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Germination and Seedling Emergence Studies in Nyala Tree (*Xanthocercis zambesiaca* Baker)

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Abstract

Studies were conducted from December 2013−January 2014 and October−November 2014 to evaluate the most effective method of breaking seed dormancy in *X. zambesica* seeds and potting media suitable for germinating the seeds. The experiments were conducted in the Botswana College of Agriculture Plant Physiology laboratory and garden under net-shade, respectively. A completely randomized design (CRD) was used with five main pre-sowing treatments (untreated seeds-control, cold water, mechanical scarification, hot water and sulphuric acid) in experiment one. Five potting growth media treatments (sand, clay, garden soil, commercial compost and mixture of the three potting media) were used in experiment two and each treatment was replicated four times for both experiments. Percentage germination, germination mean time (GMT), germination index (GRI), and percentage emergence were calculated and the data was subjected to the analysis of the variance (ANOVA). Seed germination was significantly ($p<0.01$) increased by mechanical scarification and hot water at 5 minutes 9 days after sowing compared to the control and the rest of the treatments. Germination increased for 21 days across all the treatments and in terms of absolute numbers the control was superior. A non-significant treatment effect was observed for the control, mechanical scarification, cold water and hot water at 5 minutes. GMT and GRI were significantly ($p<0.01$) higher in the control, followed by hot water at 5 minutes, mechanical scarification, cold water and sulphuric acid at 5 minutes compared to other treatments. Overall seedling emergence was consistently higher ($p<0.01$) in the commercial compost than other treatments. *X. zambesiaca* seeds are not limited by dormancy therefore can be sown without any pre-treatment. Although commercial compost enhanced seed emergence, sandy soil is recommended to resource poor tree growers due to its availability, low cost, and suitability for both large and small tree seeds.

Keywords: Germination, Germination index, Germination mean time, Potting media and seedling emergence, Pre-sowing treatments, *Xanthocercis zambesiaca*.

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Introduction

Seed germination and early seedling growth are the most critical phases of raising a successful crop (Hossain et al., 2005) and are influenced by several factors that are species specific (Murali, 1997; Gunaga et al., 2011). The propagation of most arid and semi-arid forest trees is limited by the lack of information on their germination status and nursery requirements. Poor seed germination is often attributed to a water impermeable seed coat (Teketay, 1996; Walters et al., 2004) which exerts physical dormancy (Holmes et al., 1987). Several studies demonstrated that viable dormant seeds fail to germinate, even if exposed to favourable conditions (Hilhorst, 1995; Li and Foley, 1997; Bewley, 1997).

Seed dormancy evolved differently across plant species by adapting to the prevailing local environment to trigger germination when conditions suit the establishment of a new plant (Hilhorst, 1995; Vleeshouwers, 1995; Bewley, 1997; Li and Foley, 1997; Baskin and Baskin, 2004). Dormant seeds require a dormancy breaking treatment to enhance and obtain uniform germination (Botsheleng et al., 2014). Several methods such as cold water (Eze and Orole, 1987), hot water (Awodola, 1994; Adewusi and Ladipo, 2000; Otegbeye and Momodu, 2002; Botsheleng et al., 2014; Rasebeka et al., 2014) mechanical (Botsheleng et al., 2014; Rasebeka et al., 2014) and acid treatment (Ibrahim and Otegbeye, 2004; Agbogidi et al., 2007; Botsheleng et al., 2014; Rasebeka et al., 2014) have been used in breaking seed dormancy to improve germination rate in different forest seeds. Some seed treatment methods may also be used to speed up germination in seeds with permeable coats.

In addition to seed dormancy, inadequate knowledge on suitable potting media may also be a bottleneck to producing indigenous forest tree seedlings in arid and semi-arid areas. Nursery potting media is critical in determining the quality of seedlings (Baiyeri, 2005; Sahin et al., 2005; Baiyeri and Mbah, 2006). Seedling quality is influenced by the composition of the media (Corti et al., 1998; Wilson, et al., 2001). Potting media used to raise seedlings also influences their establishment (Baiyeri, 2006), survival and productivity when planted out in the field (Baiyeri and Ndubizu, 1994; Baiyeri, 2003).

