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Germination Response of African Crabwood Seeds (*Carapa grandiflora* Sprague) as Influenced by Three Pre-Germination Treatments in the Bamenda Highlands, Cameroon

Walter Ndam Tacham[1], Christiana Ngyeti Nyikob Mbogue[1], Doreen Yetina Nkwain[1], Francoline Jong Nkemnkeng[1*], Jane Iyinji Anyi[1], Mendi Grace Anjah[2]

[1]Department of Plant Sciences, Faculty of Sciences, University of Bamenda, Bamenda, Cameroon
[2]Department of Forestry and Wildlife Technology, College of Technology, University of Bamenda, Bamenda, Cameroon
Email: *tebafranc24@yahoo.com

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Abstract

Carapa grandiflora is a multipurpose tree widely exploited for timber, traditional pharmacopoeia and handicraft for shoe making. These potentials have triggered over-exploitation of the species which might render the plant vulnerable in the nearest future. However, the germination techniques and performances are poorly understood by the local populations. Two pre-germination treatments were investigated (Mechanical and chemical scarification). Mechanical scarification consisted of nicking (T_1), manual removal of seed coat (T_2) while chemical scarification consisted of soaking the seeds in 50% concentrated sulphuric acid for 1 minute (T_3), 3 minutes (T_4) and 6 minutes (T_5). The seeds were sown in polythene bags and watering was on regular basis. Germination and early growth parameters were monitored daily. Data collected were entered into Microsoft excel and analyzed using STATGRAPHIC 2016. There was an overall germination percentage of 43.33%. Seeds soaked in 50% concentrated sulphuric acid above 1 minute failed to germinate. Nicked seeds had the best latent period followed by manual removal of seed coat while those soaked in 50% concentrated sulphuric acid for 1 minute had the least throughout the study. The germination percentage was significant ($p > 0.05$) with nicked seed having the highest (62.2 ± 2.54) followed by manual removal of seed coat (46.7 ± 2.12) while seeds soaked in 50% concentrated sulphuric acid for 1 minute were the least (30.0 ± 2.40). Early growth performance of seedlings was not significant ($p > 0.05$) though nicked seeds had better performance followed by manual removal seed coat while seeds soaked in 50% concentrated sulphuric acid were the least. Ultimately, this study showed that pre-treatments have an influence on the germination and early growth performance of *Carapa grandiflora*. Some of these pre-treatments could be adopted for the propagation, conservation and sustainable management of the species.

Subject Areas

Environmental Sciences

Keywords

Carapa grandiflora, Pre-Treatment, Germination Response, Bamenda Highlands

1. Introduction

Anthropogenic activities, current global trends in resource exploitation and climate change have caused induced pressure on plant communities. This human pressure on natural ecosystems over the last century has caused much biodiversity degradation. Unsustainable exploitation practices and lack of conservation initiatives have further exacerbated the situation. These have resulted in habitat loss, vulnerability of some species and accelerated species extinction, especially in the tropics [1] [2]. It has also made resources derived from these plants and services limited, thus a severe challenge to sustainable livelihood in the tropics. The numerous environmental hazards are a testament to these challenges, such as irregular patterns of rainfall, recurrent flood, prolonged drought and desertification, etc. However, the over-exploitation of forest resources has made some tree species to become endangered including *Carapa grandiflora*. In addition, there is inadequate knowledge of silvicultural practices for the propagation and conservation of *Carapa grandiflora* due to the recalcitrant nature of seeds.

Carapa spp is multipurpose tree of the rain-forest belt of West and Central Africa. It is an evergreen understorey tree up to 25 m; branches arise low down on the trunk which is often fluted. When cut, a gummy resin exudes, tasting bitter like quinine. Very large compound leaves at the ends of thick branchlets. Young leaves are bright red, then orange, slowly turning dark green. Fruits are a large round woody capsule 12 - 15 cm across, sometimes beaked. The fruit cracks open into 5 parts when it falls to the ground to set free 12 - 20 angular seeds, each 3 cm, shiny dark brown, pitted. The tree, suitable for timber, is planted in agroforestry systems [3]. Carapa oil is one of the most sold medicinal oils as it is used to repel mosquitoes. The oil is formed into a paste and applied topically to protect the body from mosquito bites [4]. Wood is mainly used for high-quality furniture and cabinet work, such as stairs, flooring and as veneer for furniture, interior work and plywood. It is also used as building materials. In Colombia, shoemakers prefer it for making shoe pieces [5].

