Journal of Agriculture and Environmental Sciences
June 2019, Vol. 8, No. 1, pp. 104-111
ISSN: 2334-2404 (Print), 2334-2412 (Online)

Published by American Research Institute for Policy Development
DOI: 10.15640/jaes.v8n1a10
URL: https://doi.org/10.15640/jaes.v8n1a10

Breaking seed dormancy in *Philenoptera violacea* (Klotzsch) Schrire using different pre-sowing treatment methods

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Abstract

Seeds of many woody plant species cannot germinate even if they are sown under optimal moisture, oxygen and soil conditions because of hard seed coat dormancy. A seed germination experiment to evaluate the suitability of various dormancy breaking methods in *Philenoptera violacea* seeds was conducted in the laboratory of the Department of Crop Science and Production, Botswana University of Agriculture and Natural Resources (BUAN) between November and December 2017. A completely randomized design (CRD) with 10 treatments, involving; control (untreated seeds), mechanical scarification, hot water (24 hours), boiling water (1, 3 and 5 minutes) and concentrated sulphuric acid (15, 30, 45 and 60 minutes) was used. Germination percentage, germination mean time (GMT) and germination index (GI) were significantly ($P < 0.01$) affected by seed dormancy breaking methods. The highest germination percentages were recorded in seeds immersed in concentrated sulphuric acid for 15 and 30 minutes (100%), followed by concentrated sulphuric acid 45 minutes and hot water for 24 hours (99%), mechanical scarification (98%) and concentrated sulphuric acid for 60 minutes (97%), which were significantly higher than the rest, boiling water being the least. Untreated seeds took the longest time (20.50 days) for 86% of the seeds to germinate whereas the shortest significant GMT (2.23-2.66 days) was recorded for maximum germinations (100%). Boiling water (1, 3 and 5 minutes) revealed significantly lower GI (0.00-0.02). Although, 86% of untreated seeds germinated, the present findings emphasize the importance of treating *P. violacea* seeds before sowing to promote quick and uniform germination.

Keywords: Germination percentage, germination mean time, germination index, *Philenoptera violacea,* seed dormancy, woody plant species

1. Introduction

Philenoptera violacea (Klotzsch) Schrire, commonly known as Apple leaf or Rain tree, is a small to medium-sized semi-deciduous tree that belongs to the Fabaceae family (subfamily Papilionoideae) (Palgrave, 2002). It has an open rounded crown and grows to a height of 10-18m (Venter and Venter, 1996; Palgrave, 2002). The species is found in Botswana, Democratic Republic of Congo, Tanzania, Zambia, Namibia, Zimbabwe, South Africa and Swaziland (Venter and Venter, 1996; Van Wyk and Malan, 1998). *Philenoptera violacea* occursin various types of woodlands at medium to low altitudes, frequently along rivers, and is a prominent tree in nutrient-poor savanna and alluvial soils near rivers(Palgrave, 2002). Itis a multipurpose tree species whose leaves are heavily browsed by livestock and game (Storrs, 1995; Venter and Venter, 1996; Palgrave, 2002). The tree is drought tolerant and farmers rely on it to produce leaves for livestock browsing during drought (Venter and Venter, 1996).

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The flowers falling from the tree are eaten by animals on the ground (Venter and Venter, 1996).Seeds are sometimes used as a famine food (Storrs, 1995). Roots have been used in traditional medicine to treat colds (Palgrave, 2002)and leprosy sores (Storrs, 1995). The wood is used for carvings, tool handles and dug-out canoes (Venter and Venter, 1996).

