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Germinating *Allanblackia floribunda* Seeds With Slightly Thickened Seedcoat Using Water

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Abstract: Seed dormancy is a challenge in the domestication of *Allanblackia floribunda* and this has been linked to the presence of abscisic acid (ABA) in the seeds. Recent studies have shown comparable improvement in germination when seeds with thin seedcoat (*i.e*., extracted from immature fruits) are scarified and soaked for 12 h in water or for 6 h in Fluridone (a chemical inhibitor of ABA biosynthesis). However, it is not known whether prolonged soak in water can stimulate germination in seeds with slightly thickened seedcoat. Also, the optimum soak duration in water is not known for both scarified and un-scarified seeds. Therefore, this study was conducted to determine the effect of a broad range of soak durations in water (0, 6, 12, 24 or 48 h) on the timing and percentage germination of scarified and un-scarified *Allanblackia floribunda* seeds obtained from immature fruits. The study was arranged as 2 x 5 factorial experiment and analyzed using Survival Data Analysis. The seeds extracted from immature fruits were observed to possess slightly hardened seedcoat and low in moisture content. Radicle and plumule emergence were not observed in un-scarified seeds. Among the scarified seeds, plumule emergence commenced in the control at 77 d after treatment while it was significantly ($p<0.05$) earlier (56 d) by soaking the seeds for 48 h in water. In all other treatments, plumule appearance commenced at 75-77 d. The highest hazard (plumule emergence) rate was recorded among seeds that were soaked in water for either 12 or 24 h. In these treatments, percentage germination was greater or equal to 50%. Although plumule emergence was earliest with 48 h soak, it reduced the hazard rate at later periods and so, less than 17% of the seeds germinated. The hazard rate in the control and 6 h soak treatments were similar to that observed in 48 h soak treatment. Thus, a combination of scarification and prolonged soak in water (12 or 24 h) is necessary to increase germination (earliness and rate) of *A.floribunda* seeds with slightly thickened seedcoat. This method is cheap and easy to accomplish for domestication of *Allanblackia floribunda*.

Keywords: Soak Duration, Percentage Germination, Scarified, Un-scarified, *Allanblackia floribunda*.

INTRODUCTION

Allanblackia floribunda, also known as tallow tree, belongs to the Clusiaceae family.other important members are: *Allanblackia struhlmannii*, *Allanblackia parviflora*, *Allanblackia ulugurensis*, *Allanblakia stanerana*, *Allanblackia gabonensis.* It is found abundantly in the rainforest of the Western and Central Africa [1]. This understory tree is important in traditional African medicine where it is used for the treatment of ailments including hypertension. The extracts from the bark of *Allanblackia floribunda* contains guittiferone F, an HIV inhibitor and prenylated xanthone, a natural product that acts against human epidermoid carcinoma of the nasophrynx cancer line [2]. It is also used for cooking and soap making [3]. Lately, the qualities of the seed have attracted researchers and commercial industries to the plant.

At seed and fruit maturity, the seed, which are about 40-50 per fruit, are about 2-5 cm long by 1.5-3.2 cm in diameter, embedded in the fruit's pulp and possess a hard seedcoat [4]. It contains fat that is solid at ambient temperature and the fatty acid composition of the fat is about 45-58% stearic acid and 40-51% oleic acid with only trace quantities of other fatty acids [2]. The high melting point (35 ^{o}C) of the *A. floribunda* seed fats makes Allanblackia fat more valuable than other fats since it can be used without transforming to the consistency of margarines, cocoa butter and other similar products. This quality and its composition of stearic and oleric acid has given the plant further recognition and importance in today's commerce. The Allanblackia seed oil has been approved by the European Union (EU) as safe for food production industry [5].

The demand for Allanblackia oil has placed high demand for the mature seed, making its demand higher than the supply from the natural forest and scattered stands in farms. The resultant wild harvesting of the seeds also threatens the existence of spp. due to over exploitation. It is for this reason that efforts at domesticating Allanblankia are considered as crucial for social, economic and environmental sustainability.