Previous study by Omokhua *et al.* [6] showed that seeds of the genus investigated the genus Carapa (*C. procera*) performed better following mechanical scarification (using nail cutter) with a high germination percentage (94) and early growth performance. Missanjo *et al.* [7] also observed that nicking produced the highest performance for all growth parameters studied in seeds of *Lupinus varius and Acacia polyacantha* respectively. Diémé *et al.* [8] investigated the effects of soaking seeds of *Faidherbia albida* in different concentrations of sulphuric acid for 10 minutes; though found out that 100% and 75% concentrations were effective in alleviating seed dormancy in the species. In addition, Humtsoe *et al.* [9] found out that seeds of Golden shower tree (*Cassia fistula)* perform better when treated with concentrated sulphuric acid for 2 minutes. Seed pre-treatment is species specific and there is no one type of treatment has been reported to be universally effective. It is in this light that this research is oriented to investigate the effect of seed pre-treatments on the germination and early growth performance of *Carapa grandiflora*.

2. Materials and Methods

2.1. The Study Site

The Bamenda Highlands are located between latitude 5˚4' and 7˚15'N and longitude 9˚30' and 11˚15'E. This area has a high human population density of approximately 100 - 250 people per square kilometer [10]. The Region has two distinct seasons that is the rainy season which runs from April to October and the dry season between November to March. Average rainfall is about 2400 mm with daily temperature ranging between 15˚C - 32˚C and at 1207 m above sea level [11]. This area is known to support high levels of biological diversity and endemism.

2.2. Seed Collection

Mature fruits were collected at the forest in Kedjom-Keko, Bambui and Bambili localities in Tubah Subdivision, Mezam Division. Seeds were extracted from fruits at the nursery of the Department of Plant Sciences of the University of Bamenda, Cameroon and air-dried for two weeks under natural sunlight and preserved in polythene bags. Two hundred and fifty sizeable and healthy seeds were randomly selected from lots for the research work. A water floatation test was conducted on the seeds to authenticate the viability before pre-treatment [12]. In this approach, seeds with healthy embryo and cotyledon sank and were considered viable.

2.3. Seed Pre-Treatments

Effects of seed pre-treatments on germination and early growth of *Carapa grandiflora*

Method of seed pre-treatment was adopted from Humtsoe *et al.* [9]; Sobola [13] and Diémé *et al.* [8] with some modification. Seeds were subjected to two pre-germination treatments namely mechanical scarification and chemical scarification prior to sowing. This was done as follows:

2.4. Mechanical Scarification

Nicking: The seed coat was carefully perforated using a sharp knife (T_1). Four incisions were made in each seed. Ten seeds were subjected to this treatment with three replications thus a total of 30 seeds were involved in this experiment.

Manual removal of seed coat: The entire seed coat was carefully and completely removed (T_2). Ten seeds were subjected to this treatment with three replications, thus a total of 30 seeds were involved in this experiment.

2.5. Scarification in Sulphuric Acid

The acid was diluted in distilled water to half its concentration (50%). Seeds were soaked in acid at various durations. This consisted of soaking for 1 minute (T_3); 3 minutes (T_4) and 6 minutes (T_5). Each treatment consisted of 10 seeds and three replications thus a total of 90 seeds were used in this experiment. After pre-treatment seeds were thoroughly rinsed in tap water.

There was equally a control experiment (T_0) consisting of 10 seeds, replicated three times thus a total of 30 seeds were involved. In the control, seeds were not subjected to any pre-treatments.