Sexual propagation via seeds is the most common method of raising a large number of tree seedlings (Kim et al., 2008). The method allows for genetic diversity and is relatively cheaper than asexual methods (Hartmann et al., 2011). However, seeds of many woody plant species cannot germinate even if sown under optimal moisture, oxygen and soil conditions (Olmez et al., 2008). This condition is known as dormancy and is caused by hard and impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick and fleshy seed cover (ISTA, 1993).P*hysicalexogenous dormancycaused by a hard and*impermeable seed coat or fruit enclosure, which prevents water imbibition and gaseous exchange (Linkies et al., 2009; Smýkal et al., 2014)*is common in the Fabaceae family*. Dormancy in seeds may be advantageous during seed handling because it prevents seeds from germinating during storage and other handling procedures, and induction of dormancy, for example, by drying and dark storage generally promotes storability (Schmidt, 2000). Dormancy is an adaptation that ensures seeds will germinate only when conditions are favourable for germination, seedling survival and establishment (Bachelard, 2007; Falemara et al., 2014). The physical resistance of seed coat in leguminous plants is caused by a densely packed layer of palisade cells impregnated with water-repellent compounds (Baskin and Baskin, 2004,2014; Smýkal et al., 2014). The hard seed coat only imbibes water and uptake air when the coat is disrupted particularly at the strophiole (lens) region, which is usually the weakest part of the seed coat (Moïse et al., 2005; Jaganathan et al., 2017).

For germination to take place, the seed coat should rupture to allow absorption of water and air by the seed (Mojeremane et al., 2017). Hard-coated seeds require some form of pre-treatment before sowing to break dormancy in order to obtain rapid and synchronous germination (Mojeremane et al., 2017). Seeds that have not been given pre-treatment to break dormancy may completely fail to germinate, germination may be slow or germination of individual seeds in a seed lot may take place over a long period (Schmidt, 2000).Several methods that includecold, hot and boiling water, concentrated sulphuric acid, stratification, mechanical scarification, dry heat and fire have been used successfully to break dormancy in many species with hard-coated seeds (Teketay, 1996, 1998; Uniyal et al., 2000; Aref et al., 2011; Botsheleng et al., 2014; Fredrick et al., 2017; Mojeremane et al., 2017, 2018). The methods are species specific and no single treatment has been reported to be effective across plant species (Uniyal et al., 2000; Amusa, 2011).Information on suitable methods for breaking dormancy in *P. violacea*seeds is lacking. *Philenoptera violacea* is an important multipurpose tree species and, therefore, it is important to find suitable methods for breaking seed dormancy for the species to be successfully incorporated in planting programmes.

The objective of this study was, therefore, to determine the suitability of various pre-treatment techniques on seeds germination of *Philenoptera violacea.*

2. Materials and Methods

2.1 Study site

The study was conducted in the laboratory at the Botswana University of Agriculture and Natural Resources (BUAN) from November to December 2017. BUAN is located at Sebele (23°34' S and 25°57' E, altitude of 994 m above sea level) 10 km from the centre of Gaborone, the Capital City of Botswana, along the A1 North-South Highway.

2.2 Seed collection and processing

Dry pods were harvested directly from different healthy, erect and mature mother trees at Mowana Village, North East District, Botswana,in July 2017. Mowana Village is located 34 km north east of the City of Francistown along the A1 North-South Highway. Pods were placed in paper bags and transported to the laboratory at BUAN for seed extraction. After extraction, seeds were screened to remove those showing some signs of insect damage and were,then, kept in tightly sealed bottles and stored in a cool dark place awaiting commencement of the study. Prior to the experiment, seeds were immersed in distilled water, and only those that sank and settled at the bottom were used for the experiment. Floating seeds, which represented unfilled and dead seeds, were discarded.

2.3 Seed characteristics

To assess the seed characteristics (length, width and breadth), five replications of 10 seeds were measured using a digital calliper (0-150 mm). Ten replications of 100 seeds were weighed using an electronic analytical balance (Model: PW 124) to determine the 1000 seeds weight.

2.4 Experimental design and treatments

The experiment was laid out in a completely randomized design (CRD) with 10 treatments. The seeds were subjected to 10 germination treatments described below.

2.5 Description of experimental treatments

2.5.1 Control (T1)

The control containeduntreated seeds.

2.5.2 Mechanical scarification (T2)

Seeds were scarified using a pair of scissors to remove approximately 1-2 mm of the seed coat at the distal end prior to sowing in petri dishes.