Domestication is however, faced with the challenge of seed dormancy, the physiological phenomenon that prevents viable seeds from commencing vegetative growth until a pre-programmed timing due to factors internal or external to the embryo. Naturally, only less than 10% germination over 24 months has been obtained by germinating un-scarified seeds obtained from mature fruits. Many efforts have been made to enhance germination. The World Agro Forestry Centre (ICRAF) has obtained up to 40% germination in 10 months by scarifying seeds from mature fruits and incubating them in black bags by [6]. Less than 50% germination was obtained in three months and 75% seed germination in 10 months by [7]. Recent work by [8] conducted in the Department of Crop and Soil Science, University of Port Harcourt obtained obtained remarkably high rate of (70-90%) germination, over 3-4 months by scarifying thin-coat seeds from immature fruits. Their work and that of [9] have also showed that thin-coat seeds from immature fruits express dormancy that could be linked with the presence of ABA. Although their work showed that treating scarified seeds in Fluridone for 6 h gave comparable results as treating seeds in Fluridone or no Fluridone solution (water) for 12 h, their study was not designed to identify the optimum soak duration in water for high percentage germination and earliness of germination. Also, past studies have not determined the effect of prolonged soak in water on germination of un-scarified Allanblackia seeds. Since many growth inhibiting chemicals including phenols and ABA are known to leach out of seedcoats and embryos in a suitable solvent [10], the objective of this study was to determine the effect of a wide range of soak durations in water (the universal solvent) on germination of scarified and un-scarified *Allanblackia floribunda* seeds with slightly thickened seedcoat obtained from immature fruits. It is hoped that the outcome of the study will lead to the establishment of cheap, less cumbersome and chemical free method(s) of enhancing the germination of Allanblackia seeds for rapid domestication.

MATERIALS AND METHODS

Experimental Location and Material

This experiment was carried out in the University of Port Harcourt, Nigeria. The fruits used for this experiment were gotten from Mgbu, Ndoki in Rivers State, Nigeria in the month of September. The fruits were allowed to soften during a two weeks period and then, the seeds were extracted from fruit pulps. fruits were randomly sampled for assessment of fruit and seed maturity according to method by Bewley and Black (1994). The method has been successfully used to assess Allanblackia fruits for maturity [8].

Seed Extraction and Scarification

The extracted seeds (180) were randomly allocated to one of two parts; 90 seeds were scarified while the other 90 seeds were not scarified and then tied up in a black polythene bag awaiting treatment application.

Weighing of Seeds and Sterilization

The seeds were individually weighed. Eighteen grooves were made on each of the plastic containers used in this study. The grooves were important in ensuring that each seed was kept in place and did not make surface contact. The containers were washed, and surface sterilized with 5% sodium hypochlorite while the seeds were sterilized using 0.5% sodium hypochlorite solution and quickly rinsed thrice with distilled water. All surfaces were also surface sterilized to limit contamination.

Only viable seeds, assessed using flotation test, were used in this study.

Treatment Application

The pH of the distilled water used in this study was standardized prior to soaking of seeds. Sterile scarified and un-scarified seeds were then soaked for 0, 6, 12, 24 or 48 h. Each soak duration was a treatment. Thus, there were five treatments. The eighteen treated seeds per treatment were placed in the grooves on the containers and then, the lids and containers were moistened. The containers were then carefully tied up in a black polythene bag.

Experimental Design

The experiment was a two-factor experiment: Factor one; was seedcoat conditioning at two levels (scarified and un-scarified) while Factor two; was soak duration at five levels (0, 6, 12, 24, 48). Thus, the experimental design was a 2 x 5 factorial arranged as Complete Randomized Design (CRD). Each treatment combination was replicated 18 times with each seed serving as a replicate.

