sulphuric acid for 15 minutes, boiling water for 3 and 5 minutes were significantly (P<0.0001) higher compared to the control. The GMT ranged between 2.00 to 10.53 and the maximum was observed in seeds soaked in sulphuric acid for 15 minutes and minimum in the control seeds. The GI of all the treated seeds was significantly (P< 0.0001) higher compared to the control seeds, except the sulphuric acid 15 minutes and the boiling water 5 minutes (Table 2).

Table 2. Effects of seed pre-treatment on the germination *P. africanum*. Means followed by same letter in rows are not significantly different at 5% level using Tukey's Studentized range. GP= germination, MGT= mean germination time, GI =germination index, min= minutes, hrs= hours.

Provenances	Parameters	Treatments										P value
		Control	Mechanical scarification	Hot water	Sulphuric acid				Boiling water			
				24 hrs	15 min	30 min	45 min	60 min	1 min	3 min	5 min	
Butale	GP (%)	30^{b}	85^{a}	83^{a}	82^{a}	85^{a}	87^{a}	84^{a}	1^{c}	0^{c}	0^{c}	0.0001
	MGT(days)	6.60^{a}	3.98^{ab}	3.31^{b}	6.58^{a}	4.10^{ab}	3.54^{ab}	3.31^{b}	2.00^{bc}	0.00^{c}	0.00^{c}	0.0001
	GI	0.37^{b}	1.13^{a}	1.11^{a}	1.10^{a}	1.14^{a}	1.17^{a}	1.12^{a}	0.01^{c}	0.00^{c}	0.00^{c}	0.0001
Gaborone	GP (%)	3^{e}	88^{a}	85^{ab}	18^{d}	75^{b}	87^{ab}	86^{ab}	87^{ab}	32^{c}	24^{cd}	0.0001
	MGT(days)	2.00^{d}	4.09^{cd}	2.10^{d}	10.43^{a}	4.05^{cd}	4.26^{cd}	3.69^{cd}	4.22^{cd}	7.09^{b}	5.06^{bc}	0.0001
	GI	0.36^{e}	1.19^{a}	1.14^{ab}	0.21^{d}	0.99^{b}	1.17^{a}	1.15^{a}	1.17^{a}	0.39^{c}	0.29^{cd}	0.0001

Discussion

Different approaches of breaking seed dormancy in order to enhance germination rate in many tropical species with hard see coats have been used by different researchers (Teketay, 1996, 1998; 2005; Walters *et al.,* 2004; Aref *et al.,* 2011; Frederic *et al.,* 2016; Amoakoh *et al.,* 2017; Mojeremane *et al.,* 2017). These approaches are species specific and no single method is effective across plant species (Uniyal *et al.,* 2000; Amusa, 2011). Tadros *et al. (*2011) reported that the hard coat helps seeds to withstand tough environmental conditions such as heat from direct sunlight, digestive enzymes of animals, severe drought and mechanical damage. Hard coated seeds enrich the soil seed bank and ensure that germination take place during a period of time when environmental conditions are suitable for the survival and growth of seedlings (Morgan, 1998; Wang *et al.,* 1998). Seeds with hard coats are common in arid and semi-arid environments and affect the germination patterns and the planting of several species. Koornneef *et al.* (2002) reported that seed dormancy and germination are controlled by developmental and environmental factors.

Pre-treatment methods used in *P. africanum* significantly affected the GP, GMT and GI of seeds from the two locations. Mechanical scarification, hot water and all sulphuric acid soaking times were more effective in softening or cracking the seed coat of *P. africanum* seeds from Butale. Soaking seeds in boiling water (1, 3 and 5 minutes) was not effective in improving germination seeds from Butale.

The GMT for Butale seeds ranged from 3.31- 6.68 days, whereas the GI ranged between 1.10 and 1.7. The germination of Gaborone seeds was enhanced by mechanical scarification, soaking in hot water, sulphuric acid (30, 45 and 60 minutes) and boiling water (1 minute). Soaking seeds in boiling water for 3 and 5 minutes failed to improve germination. The GMT ranged between 2.10 and 4.09 days, while the GI ranged between 0.99 and 1.19.