A total of 180 seeds were used for the research work. Polythene bags were filled with forest topsoil and placed in a shed measuring 8 m × 8 m. Blocks, plots and subplots were placed 30 cm apart to enable free movement during watering and nursery care. Seeds were sown in polythene bags with respect to pre-treatment that is one seed per polythene bag. Sowing was at an estimated uniform depth of 3 cm. After sowing, watering was done daily while nursery care, once a month. Germination parameters were evaluated daily for a period of 3 months. Early growth parameters were also evaluated monthly for 5 months thus the experiment lasted for 8 months. The experimental design was complete block design.

2.6. Data Collection and Analysis

Data collected were latent period, germination percentage, number of leaves, collar diameter and shoot height.

Latent period (L): This is the number of days taken for the first seed to germinate [14]. This was evaluated daily until no germination observed for a period of three month. Some seeds were randomly selected removed from the soil for observation. Seed were considered to have germinated following emergence of the radicle with length of at least 5 mm.

Germination percentage (GP): This is the percentage ratio of the total number of seeds germinated to the total number of seeds sown [15]. This can be expressed as follows:

$$GP = \frac{N}{Tn} \times 100 \quad (1)$$

where N is the number of seeds germinated and Tn: Total number of seeds sown.

Collar diameter was measured by placing the calliper 10 cm above the ground along the seedling and value recorded.

Height of shoot was measured by placing a measuring tape from the soil level to the tip of the shoot.

Numbers of leaves were counted on the seedling.

Data was analyzed using the statistical programme STATGRAPHIC 18, where the least significant differences (LSD) between the mean were detected and separated using the Duncan's New Multiple Range Test (DNMRT) at $p \leq 0.05$. Data were presented on tables.

3. Result

Our findings show that out of 180 seeds sown, 78 germinated given an overall percentage germination of 43.33%. Seeds soaked in 50% concentrated sulphuric acid above 1 minute failed to germinate while those that were nicked had the best latent period, germination percentage and early growth performance.

3.1. Effects of Pre-Germination Treatments on Latent Period and Germination Percentage of *Carapa grandiflora*

Latent period was not significant though seeds that were nicked (T_1) had the best (30.6 days) followed by those with seed coat manually removed (34.2 days) while seeds that were soaked in 50% concentrated sulphuric acid for 45s (56.8 days) were the least.

In addition, results for germination percentage were significant with seeds nicked having the highest germination percentage (62.2%) followed by seed with coat manually removed (46.7) while seeds soaked in 50% concentrated sulphuric acid at 1 minute were the least (Table 1).

Table 1. Effect of seeds pre-treatment on latent period and germination percentage.

Treatments	Latent period L/days	Germination percentage
T_0	39.9 ± 30.1^{a}	34.4 ± 3.27^{ab}
T_1	30.6 ± 13.2^{a}	62.2 ± 2.54^{b}
T_2	34.2 ± 13.1^{a}	46.7 ± 2.12^{ab}
T_3	56.8 ± 43.3^{a}	30.0 ± 2.40^{a}

Values (mean ± SE) with different letters in columns (superfix) indicate significant difference ($P > 0.05$).

3.2. Effects of Seeds Pre-Germination Treatment on Shoot Height of Seedlings

There was no significant difference in height of seedlings in all the pre-germination treatment. Seeds with coat manually removed (T_1) had the highest shoot height followed by those nicked (T_2) while seeds soaked in 50% concentrated sulphuric acid had the least height (T_4). These results are shown in Table 2.

Table 2. Effects of seeds pre-germination treatment shoot height of seedlings.

Treatment	SH1	SH2	SH3	SH4	SH5
0	14.0 ± 10.85[a]	15.0 ± 11.56[a]	15.89 ± 12.01[a]	16.78 ± 12.80[a]	18.11 ± 13.68[a]
1	17.67 ± 7.16[a]	18.44 ± 7.52[a]	20.44 ± 8.08[a]	21.89 ± 8.56[a]	23.33 ± 8.97[a]
2	15.44 ± 6.48[a]	16.44 ± 7.00[a]	18.78 ± 7.48[a]	20.56 ± 8.18[a]	22.56 ± 8.833[a]
3	13.22 ± 10.11[a]	14.44 ± 11.01[a]	15.56 ± 11.85[a]	17.33 ± 13.07[a]	18.44 ± 13.90[a]

Values (mean ± SE) with the same letters in the column (superfix) are not significantly different (P > 0.05).