Xanthocercis zambesiaca belongs to the family Fabaceae (alt. Leguminosae), subfamily Faboideae and also placed in Papilionoideae (Palmer and Pitman, 1972; Drummond, 1972). It is commonly known as the nyala tree (Van Wyk and Van Wyk, 1997; Van Wyk, 2001; Palgrave, 2002) and has an easterly distribution in Africa (Van Wyk, 2001) occurring in Botswana, Malawi, Mozambique, South Africa, Zambia and Zimbabwe (Palmer and Pitman, 1972; Drummond, 1972; Brummitt et al., 2007). *X. zambesiaca* is evergreen to semi-deciduous tree with a short single stem, heavy rounded crown and somewhat dropping leaves (Van Wyk and Van Wyk, 1997; Van Wyk, 2001; Palgrave, 2002; Brummitt et al., 2007). It grows to a height of 25−30 m (Palgrave 2002; Brummitt et al., 2007) and thrives well at low altitudes in hot areas, in the rich alluvial soils of the major river valleys, on river banks and beside lakes, sometimes in drier places, often associated with termite mounds (Brummitt et al., 2007). The tree grows slowly compared to other forest trees and tolerates very mild frost (Palgrave, 2002).

Fruits are green and turn dark brown when ripe and can stay on the tree for the whole year (Palgrave, 2002). They are eaten fresh or dried and pounded to make porridge (Palmer and Pitman, 1972; Palgrave, 2002). Fruits are also eaten by birds and game (Palmer and Pitman, 1972; Van Wyk and Van Wyk, 1997; Van Wyk, 2001). Leaves are browsed by livestock and game (Palmer and Pitman, 1972; Van Wyk and Van Wyk, 1997). Extracts of leaves have a great potential as anti-tuberculosis agent (Mmushi et al., 2010). The white wood is hard, heavy with an attractive fine-texture, works well but irritate the nose and throat when handled (Palmer and Pitman, 1972; Van Wyk and Van Wyk, 1997; Palgrave, 2002).

X. zambesiaca is one of the least known trees around the world. In Botswana the tree is endemic to the eastern part of the country. It has potential to be propagated in tree nurseries and planted as an ornamental or shade tree in gardens and parks. *X. zambesiaca* is not commonly propagated in tree nurseries due to inadequate information on its seed germination status and nursery requirements. To fill the knowledge gap, the present study was undertaken to evaluate: (1) influence of pre-sowing treatments on germination of *X. zambesiaca* seeds and (2) the influence of potting media on the emergence of *X. zambesiaca* seeds.

Materials and Methods

Study Site

The study was conducted at Botswana College of Agriculture (BCA), Gaborone. The College is located at Sebele Content Farm, approximately 10 km north of the centre of Gaborone along the north-south A1 highway. The germination study was conducted in the Department of Crop Science and Production Laboratory and the potting media study under a shade-net at College gardens. Seeds were obtained from Botswana National Tree Seed Centre, Department of Forestry and Range Resources, Ministry of Environment, Wildlife and Tourism, Gaborone.

Experimental Design

Seed Germination Study

The experiment was conducted between January and February 2014 using a completely randomized design (CRD) with five main treatments: (1) untreated seeds or control, (2) cold water, (3) mechanical scarification, (4) hot water, and (5) sulphuric acid. Each treatment was replicated four times. The seeds were tested for viability by floating them in tap water prior to the experiment and those that floated were removed as they may not have been viable and those that sank and settled at the bottom were used in the experiment (Rasebeka et al., 2014; Botsheleng et al., 2014). The seeds were too big to fit in petri-dishes and therefore germinated in 600 ml glass beakers. Ten

(10) seeds were sown in forty four (44) glass beakers and covered with a germination mix (hygromix) which was kept moist throughout the experiment by adding distilled water whenever required. A total of 440 seeds were used in this experiment.

Pre-sowing Treatments

The control seeds were not treated, cold water treated seeds were soaked in distilled water (25°C) for 24 hours prior to sowing. Mechanical scarification was carried out as described by Emongor et al. (2004). A pair of scissors was used to cut seeds about 1 mm from the helium (Karaguzel et al., 2004; Okunlola et al., 2011). Sulphuric acid treated seeds were equally divided into four 100 ml heat-resistant non-corrosive glass beakers. Concentrated sulphuric acid (98%) was slowly poured on the side of the beaker to cover all the seeds. The seeds were left for 5, 10, 15 and 20 minutes, after which they were removed and the acid drained off into another beaker. Seeds were then washed and rinsed thoroughly in tap water and distilled water, to remove all the acid before sowing. The hot water treated seeds followed the procedure used for the sulphuric seeds. The seed in the four beakers were soaked in boiling water for 5, 10, 15 and 20 minutes (Likoswe et al., 2008).