The results of this study are in conformity with research done on other species which reported that mechanical scarification (Likoswe *et al.,* 2008; Aref *et al.,* 2011; Mojeremane *et al.,* 2017), hot water (Sadeghi *et al.,* 2009), sulphuric acid (Likoswe *et al.,* 2008; Pipinis *et al.,* 2011; Mojeremane *et al.*, 2017) are effective methods of improving germination of species with hard impermeable seed coat. The enhanced germination observed in these treatments could be attributed to the uptake of water and oxygen following softening or cracking of the seed coat. Other studies found that boiling water improved germination of hard seed coat species by uplifting water and O_2 permeability of the testa (Teketay, 1998; Aydin and Uzun, 2001). However, in this present study, all the boiling water soaking times failed to improve the germination of seeds from Butale. This is in agreement with Kahaka (2017) who observed that soaking *Dicrostachys cinerea* and *Senegalia erubescens* seeds in boiling water for 1, 3 and 5 minutes was not effective in improving germination. Kahaka (2017) attributed lack of improved germination to the sensitivity of the seeds to long soaking periods which probably caused damaged to the embryo and this could also be true for *P. africanum* seeds.

Conclusion

The results of these experiments showed that *P. africanum* seeds exhibited hard seed coat dormancy. Soaking seeds in the sulphuric acid and hot water significantly increased seed germination. Mechanical scarification was found to be the best non-chemical treatment to overcome this coat imposed dormancy in the seeds of *P. africanum*. Mechanical scarification and hot water methods are recommended for use by tree growers and farmers because the acid is expensive and need to be used by trained people.

Acknowledgement

The authors want to acknowledge the Botswana University of Agriculture and Natural Resources for permission to use their facilities for the research.

References

Amoakoh OA, Nortey DDN, Sagoe F, Amoako PK, Jallah CK. 2017. Effect of presowing treatments on germination and early growth performance of *Puoteria campachiana*. Forest Science and Technology **13(2),** 83-86.

Amusa TO. 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex Pers). Journal of Horticulture and Forestry **3(4),** 96-103.

Analytical Software. 2013. STATISTIX 10 for Windows. Tallahassee, Florida, US.

Aref IM, Atta HAE, Shahrani TA, Mohamed AI. 2011. Effects of seed pre-treatment and seed source on germination of five Acacia spp. African Journal of Biotechnology **10(71),** 15901-15910.

Aydin I, Uzun, F. 2001. Effects of some applications on germination rate of Gelemen clover seeds gathered from natural vegetation in Samsun. Pakistan Journal of Biological Sciences **4(2),** 181-183.

Bizimenyera SE, Swan GE, Chikoto H, Eloff JN. 2005. Rationale for using *Peltophorum Africanum* (Fabaceae) extracts in veterinary medicine. Journal of South African Veterinary Association **76(2),** 54-581.

Bosch CH. 2006. *Peltophorum africanum* Sond. In: Schmeltzer GH, Gurib-Fakim A. (Eds). Prota 11(1): Medicinal plants/Plantes médicinales 1. (CD-Rom). PROTA, Wageningen, Netherlands.

Botsheleng B, Mathowa T, Mojeremane W. 2014. Effects of pre-treatments methods on the germination of Pod mahogany (*Afzelia quanzensis*) and mukusi (*Baikiaea plurijuga*) seeds. International Journal of Innovative Research in Science Engineering and Technology **3(1),** 8108-8113.

Chinsembu CK, Hedimbi M, Mukaru CW. 2011. Putative medicinal properties of plants from the Kavango region, Namibia. Journal of Medicinal Plant Research **5(31),** 6787-6797.

Fredrick C, Muthuri C, Ngamau K, Sinclair F. 2016. Provenance and pre-treatment effect on seed germination of six provenances of *Faidherbia albida* (Delile) A. Chev. Agroforestry Systems.
DOI: 10.1007/s10457-016-9974-3

Kahaka P. 2017. The effect of pre-sowing treatments on the germination of *Dicrostachys cinerea*, *Senegalia erubescens* and *Vachellia nilotica* from Botswana. Unpublished BSc report. Department of Crop Science and Production, BUAN.

Koornneef M, Bentsink L, Hilhorst H. 2002. Seed dormancy and germination. Current Opinion in Plant Biology **5(1),** 33-36.

Likoswe MG, Njoloma JP, Mwase WF, Chilima CZ. 2008. Effect of seed collection times and pre-treatment methods on germination of *Terminalia sericea* Burch. ex DC. African Journal of Biotechnology **7(16),** 2840-2846.

Maroyi A. 2011. Ethnobotanical study of medicinal plants used by people in Nhema communal area, Zimbabwe. Journal of Ethnopharmacology **136(1)**, 347-354.