Data Collection and Analysis

Data collected for this experiment included date of seed rot, date of germination; radical and plumule appearance. The seeds were observed weekly for the above. At the end of the experiment, data on early seedling development was also collected as additional data to explain treatment effect. Such data include: number of leaves, length of plumule and radical.

Data collected was used to calculate the duration from treatment to plumule and radical appearance. Data

was analyzed using Genstat release 10.3DE 17th Edition Copyright 2014, VSN international Ltd. The survival data analysis was used to analyze the effect of soak on plumule and radical using Kaplan Meier estimate. Means were compared using standard error of differences (SED). A

Survival data analysis is a set of methods for analyzing data where the outcome variable is the time until the occurrences of an event of study (example death, germination time, appearance of shoot, etc). This programme is most suitable on the analysis of duration from a defined time to the occurrences of a named event with a possibility that some individuals in the study may survive longer than the period of study [11]. Thus, for such individuals, the duration to the named event is unknown. Therefore, censored value will be given, the observation are called censored because the information about their survival time is not complete. Data for seeds that did not germinate were given a censored value of zero (0).

Plumule and radicle length data was analyzed using standard deviation and the leaf counts were transformed using the square root transformation. Analysis of data on number of leaves was analysed using the factorial analysis; general treatment structure (no blocking).

RESULTS AND DISCUSSION

Fruit Maturity Assessment

The assessment of the Allanblackia fruits for maturity showed that:

- *Colour:* the fruits were greenish brown in colour suggesting that the fruits were immature but close advancing towards maturity. Immature fruits are usually greenish in colour.
- *Hydration/Dehydration:* The fruits were slightly dehydrated and the pulp was slimy. This showed that the fruits were immature as at when they were used for this experiment.
- *Dehiscence and Abscission:* The immature fruits had no opening or splits and the pedicle was still attached to the fruit, this shows that the fruits were forcefully harvested and so immature.
- *Fruit Hardness:* The fruit was difficult to cut through this shows that the fruit was not fully mature.
- *Pulp Loosening:* The pulp was not loosed it was still very much attached to the fruit.

Seed Maturity Assessment

The seed assessment maturity carried out based on the following criteria showed that:

- *Hardness of Seedcoat:* the seedcoat was slightly hardened and relatively difficult to remove suggesting that the seeds were not fully mature (*i.e*, seeds did not possess thickened, hard seedcoat). The removal of the slightly thickened seedcoat was done mechanically.
- *Endosperm and Embryo Maturity:* Using the cutting test, it was observed that the endosperm was slimy and hard, indicating that the seeds were not mature but approaching maturity. The embryo was also present.
- *Germinability:* Flotation test revealed that the seeds were viable.

Thus, these assessments indicate that the fruits used in this study were immature while the seeds extracted from them were approaching an advanced stage of seed maturity; possessed mature embryo (*i.e*., had the inherent capacity to germinate (viable) and slightly hardened seedcoat.

Seed Moisture Content

Seed weight (fresh weight basis/seed) after extraction ranged from 7-9.3 g in un-scarified seeds while it ranged from 5-6.9 g in scarified. The seeds dry weight for un-scarified and scarified seeds was 6.16 g and 5.15 g respectively. Percentage moisture content of the seeds ranged from 7.2%-10.2% for un-scarified and scarified seeds respectively. Differences in seed weight were due to scarification of seeds.

The low moisture content of these seeds and the presence of slightly hardened seedcoat suggests that the seed were closer to being fully mature than being immature. Also, when compared to the moisture content of the seeds reported by [8]: 91-94%, it is apparent that the seeds used in this study had commenced the desiccation process which occurs during the last phase of seed development [12].

Effect of Treatments on Radicle Emergence

In this experiment, the un-scarified seeds did not initiate radicles. The first radicle appeared 49 d after a 48 hrs soak of scarified seeds in water (Fig 1). This was significantly earlier ($p>0.05$) than that in the control and all other treatments. Radicle emergence was most delayed in the control. In the control, the first radicle emergence occurred 98 d after treatment. A 24 and 12 h soak led to radicle emergence at 70 and 84 d after soaking respectively.