Potting Media and Seedling Emergence Studies

The experiment was conducted from September to November 2014 and laid out in a completely randomized design with five treatments (potting media) each replicated four times. The four potting media were; sandy soil, clay soil, garden soil, commercial compost and a mixture of four potting media in ratio 1:1, v/v. Four plastic pots (30 cm, inside diameter) each with 10 seeds were used per treatment (20 pots, 200 seeds). Based on the results shown in Table 2 seeds were not pre-treated. Pots were watered daily and weeds were carefully removed manually whenever they appeared throughout the duration of the study.

The characteristics of the different potting media are shown in Table 1. The pH of the potting media was measured from a soil-water suspension (1:1, v/v) by a pH meter (model: Hannah HI 110). The cation exchange capacity (CEC) was determined by the ammonium acetate saturation method (Schollenberger and Simon, 1945). The total nitrogen (N) content was determined by the Kjeldahl method (Bremner, 1965) and measured on a distillation unit (model: BuchiK-350). Available phosphorus (P) was determined by the Bray II method (Bray and Kurt, 1945) and measured on an inductively coupled plasma-optical emission spectrophotometer (model: Perkinelmer DV 2100). Exchangeable potassium (K^+) was measured by a flame photometer (model: Sherwood 410) after extraction with 1 N ammonium acetate pH 7.0

Table 1. *Physical and Chemical Properties of Potting Media*

Potting media	**pH (H_2O)**	**CEC (cmol kg^{-1})**	**Total N (%)**	**Available P (ppm)**	**Exchangeable K^+ (%)**	**Textural class**		
						Sand (%)	Clay (%)	Silt (%)
Sandy	6.20	0.37^{b}	0.07^{d}	1.60^{c}	0.20^{d}	91	2	7
Clay	5.30	1.17^{a}	0.36^{c}	8.00^{c}	1.52^{c}	39	40	21
Top garden soil	6.30	1.61^{a}	0.87^{b}	7.00^{c}	1.78^{bc}	87	7	6
Commercial compost	5.00	-	0.74^{b}	98.00^{a}	2.98^{a}	-	-	-
Mixture	6.30	1.50^{a}	1.25^{a}	23.00^{b}	2.07^{b}	91.5	4.5	4
Sig.	ns	*	**	**	**	-	-	-
LSD 0.05	ns	0.78	0.26	7.33	0.41	-	-	-
CV (%)	14.86	36.53	21.51	14.65	13.10	-	-	-

** Highly significant at $p<0.01$, * significant at $p<0.05$, ^{ns} non-significant at $p>0.05$. Means separated by Least Significance Difference (LSD) Test at $p\leq 0.05$, means within columns followed by the same letters are not significantly different, ppm denotes parts per million.

Germination and Seedling Emergence Parameters

The following germination parameters were determined daily, but the results given here are cumulatively presented in 3 days intervals;

GP = (germinated seeds/total tested seeds) × 100 % (1)

The germination mean time was determined as described by Scott *et al.* (1984) as;

(GMT days): = ΣTi Ni/S (2)

Where Ti is the number of days from the beginning of the experiment, Ni the number of seeds germinated per day and S is the total number of seeds germinated.

The germination index (GRI) was calculated for each treatment using the following equation;

GRI = (G1/1) + (G2/2) +......+ (Gx/x), (3)

Where G is the germination day 1, 2…, and x represents the corresponding day of germination (Esechie, 1994).

Seedling emergence was determined daily, but the results given here are cumulatively presented in 2 days intervals;

SE = (emerged seedlings/total seed sown) × 100 % (4)

Data Analysis

The data collected was subjected to an analysis of variance (ANOVA) using the STATISTIX-8 program. Treatments means were separated using the Least Significant Difference (LSD) and Tukey's Studentized Range (HSD) Tests at $p\leq 0.05$.