2.5.3 Hot water (T3)

For the hot water treatment, seeds were put into four separate coffee filter papers which were clipped tightly to prevent seeds from falling out and placed in a beaker. Boiling water (98.8°C) was poured in the beaker with seeds and left to cool down to room temperature for 24 hours.

2.5.4 Boiling water treatments (T4-T6)

In the boiling water treatments, seeds of each treatment were put into four separate coffee filter papers and immersed in a cooking pot with boiling water on a heat source for 1 (T4), 3 (T5)and 5(T6) minutes, respectively. After each boiling time, seeds were removed from the pot and immersed in a small bucket containing cold distilled water to cool them down for few minutes before sowing.

2.5.5 Concentrated sulphuric acid (98.8%) treatments (T7-T10)

In the concentrated sulphuric acid treatments, four replications of 25 seeds of each treatment were put into heat resistant non-corrosive glass beakers. The acid was added slowly to a level covering all the seeds. After each immersion time 15 (T7), 30 (T8), 45 (T9) and 60(T10) minutes, seeds were sieved out using an acid resistant sieve and the acid drained off simultaneously into the beaker. Seeds were thoroughly washed in running tap and distilled water to remove all the acid to avoid further scarification of seeds and for safe handing.

Each treatment had 100 seeds replicated four times with 25 seeds in each replication. Seeds were germinated in petri dishes lined with cotton wool, kept continuously moist by spraying with distilled water. Ambient air in the laboratory varied from 25-30°C.

2.6 Seed germination counts

Germination was defined as the emergence of the radicle and was recorded daily for a period of 30 days. Counted germinated seeds were discarded after recording. Seeds that had not germinated at the end of the experiment were tested for viability using a cutting test.

2.7 Statistical analyses

Data collected on the germinating seeds were used to calculate:

(i) Germination percentage (GP) for each treatment using the equation: GP (%) = (germinated seeds/total seeds tested) ×100;

(ii) Germination mean time (GMT) using the equation: GMT (days) = $\sum T_i N_i/S$(Scott et al., 1984), where, T_i = number of days from the beginning of the experiment; N_i= number of seeds that germinated per day and S = Total number of seeds that germinated; and

(iii) Germination index (GI) using the equation: GI = $(G_1/1) + (G_2/2) + \ldots + (G_X/x)$(Esechie, 1994), where, G = germination day 1, 2…, and x = the corresponding day of germination.

Data were analysed using both descriptive statistics and One-Way ANOVA using Statistix 10 Analytical Software (2013) after they were first arcsine transformed to meet the normality assumption. Tukey's Honestly Significantly Difference (HSD) Test was used to separate means with statistically significant differences.

3. Results

3.1 Mean seed size and weight

The mean seed length, width, breadth and 1000 seeds weight were 15.26 ± 0.19 mm, 8.08 ± 0.27 mm, 3.63 ± 0.07 mm and 30.84 ± 0.2 mm.

3.2 Germination

The results show that germination percentage (GP) was significantly (P <0.01) affected by different seed dormancy breaking methods (Table 1). The highest GP was recorded from seeds treated in concentrated sulphuric acid for15 and 30 minutes(100%), followed by hot water and concentrated sulphuric acid for45 minutes (99%), mechanical scarification (98%) and concentrated sulphuric acid for60 minutes (97%). No statistical differences were observed among these treatments. However, germination percentages of the above treatments were significantly higher than the 86% recorded from the control and germination percentages in the boiling water treatments (1, 3 and 5 minutes),which ranged from 0-2% (Table 1).