Although soaking seeds for 48 h gave the earliest radicle emergence, it caused significant decline in rate of sprouting after about 77 d. Soaking seeds for 6 h in water led to the highest hazard (radicle emergence), and this was closely followed by soaking of seeds in water for 12 h. By the end of the study (i.e., at 112 d) however, just over 50 % of the scarified seeds initiated radicles by soaking them in water for 6 h. The 12, 24 and 48 h soak treatments led to 33 and 27% radicle emergence by 112 d after treatment respectively.

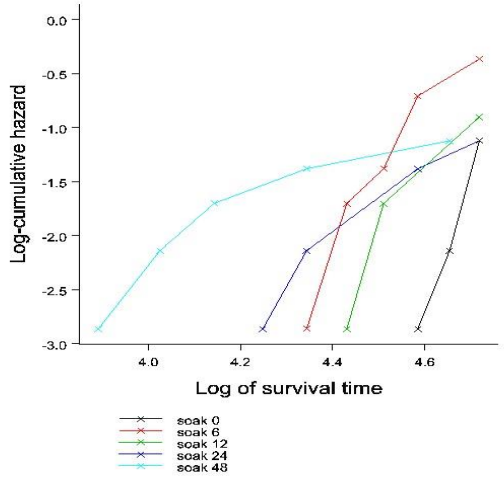

Figure 1. Effect of different soak durations in water on radicle emergence for scarified seeds. Logrank statistic 3.17 p>0.001

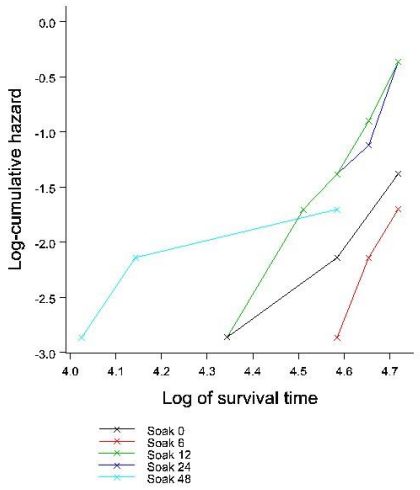

Figure 2. The Effect of Different soak Duration in Water on Plumule Emergence of Scarified Seeds. Logrank statistic 9.52 p>0.05

The Effect of Treatments on Plumule Emergence

Plumule emergence was not observed in un-scarified seeds. Among the scarified seeds, plumule emergence commenced in the control at 77 d after treatment while it was significantly ($p<0.05$) earlier (56 d) by soaking the seeds for 48 h in water. All other treatments, plumule appearance was first observed at 75-77 d and this did not differ significantly from the control.

The highest hazard (plumule emergence) rate was recorded among seeds that had been soaked in water for either 12 or 24 h (Fig. 2). In these treatments, percentage germination was greater or equal to 50%. Although plumule emergence was earliest with 48 h soak, it reduced the hazard rate at later periods and so, less 17% of the seeds germinated. The hazard rate in the control and 6 h soak treatments was similar to that observed in the 48 h soak treatment.

Effect of Treatment on Early Seeding Growth

Seedling growth was evaluated at the end of the study (112 days after seed treatment). The results showed that the highest number of leaves was obtained by soaking seeds in 48 h. Generally, however, plumule length, radicle length and number of leaves per plant increased with longer seed soak durations.