Results and Discussion

Seed Germination Study

Studies conducted on other species have shown that pre-sowing treatments enhances seed germination (MacDonald et al., 2002; McDonald and Omoruyi, 2003; Aliero, 2004; Hossain et al., 2005, Likoswe et al., 2008; Okunlola et al., 2011, Anand et al., 2012; Botsheleng et al., 2014; Missanjo et al., 2014; Rasebeka et al., 2014). Our results (Table 2) presented in 3 day intervals show that the mean germination percentage of the mechanical scarified seeds was significantly higher ($p<0.01$) than other treatments 9 days after sowing, but statistically at par with seeds immersed in hot water for 5 minutes. Mechanical scarification breaks the physical dormancy of the seeds with hard coats that inhibit water uptake and gas exchange (Doran et al., 1983; Hossain et al., 2005; Azad et al., 2010, 2011; Mwase and Mvula, 2011). Once the hard seed coat is broken, water and air are able to enter the seed and stimulate germination (Likoswe et al., 2008) which could have occurred 9 days after sowing *X. zambesiaca* seeds. The results also show that 9 days after sowing the seeds immersed in hot water and sulphuric acid for 5 minutes had a mean germination percentage significantly higher ($p<0.01$) than the control. This is in agreement with results of others who reported that immersing seeds in hot water (Teketay, 1996; Rasebeka et al., 2014) and sulphuric acid (MacDonald and Omoruyi, 2003; Aliero, 2004; Likoswe et al, 2008; Rasebeka et al., 2014) enhances their germination. Table 2 shows that between 12 and 15 days after sowing the mean germination percentages among the control, cold water, mechanical, hot water (5 min) and sulphuric acid (5 min) treated seeds were statistically at par. However, seeds treated in sulphuric acid for 5 min and the control had a slightly higher mean germination percentage. During the same period seeds treated in hot water (15 and 20 min), and sulphuric acid (10, 15 and 20 min) had significantly lower ($p<0.01$) mean germination percentages.

There was no significant difference observed in the mean germination percentages among the control, cold water, mechanical and hot water (5 min) treated seeds 18−21days after sowing. However, the highest mean germination percentage (80%) was recorded in the control seeds. The overall, comparison among the various treatments means showed that the control increased germination over all other treatments, indicating that *X. zambesiaca* seeds are not hindered by dormancy. The seed coat of *X. zambesiaca* is water permeable and does not require a pre-sowing treatment. Lower mean germination percentages observed in the hot water and sulphuric acid treated seeds probably show that the embryo was damaged as reported in other studies (MacDonald et al., 2002; Likoswe et al., 2008; Botsheleng et al., 2014).

No significant differences were observed in GMT among seeds in the control cold water, mechanical scarification, hot water (5min) and sulphuric acid (5 min). However the, the control had GMT of 16 days which was slightly higher. The highest GRI was recorded in the control, cold water, mechanical scarification and hot water (Table 2).

Table 2. *Effect of Different Seed Pre-treatments on X. zambesiaca Seed Germination*

Treatments	**Mean germination percentages**					**GMT**	**GRI**
	9 days	12 days	15 days	18 days	21 days		
Control	2.50^{d}	40.00^{ab}	65.00^{a}	80.00^{a}	80.00^{a}	16.80^{a}	0.66^{ab}
Cold water	2.50^{d}	32.50^{abc}	52.50^{ab}	62.00^{ab}	65.00^{ab}	13.13^{abc}	0.51^{abc}
Mechanical	37.50^{a}	45.00^{ab}	60.00^{a}	65.00^{ab}	65.00^{ab}	13.73^{ab}	0.71^{a}
Hot water							
5 min	32.50^{ab}	37.00^{ab}	60.00^{a}	67.50^{ab}	67.50^{ab}	14.18^{ab}	0.64^{ab}
10 min	7.50^{d}	40.00^{ab}	45.00^{ab}	45.00^{bcd}	45.00^{bcd}	9.45^{bcd}	0.44^{bcde}
15 min	7.50^{d}	15.00^{cde}	17.50^{cd}	17.50^{ef}	17.50^{ef}	3.68^{def}	0.18^{efg}
20 min	2.50^{d}	2.50^{e}	2.50^{d}	2.50^{f}	2.50^{f}	0.53^{f}	0.03^{g}
Sulphuric acid (98%)							
5 min	22.50^{bc}	47.50^{a}	50.00^{ab}	52.50^{bc}	52.50^{bc}	11.03^{abc}	0.45^{bcd}
10 min	12.50^{cd}	27.50^{bcd}	35.00^{bc}	35.00^{cde}	35.00^{cde}	7.40^{cde}	0.35^{cdef}
15 min	10.00^{d}	17.50^{cde}	20.00^{cd}	20.00^{dee}	20.00^{def}	4.20^{def}	0.21^{defg}
20 min	12.50^{cd}	12.50^{de}	15.00^{cd}	15.00^{ef}	15.00^{ef}	3.15^{ef}	0.16^{fg}
Sig.	****	****	****	****	****	****	****
HSD (0.05)	11.64	18.64	20.92	26.11	26.87	5.90	0.26
CV (%)	34.96	26.46	22.32	25.44	26.04	27.34	26.58

**** Highly significant at $p<0.01$. Means separated using Tukey's studentized range (HSD) test at $p\leq0.05$, means within columns followed by the same letters are not significantly different. Where GMT is germination mean time and GRI is germination index.

