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SEED BIOLOGY OF ERYTHROPHLEUM SUAVEOLENS (GUILL. AND PERR.) BRENAN: A THREATENED MEDICINAL PLANT

Research

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ABSTRACT
Background: *Erythrophleum suaveolens* (Guill. & Perr.) Brenan is a highly threatened medicinal plant. The plant population is at a blink of extinction due to high exploitation for medicinal uses along with seed dormancy. Therefore, there is an urgent need to conserve this medicinal plant both in situ and ex situ. This study was conducted to understand the seed biology of this important species so as to work the conservation strategy.

Methods: An experiment was conducted at a green house. Seeds were subjected to ten presowing seed treatments methods, namely, immersion in hot water (100°C) for 12 hours, 24 hours, and 36 hours, immersion in cold water at room temperature for 24 hours, immersion in concentrated sulfuric acid (0.3M H_2SO_4) for 1 minute, 5 minutes and 10 minutes, scarification by mechanically nicking using scalpel, scarification by mechanically nicking using scalpel followed by immersion in cold water at room temperature for 24 hours, and a control where seeds were sown without any treatment.

Results: The results showed that seeds of *E. suaveolens* exhibits long dormancy and demands pre-treatment. Scarified seeds through nicking attained a germination of 93%, a high germination rate of 9.8 seeds per day and a high germination speed index (GSI) of 11.4.

Conclusion: It is recommended to use scarification by nicking as a pretreatment method on *E. suaveolens* seeds in order to enhance germination percentage, rate and speed for conservation of the species.

KEY WORDS:*Erythrophleum suaveolens*, Dormancy, Medicinal plant; Seed biology; Threatened plant

INTRODUCTION
Knowledge on seed biology is one of the key factor in the achievement of plant conservation. Thus, understanding the process of seed growth and its regulation is crucial in the efforts to increase production [1]. However, seed dormancy which result into low germination rate imposes challenges on the increase and survival of plant species [2]. Germination of seed is influenced by intrinsic and extrinsic factors and are species specific [3, 4].

Therefore, research on seed germination for a particular tree species is necessary.

Erythrophleum suaveolens (Guill. & Perr.) Brenan is an important medicinal plant belonging to the family of Fabaceae. It grows up to the average height of 30m, with a spreading branching, forming a rounded crown. It produces fruits of an oval flat shape in wood pod. Its bark is rough and blackish in colour. The leaves are green in colour and have an ovate shape. The flowers have fluffy spikes and are cream yellow coloured [5, 6]. The species occurs in moist semi-deciduous forest and wooded grassland. It can be found in west and south tropical Africa. It is also found in tropical Asia where it has been introduced as an ornamental plant [5].

The tree species commonly known as "Red-water Tree" is well distributed in the religions of Malawi and it has been prioritized as one of the species for conservation in Malawi to enhance its contribution to the livelihood of communities. The tree species contains erthrophlein alkaloid elements that are used as traditional medicine to treat different diseases. For example, the crashed bark is used to treat swellings caused by filariasis [5]; the dried powdered bark is taken as snuff to cure headache; diluted decoction of the roots is used as an anthelminthic, especially against tape worms; decoction of the roots and bark is applied to sooth general body pains [7]; while the powdered bark is mixed with palm oil and after boiling it, it is mixed with the seed of maize, cow pea or cotton which effectively reduces pest damage to the seed [8].

Due to its herbal medicinal uses, *E. suaveolens* is a threatened species at risk of extinction. Furthermore, forest clearing for agriculture or developmental activities and illegal felling of trees for charcoal production highly pose a threat of extinction of the species. Seed dormancy of the species also contributes to its low population [5]. Therefore, there is an urgent need to conserve this medicinal plant both in situ and ex situ. This study was conducted to understand the seed biology of *E. suaveolens* so as to work out the conservation strategy.