Table 1. Effect of Duration of Soak in water on the Number of Leaves, Plumule Length and Radical Length of Allanblackia Seedlings at 112 days

Duration (h)	Plumule length (cm)	Radicle length (cm)	Number of leaves (Square Root Transformation)
0	0.5 ± 0.3	1.3 ± 0.8	0.6
6	1.4 ± 0.9	4 ± 2.7	0.9
12	3.1 ±2.2	5.1 ± 3.9	1.3
24	0.7 ± 0.4	4.4 ± 3.1	1.3
48	5.2 ±3.5	7.2 ±5.0	5.4

This study has shown that the Allanblackia fruits, which were collected in the month of September in Rivers State, Nigeria, were immature but the seeds extracted from them were in the advanced stages of seed maturity since they possessed mature embryos (*i.e.*, were germinable or viable) and had low moisture content with slightly hardened seedcoat. The desiccation process that leads to the formation of hard seedcoat commences in the last stage of seed development. Also, seeds with such slightly thickened seedcoat can be induced to germinate earlier than what obtains under nature. Naturally, the duration to germination of mature Allanblackia seeds is very long (6 to 25 months) with only less than 10% germination is obtainable. This situation is a huge inherence to the domestication process of Allanblackia.

An important finding in this study is the observation that prolonged soaking of un-scarified seeds with slightly thickened seedcoat in water does not lead to germination. However, scarifying same set of seeds leads to both early (56-77 d) and high rate (up to 50%)

of germination. This inability of treatments to germinate un-scarified Allanblackia seeds is not new. Even un-scarified seeds with thin seedcoat and harvested in the month of August could not been induced to germinate [8, 9]. These confirm that dormancy in Allanblackia commences well before the formation of the hard seedcoat and so, other factors (physiological/chemical) may control dormancy in Allanblackia.

Also, this study has shown that the removal of the effect of even a slightly hardened seed coat is necessary for early (77 days after treatment) commencement of germination when compared to that of un-scarified seeds, which did not germinate even at the end of the study (*i.e.*, 112 days after treatment). Thus, scarification is the necessary first step whether seed coat is thin [8], slightly thickened (as in this study) or thickened/hardened [6, 7].

Although scarification of slightly thickened seeds of Allanblackia leads to earlier germination compared to what happens under nature, scarification alone leads to very low rate of germination (<17%). Increased germinated (50%) is however, encouraged by soaking the scarified seeds in the universal solvent, water, for 12 or 24 h. These durations are considered optimum in this case, since they caused early commencement of germination (75-77 d), with greater rate of germination and had positive impacts on early seedling growth compared to values obtained when the seeds where soaked for periods less or greater than 12-24 h. The optimum soak duration in water being proposed here agrees relatively with that (6-12 h) found to support early germination in Allanblackia seeds with thin seedcoat [9]. The percentage germination obtained at the proposed optimum soak duration in this study was however lower than that obtained by soaking thin seedcoat seeds in water for 6 h (*i.e.*,70-100% germination in less than 100 d). This difference may be due to differences in depth of dormancy with the thin seedcoat seeds being less dormant than the slightly thickened seeds [13]. Although it is not clear why 48 h soak induced the earliest start of germination but only 17% germination, soaking seeds in solvents such as water is thought to stimulate germination by absorbing the water soluble growth inhibiting chemicals (e.g., ABA) contained in the seeds.

CONCLUSIONS

This study utilised *Allanblackia* seeds that possessed slightly hard seedcoat; implying that they were closer to being fully mature than immature. This study has shown that the presence of even slightly hard seedcoat inhibits germination of viable *Allanblackia* seeds. Also, it is recommended that germination in *Allanblackia* be measured as duration to plumule emergence rather than timing of radicle emergence. Furthermore, seeds with slightly thicken seedcoat will not germination regardless of the soak duration in water. Thus, scarification is the necessary first step to inducing germination when seedcoat is present. Lastly, the study recommends the combination of scarification and soaking in water within the proposed optimum soak duration (12 or 24 h) for the induction of at least 50% (in 75-77 d) germination in *Allanblackia* seeds with slightly thickened seedcoat. For greater effects on germination (>70% germination in less than 3-4 months), it is recommended that *Allanblackia* be germinated from viable seeds that have thin seedcoat (not hard nor slightly hardened) and high in moisture content. The method described here provides a cheap and easy method of germinating large number of seeds, which is important in the domestication efforts this highly valuable plant.

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