Growth Media and Seedling Emergence Studies

The physical and chemical properties in Table 1 show that the potting media are slightly acidic (pH 5.0−6.3). Commercial compost had the highest phosphorus (98.0%) and exchangeable K^{+} (2.98) which probably contributed to the medium's outstanding performance in Table 3. The quality of nursery seedlings is greatly dependent on the media's environment where the plants are raised (Adediran, 2005). Results presented in Table 3 show a high significant difference ($p<0.01$) among all the potting media in the number of days to emergence (DTE). The least number of DTE of 25 days were observed in the commercial compost, followed by 27 days for sand, while the garden soil media had the highest number of DTE of 31. Overall, the seedling emergence was consistently higher ($p<0.01$) in the commercial compost than other media. The results on percentage seedling emergence show that 72.50% of the seeds sown in commercial compost emerged followed by sand (47.50%), mixture (37.50%) and garden soil (35.00%) which was statistically at par. Clay soil had significantly the lowest ($p<0.01$) number (25%) of seedlings emergence at the end of study. This was not surprising because clay soils have poor drainage and the soil tends to stay wet and soggy after irrigation. Due to poor drainage they have pore spaces filled with water and therefore little oxygen is available to seeds and seedling roots and this probably affected seedling emergence in the present study.

Studies have shown that compost and composted materials provide nutrients, increase cation exchange capacity, improve water holding capacity (Tomati et al., 1990; Argo and Biernbaum, 1997) and are well aerated (Argo and Biernbaum, 1997). These could have contributed to the outstanding performance observed in Table 3 for the commercial compost.

The sand was the second best after the commercial compost because it has loosely aggregated particles that allow free exchange of gases between the potting media and the seed embryo (Pahla et al., 2014). The amount of oxygen in the substrate affects the success of raising containerized plants in containers (Aendekerk, 1992). Oxygen is essential for respiratory purposes in germinating seeds.

Table 3. *Effect of Potting Media on Seedling Emergence of X. Zambesiaca Seeds*

Treatment	Mean seedling emergence (%) (DAS)							
	Day 25	Day 27	Day 29	Day 31	Day 33	Day 35	Day 37	Day 39
Sand	0.00^b	2.50^b	7.50^b	12.50^b	12.50^b	27.50^b	35.00^b	47.50^b
Clay	0.00^b	0.00^b	2.50^c	10.00^b	12.50^b	12.50^c	20.00^c	25.00^c
Garden soil	0.00^b	0.00^b	0.00^c	2.50^c	15.00^b	32.50^b	32.50^b	35.00^b
Commercial compost	32.50^a	50.00^a	57.50^a	65.00^a	67.50^a	67.50^a	72.50^a	72.50^a
Mixture	0.00^b	0.00^b	2.50^c	10.00^b	15.00^b	22.50^b	22.50^c	37.50^b
Sig.	****	****	****	****	****	****	****	****
HSD (0.05)	4.88	9.35	14.92	21.47	21.29	22.38	24.74	19.74
CV (%)	34.40	40.78	48.80	49.16	39.78	31.53	31.04	20.77

**** Highly significant at $p<0.01$. Means separated using Tukey's studentized range (HSD) test at $p\leq0.05$, means within columns followed by the same letters are not significantly different. Where Day are days after sowing (DAS).

Conclusions

Based on the results of the study, it could be concluded that *X. zambesiaca* seeds are water permeable and do not require pre-sowing treatment. *X. zambesiaca* is a slow growing tree whose seedlings took over 3 weeks to emerge. The commercial compost proved to be the best potting media for germinating *X. zambesiaca* followed by sandy soil. Although the highest seedling emergence was recorded in the commercial compost, sand soil is the most suitable germination media for resource limited tree growers in arid and semi-arid areas due to its availability, low cost, and suitability for both large and small tree seeds. Commercial compost can be used by farmers/or nursery owners in commercial production.

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