Mazimba P. 2014. Pharmacology and phytochemistry studies in *Peltophorum africanum*. Bulletin of Faculty Pharmacy, Cairo University **52(1),** 145-143.

Mojeremane W, Mathowa T, Teketay D, Stimela T, Kopong I, Rampart M. 2017. Presowing seed treatment methods to overcome dormancy in seeds of *Vachellia rehmanniana* Schinz. Agriculture and Forestry **63(2),** 171-181.

Morgan JM. 1998. Comparative germination responses of 28 temperate grassland species. Australian Journal of Botany **46(2),** 209-219.

Palgrave KC. 1983. Tree of southern Africa. Struik Publishers, Cape Town. 959pp.

Pipinis E, Milios E, Smiris P, Gioumousidis C. 2011. Effect of acid scarification and cold moist stratification on germination of *Cercis siliquastrum* L. Turkish Journal of Agriculture and Forestry **35(3),** 259-264.

Rasebeka L, Mathowa, T, Mojeremane W. 2014. Effect of seed pre-sowing treatment on germination of three *Acacia* species indigenous to Botswana. International Journal of Plant and Soil Science **3(1),** 62-70.

Sadeghi S, Ashrafi SZ, Tabatabai MF, Alizade HM. 2009. Study methods of dormancy breaking and germination of common madder (*Rubia tinctorum* L.) seed in laboratory conditions. Botany Research International **2(1),** 7-10.

Schütz W, Rave G. 1999. The effect of cold stratification and light on the seed germination of temperate sedges (Carex) from various habitats and implications for regenerative strategies. Plant Ecology **144(2),** 215-230.

Setshogo MP, Venter F. 2003. Trees of Botswana: names and distribution. Southern African Botanical Diversity Network Report 18, Pretoria 162p.

Storrs AG. 1995. Know your trees. Regional Conservation Unit, Nairobi 380p.

Tadros MJ, Samarah NH, Alqudah AM. 2011. Effect of different pre-sowing seed treatments on the germination of *Leucaena leucocephala* (Lam.) and *Acacia farnesiana* (L.). New Forests **42(3),** 397-407.

Teketay D. 1996. Germination Ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecology and Management **80(1-3),** 209-223.

Teketay D. 1998. Germination of *Acacia origena*, *A. pilespina* and *Pterolobium stellatum* in response to different pre-sowing seed treatments, temperature and light. Journal of Arid Environments **38(4),** 551-560.

Teketay D. 2005. Seed and regeneration ecology in dry Afromontane forests of Ethiopia: I. Seed production-population structures. Tropical Ecology **46(1),** 29-44.

Theo A, Masebe T, Suzuki Y, Kikuchi H, Wada S, Obi CL, Bessong O, Usuzawa M, Oshima Y, Hattori T. 2009. *Peltophorum africanum*, a traditional South African medicinal plant contains an anti HIV-1 constituent betulinic acid. Tohoku Journal of Experimental Medicine **217(2),** 93-99.

Uniyal AK, Bhatt BP, Todaria NP. 2000. Provenance characteristics and pre-treatment effects on seed germination of *Grewia oppositifolia* Roxb. A promising agroforestry crop of Garhwal Himalaya, India. International Tree Crops Journal **10(3),** 203-213.

Van Wyk B, Van Wyk P. 1997. Field guide to trees of southern Africa. 2. Ed. Struik Publishers 720pp.

Van Wyk B-E, Gericke N. 2000. People's plants: A guide to useful plants of southern Africa. Briza Publications. Cape Town 351pp.

Venter F, Venter JA. 1996. Making the most of indigenous trees. 3rd Edition. Briza Publications, Pretoria 368pp.

Walters M, Midgley JJ, Somers MJ. 2004. Effects of fire and fire intensity on the germination and establishment of *Acacia karoo, Acacia nilotica, Acacia luederitzii* and *Dichrostachys cinerea* in the field. BMC Ecology **4,** 3. doi: 10.1186/1472-6785-4-3.

Wang ZL, Wang G, Liu XM. 1998. Germination strategy of the temperate sandy desert annual chenopod *Agiophylium squarrosum*. Journal of Arid Environment **40,** 69-76.

Yang J, Lovett-Doust, Lovett-Dous L. 1999. Seed germination patterns in green dragon (*Arisaema dracontium*, Araceae). American Journal of Botany **6(8),** 1160-1167.

ZAR J. 2010. Bio-statistical analysis. 5th Edition Prentice-Hall Inc., New Jersey 944pp.