MATERIALS AND METHODS

Seed Acquisition and Study Site

Erythrophleum suaveolens seed from Mwabvi Wildlife Reserve, Nsanje (16°42'S, 35°04'E, and about 150m above sea level), was supplied by Forestry Research Institute of Malawi (FRIM) in March 2014. The study was conducted in Malawi at Mzuzu University green house. Mzuzu University is located at latitude $11^0$28'S, longitude $34^0$01'E and altitude 1270 m above sea level. It is in silvicultural zone M [9]. The mean annual temperature ranges from 13.5^0C to 24^0C, with mean annual rainfall of 1150 mm. It is situated about 360 km north of Lilongwe the capital.

Experimental Design and Treatments

There were ten treatments in the experiment:

T1: Immersion of seeds in hot water (100^0C) for 12 hours
T2: Immersion of seeds in hot water (100^0C) for 24 hours
T3: Immersion of seeds in hot water (100^0C) for 36 hours
T4: Immersion of seeds in cold water at room temperature for 24 hours
T5: Immersion of seeds in concentrated sulphuric acid (0.3 M H_2SO_4) for 1 minute
T6: Immersion of seeds in concentrated sulphuric acid (0.3 M H_2SO_4) for 5 minutes
T7: Immersion of seeds in concentrated sulphuric acid (0.3 M H_2SO_4) for 10 minutes
T8: Scarification by mechanically nicking using scalpel
T9: Scarification by mechanically nicking using scalpel followed by immersion in cold water at room temperature for 24 hours
T10: Control (Seeds that were left intact)

The experiment was laid out in a completely randomized design. Each treatment had a total of 100 seeds of equal size, weight and length, which were replicated four times with 25 seeds per replicate. Therefore, a total of 1000 seeds were used in the whole experiment.

Seeds were sown on 2nd April 2014 in 10 cm by 6 cm black polythene tubes filled with soil collected from natural woodland (*Brachystegia* stand). Soil from natural woodland was used because it is free from *E. suaveolens* seeds. One seed was sown per tube at a depth of 2 cm, as recommended by Abideen et al. [10]. Watering was done twice a day (morning and evening) as per requirement to maintain adequate moisture necessary for germination. Any seed that germinate more than 30 days is said to have dormancy [11], as such the experiment was set for a period of 31 days.

DATA COLLECTION AND ANALYSIS

Germination was recorded daily for a period of 31 days from the day of sowing. The emergence of radical was used as the marker for seed germination. Daily germination was summed up to obtained cumulative germination for each treatment. Germination percentage was calculated by dividing the total number of seeds that germinated in each replicate by the number of seeds sown in that particular replicate multiplied by 100 [12]. Germi-

nation percentages were transformed into arcsine values in order to normalize the data [13]. The transformed germination percentages were then subjected to one-way Analysis of Variance (ANOVA) using GenStat 17 [14] at 5% significance level. Germination rate (GR) and germination speed index (GSI) were calculated using the following formula's respectively [15]:

$$GR = \sum_{i=1}^{n} n_i / t_i$$

$$GSI = \sum_{i=1}^{n} n_i t_i / N$$

where n_i is the number of germinated seeds in day t_i; N is the total number of seeds sown.

RESULTS AND DISCUSSION

Germination Percentage and Rate

Summary of the results on germination percentage and germination rate for different treatments are presented in Table 1. The results show that there were significant (P<0.05) differences on seed germination among the pretreatments with T8 and T9 attaining the highest germination percent of 93% and 92%, respectively. The rest of the pretreatment methods were ineffective with germination percentage ranging from 0-38%. Similar trend was also observed for germinate rate.

High germination in treatments T8 and T9 may be attributed to the fact that each seed was given individual treatment according to the thickness of its seed coat. Thus, these treatments created a weak site on each seed by breaking part of the seed coat which enhanced quick absorption of moisture and air resulting in fast germination [16]. The present results are in agreement to those in literature [17-19]. Schmidt [17] reported that mechanical scarification followed by soaking in cold water for 24 hours yields tangible results. In support to these findings, Kobmoo and Hellum [18] found that germination after mechanical scarification treatment of *Cassia siamea* was 99.75%. Missanjo et al. [19] also reported that mechanical scarification by nicking of *Albizia lebbeck* gave the highest germination of 80.3%.