Table 1.Effect of different pre-sowing treatment methods on the germination of seeds of *P. violacea.*

Treatment No.	Method of breaking dormancy	Germination (%)	GMT (days)	GI
T1	Untreated seeds (control)	86.00^{b}	20.50^{a}	0.72^{b}
T2	Mechanical scarification	98.00^{a}	10.23^{b}	0.82^{a}
T3	Hot water for 24 hours	99.00^{a}	7.38^{bc}	0.82^{a}
T4	Boiling water for 1 minute	2.00^{c}	7.50^{bc}	0.02^{c}
T5	Boiling water for 3 minutes	0.00^{c}	0.00^{c}	0.00^{c}
T6	Boiling water for 5 minutes	1.00^{c}	2.50^{bc}	0.01^{c}
T7	Sulphuric acid (98%) 15 minutes	100.00^{a}	2.66^{bc}	0.83^{a}
T8	Sulphuric acid (98%) 30 minutes	100.00^{a}	2.23^{bc}	0.83^{a}
T9	Sulphuric acid (98%) 45 minutes	99.00^{a}	3.20^{bc}	0.82^{a}
T10	Sulphuric acid (98%) 60 minutes	97.00^{a}	3.41^{bc}	0.81^{a}
Significance		**	**	**
HSD (0.05)		5.57	8.72	0.04

*** Highly significant at P <0.01. Means separated using Tukey's HSD Test at P ≤0.05; means within columns followed by the same letters are not significantly different.*

3.3 Germination mean time and germination index

Germination mean time (GMT)*was significantly (P <0.01) affected by different seed dormancy breaking methods (Table 1). Seeds immersed in sulphuric acid for 15 and 30 minutes gave the shortest GMT of 2.23 and 2.66 days, which was the fastest germination and had the highest germination rate of 100%. However, the GMT for the above treatments was not significantly different when compared with hot water, mechanical scarification and sulphuric acid at 45and 60minutes. Untreated seeds (control)*had significantly (P <0.01) higher (20.50 days) GMTthan seeds treated withboiling water for1, 3 and 5 minutes(0.00-2.00 days) GMT (Table 1).The pre-sowing seed treatment methods significantly (P <0.01) affected seed germination index (GI).*The highestGI* were recorded in seeds treated with sulphuric acid for 15 and 30 minutes,but these were not statistical different when compared with those from seeds treated with mechanical scarification, hot water, sulphuric acid (45 and 60 minutes).As expected,seeds treated with boiling water for 1 and 5 minutes recorded the lowest GI (Table 1).

4. Discussion

Most arid and semi-arid tree species cannot germinate promptly when subjected to conditions favourable for germination due to hard seed coat impermeable to water (Botsheleng et al., 2014).The hard seed coat is caused by one or more water-impermeable layers of palisades (Baskin and Baskin, 2004),which ensures that seed germination takes place only when environmental conditions are favourable for the growth and survival of young seedlings (Bachelard, 2007; Luna et al., 2009; Falemara et al., 2014). Hard coated seeds require some physical or chemical treatment to break dormancy in order to obtain maximum and rapid germinationthat is uniform (Aref et al., 2011; Botsheleng et al., 2014).

Numerous studies have demonstrated that pre-treating seeds to break the hard seed coat significantly enhances germination in various tree species (Teketay, 1996, 1998; Hossain et al., 2005; Chaodumrikul et al., 2016; Mojeremane et al., 2017, 2018).

In the present study, the highest germination percentage was observed in seeds treated in sulphuric acid for *15, 30, 45 and 60 minutes, hot water treatment and mechanical scarification.* These treatments are likely to have quickly softened or disrupted the impermeable cover and allowed imbibition by the embryo. Maximum germination recorded in these treatments could probably be attributed to the uptake of water and gaseous exchangedue to softening or cracking of the seed coat (Teketay, 2005; Azad et al., 2011) which triggered the germination process (Teketay, 2005; Rasebeka et al., 2014). Mechanical scarification has been reported to break physical dormancy rendered by hard seed coats by enhancing gases and water uptake especially in leguminous species (Missanjo et al., 2014; Mojeremane et al., 2017, 2018).Earlier germination in nicked *P. violacea* seeds in the present study could be due to cracks made on the seed creating entry of water and exchange of gases, resulting in enzymatic hydrolysis and, thus, transforming the embryo into a seedling. This is in agreement with others who demonstrated that nicking part of the hard seed coat, which prevents imbibition and gaseous exchange (Teketay, 2005; Azad et al.,2011) triggered the germination process (Teketay, 2005; Olatunji et al*.,* 2013).Mojeremane et al. (2018) nicked the seed coat of *Peltophorum africanum* Sond. andobserved 85 and 88% germination compared with 30% in the untreated seeds. Nicking has been used effectively to break dormancy in many species by partially removing the seed coat on the side of the hilum, opposite the radicle to speed up germination (Kaliniewicz and Tylek, 2018). It is performed carefully on the side away from the micropyle to avoid damaging the embryo (Mwase and Mvula, 2011). Nicking is a time-consuming exercise and may not be a suitable treatment for propagating large numbers of nursery seedlings (Baskin and Baskin, 2014).