Hanson and Johnson [20] reported a high germination percentage for hot water treatment. It is suggested that hot water treatment softens the cuticle and sometimes part of the palisade layers of the seed coat which effectively break dormancy. In contrast, seeds which were treated with hot water in this study gave a poor germination percentage of 20% maximum. Low germination in this study could mean that the hot water pretreatment failed to effectively break the seed coat dormancy as it failed to soften the cuticle layer of seed which would make the seed coat to be permeable thereby enhancing water imbibition's and exchange of gases (oxygen and carbon dioxide).

The idea of soaking seeds before sowing is aimed at shortening the lag phase in germination so as to enhance seedling establishment [17]. Fenner and Thomson [21] reported that *Cassia nigrescens* seed soaked in cold water for 12 to 24 hours attained 60% in germination. However, results attained by seeds soaked in cold water for 24 hours (T4) in this study, registered poor germination percentage (10%). Failure of T4 to attain high germination percentage could be that the *E. suaveolens* seeds were not given an optimal soaking period to overcome the seed coat dormancy.

Seeds that were treated with concentrated sulphuric acid for 10 minutes had the maximum germination of 38% (T7). Although this was not very efficient outcome, the ability of the seed to germinate in T7 mirrors Nikoleave [22] who explained that sulphuric acid is thought to disrupt the seed coat and expose the lumens of the macrosclereids cells, thereby, permitting imbibition's of water, which triggers germination. However, Kobmoo and Hellum [18] explained that there are variations in which seed respond to different soaking period in sulphuric acid for a particular concentration. This could be applied to the current study where poor results were obtained in seeds which were soaked in sulphuric acid for 1 minute (T5) and 5 minutes (T6) where no single seed germinated. This could be attributed to short period of soaking which did not soften the seed coat. The present results are supported by Schmidt [17] who explained that insufficient soaking of seed in sulphuric acid may not be effective enough in breaking the seed dormancy as it just makes the seed coat glossy without disrupting the seed coat.

Control treatment also performed poorly since no seed germinated. This could mean that *E. suaveolens* has a very thick seed coat in which germination cannot easily take place under normal circumstances. Yi et al. [23] reported that, under natural conditions, germination of *Erythrophleum fordii* seeds is very difficult. As such dormancy breaking should be done in raising *E. suaveolens* species to enhance germination.

Basing on germination percentages and the germination rate attained in this study, T8 and T9 are considered to be the most effective and practical methods of achieving high germination percentage of *E. suaveolens.*

Treatment	Germination (%)±s.e	Germination Rate (Seeds per day)±s.e
T1	0 ± 0^{d}	0 ± 0^{c}
T2	$20\pm2^{b,c}$	$1.1\pm0.2^{b,c}$
T3	$10\pm1^{c,d}$	0.8 ± 0.1^{c}
T4	$10\pm1^{c,d}$	0.8 ± 0.1^{c}
T5	0 ± 0^{d}	0 ± 0^{c}
T6	0 ± 0^{d}	0 ± 0^{c}
T7	38 ± 3^{b}	2.5 ± 0.8^{b}
T8	93 ± 2^{a}	9.8 ± 2.3^{a}
T9	92 ± 2^{a}	7.9 ± 1.9^{a}
T10	0 ± 0^{d}	0 ± 0^{b}

Note: Means followed by the same superscript within a column significantly differ ($P<0.05$); s.e.=standard error; T1=Immersion of seeds in hot water (100^{0}C) for 12 hours; T2=Immersion of seeds in hot water (100^{0}C) for 24 hours; T3=Immersion of seeds in hot water (100^{0}C) for 36 hours; T4= Immersion of seeds in cold water at room temperature for 24 hours; T5= Immersion of seeds in concentrated sulphuric acid for 1 minute; T6=Immersion of seeds in concentrated sulphuric acid for 5 minutes; T7=Immersion of seeds in concentrated sulphuric acid for 10 minutes; T8=Scarification of seeds by nicking using scalpel; T9=Scarification of seeds by nicking with scalpel followed by immersion of seeds in cold water at room temperature for 24 hours; T10=Control