Concentrated sulphuric acid(H_2SO_4) treatments enhanced germination of *Philenoptera violacea* seeds and also exhibited lowest GMT time and highest GI. It took *2.23-3.41 days forP. violacea* seeds*treated in concentrated $H_2SO_4$to reach maximum germination.* This is in agreement with others who usedH_2SO_4 treatments to overcome dormancy in seeds of many tree species*(Teketay, 1996;* Agbogidi et al., 2007; Likoswe et al.,2008; Amusa, 2011;Aref et al.,2011; Li et al., 2013; Fredrick et al., 2017; Mojeremane et al., 2017, 2018). For example, Amusa (2011) reported that *concentrated H_2SO_4 disrupts and exposes the* lumens of the macrosclereids cells *by disrupting the hard seed coat, thereby, allowing uptake of water to induce the germination process.*Agbogidi et al. (2007) found that H_2SO_4 reduced the germination period of *Dacryodes edulis* [G. Don] Lam. HJ., considerably and concluded that it was the best method, though dangerous to handle.

Soaking seeds in boiled water for 24hours enhanced germination of *P. violacea* seeds (Table 1), which is consistent with results reported for other tree species (Tigabu and Oden, 2001; Teketay, 2005; Tadros et al., 2011; Mojeremane et al., 2018). Mojeremane et al. (2018) reported 83 and 85% germinationin *P. africanum* seed scarified in hot water. Contrary to present findings, the ineffectiveness of hot water in enhancing germination has been reported for some plant species (Teketay, 1996; 1998; Botsheleng et al., 2014; Mojeremane et al., 2017), suggesting that the effects of these treatmentsvary across species (Tigabu and Oden, 2001; Teketay, 2005).The untreated seeds recorded a maximum germination of 86%in 20.50 days after sowing. This could suggest that *P. violacea*seeds are not hindered by seed coatdormancy and some treatments may only be required to speed rapid and uniform germination.

Boiling water treatments (T4-T6)recorded the lowest germination. It is possible that*P. violacea* seeds are not characterized by very hard seed coat and, therefore, boiling them might have destroyed the seed embryo. Inspection conducted at the end of the study showed that all seeds were rotten suggesting that the treatments are not suitable for this species. Kahaka (2017) also reported low germination in *Dicrostachys cinerea* and *Senegalia erubescens* seeds treated in boiling water for 1, 3 and 5 minutes.

5. Conclusions

It took 20.50 days to attain maximum germination percentage of 86 in untreated seeds. Therefore, these findings emphasize the importance of treating *P. violacea* seeds before sowing in pots/seedbeds topromote quick and uniform germination. C*oncentrated H_2SO_4proved to be an excellent seed coat softener in enhancing uniform germination. However, this method cannot be used by farmers because of the difficulty of obtaining the acid, possibility of accidents during handling and the proper disposal of the generated wastes. Mechanical scarification was also effective in promoting uniform germination, but* it is a laborious process requiring individual handling of each seed by farmers. Mechanical scarification is suitable for breaking dormancy in small quantities of seeds.

Therefore, soaking *seeds in boiled* water and leaving them to cool for 24 hours to room temperature is recommended as it is a practical, safer, simple and low-cost method that farmers can use to promote rapid and uniform germination in *P. violacea.*

6. Acknowledgement

The authors are grateful to the Department of Tertiary Education Financing, Ministry of Tertiary Education, Research Science and Technology for providing research grant to Othatile Thato Setlhabetsi and Botswana University of Agriculture and Natural Resources for providing research facilities.

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