Table 1: Effect of Pretreatment Methods on Germination of *E. suaveolens* seeds at 31 days after sowing

Germination Speed Index

Germination speed index (GSI) of *E. suaveolens* seeds from different pretreatments are presented in Figure 1. The results indicate that T8 showed superior GSI followed by T9. Seeds in treatments T8 and T9 germinated rapidly and vigorously. Within ten days, these treatments registered germination above 50%. Schmidt [17] argued that high germination speed index predicts seedling survival in the field. Therefore, treatments T8 and T9 could be recommended in nursery silviculture for production of high quality seedlings which will attain high establishment and high survival rate in the field.

Conclusion

Erythrophleum suaveolens is an economically essential medicinal plant and the species is at risk of extinction. The present results showed that seeds of *E. suaveolens* exhibits long dormancy and demands pre-treatment. Scarified seeds through nicking attained a high germination percentage, high germination rate and high germination speed index. Therefore, these findings from the seed biology study would help the commercial growers for propagation of the plant through its seed. The findings could also be of great use to pharmaceutical industries for the development of new antimicrobial drugs to address unmet therapeutic needs. Furthermore, cultivation of this plant in favourable growth areas should be encouraged to the locals so as to meet the needs for local medicinal usage.

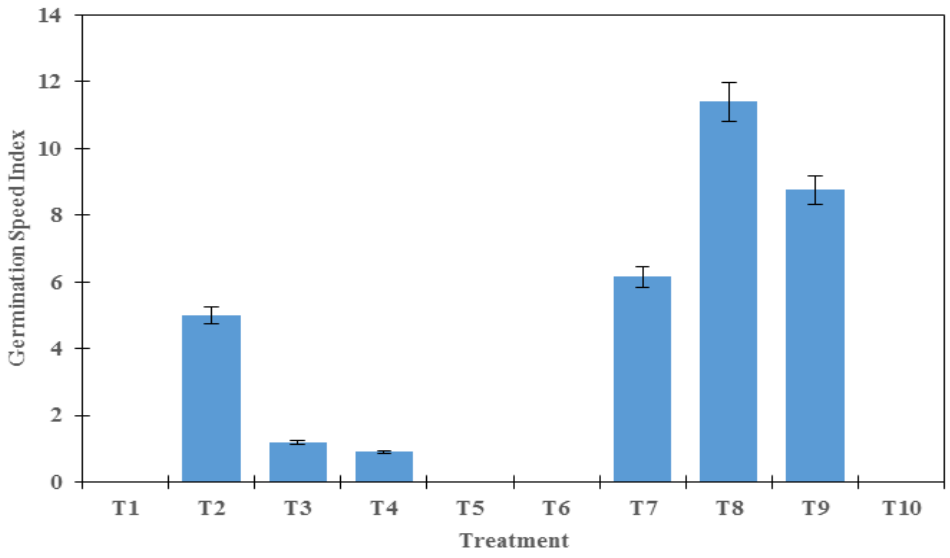

Figure 1: Germination speed index of *E. suaveolens* seeds for different pretreatments methods

T1=Immersion of seeds in hot water (100^0C) for 12 hours; T2=Immersion of seeds in hot water (100^0C) for 24 hours; T3=Immersion of seeds in hot water (100^0C) for 36 hours; T4= Immersion of seeds in cold water at room temperature for 24 hours; T5= Immersion of seeds in concentrated sulphuric acid for 1 minute; T6=Immersion of seeds in concentrated sulphuric acid for 5 minutes; T7=Immersion of seeds in concentrated sulphuric acid for 10 minutes; T8=Scarification of seeds by nicking using scalpel; T9=Scarification of seeds by nicking with scalpel followed by immersion of seeds in cold water at room temperature for 24 hours; T10=Control

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