3.3. Effects of Seeds Pre-Germination Treatment on Collar Diameter of Seedlings

There was no significant difference in collar diameter of seedlings in all the pre-germination treatment. Nicked seeds had the highest collar diameter followed by manual removal of seed coat while seeds socked in 50% concentrated sulphuric acid were the least (Table 3).

Table 3. Effects of seed pre-germination treatment on collar diameter of seedlings.

Treatment	CD1	CD2	CD3	CD4	CD5
0	4.42 ± 3.37[a]	4.65 ± 3.55[a]	4.94 ± 3.75[a]	5.28 ± 3.99[a]	5.63 ± 4.24[a]
1	6.34 ± 2.45[a]	6.61 ± 2.56[a]	6.96 ± 2.67[a]	7.39 ± 2.81[a]	7.9 ± 2.98[a]
2	5.46 ± 2.07[a]	5.97 ± 2.26[a]	6.43 ± 2.45[a]	6.97 ± 2.64[a]	7.41 ± 2.81[a]
3	3.93 ± 3.02[a]	4.17 ± 3.16[a]	4.56 ± 3.46[a]	4.88 ± 3.68[a]	5.31 ± 3.99[a]

Values (mean ± SE) with the same letters in the column (superfix) are not significantly different (P > 0.05).

3.4. Effects of Seeds Pre-Germination Treatment on Number of Leaves

There was no significant difference in the number of leaves of seedlings in all the pre-germination treatment. That notwithstanding, nicked seeds had the highest number of leaves with respect to the different months followed by manual removal seed of seed coats while seeds socked in 50% concentrated sulphuric acid were the least (Table 4).

Table 4. Effects of seed pre-germination treatment number of leaves of seedlings.

Treatment	NL1	NL2	NL3	NL4	NL5
0	2.44 ± 2.12[a]	2.89 ± 2.42[a]	3.33 ± 2.73[a]	4.44 ± 3.43[a]	5.11 ± 3.92[a]
1	3.44 ± 1.59[a]	4.11 ± 1.83[a]	5.11 ± 2.14[a]	5.44 ± 2.24[a]	6.44 ± 2.555[a]
2	3.0 ± 1.22[a]	3.78 ± 1.48[a]	4.56 ± 1.81[a]	5.11 ± 1.96[a]	6.11 ± 2.42[a]
3	1.89 ± 1.69[a]	2.44 ± 2.01[a]	3.11 ± 2.62[a]	3.44 ± 2.74[a]	4.22 ± 3.34[a]

Values (mean ± SE) with the same letters in the column (superfix) are not significantly different (P > 0.05).

4. Discussion

Breaking of seed dormancy through appropriate, cheap and easily handled methods of pre-treatment remains a very important tool for rapid propagation of endangered species by local people. Recalcitrant seeds are common in tropical forest and do not germinate readily even under favorable conditions, hence the need for pre-treating seeds [16]. They are intolerant to desiccation, losing viability rapidly when their moisture content falls below a relatively high threshold, usually 20% - 30% and are common in tropical and subtropical regions [17]. The irregular germination pattern at times enables seeds to withstand challenging and unfavorable environmental conditions.

Studies have shown that different methods of pre-treatments can enhance germination rate and speed up germination process [18] [19]. The result of this study shows that there was no significant variation ($p \geq 0.05$) among pre-treatments in seed germination percentage, emergence and early growth performance. Germination started on the thirtieth day after sowing in nicked seeds and lasted for eight weeks in seeds soaked in 50% concentrated sulphuric acid. Mechanical scarification exhibited better germination performance compared to other pre-treatments. According to Azad *et al.* [20], nicking is known to break physical dormancy of seeds with hard coats which inhibits water uptake and gases. Missanjo *et al.* [7] also noted that earlier germination of nicked seeds is as a result of cracks or cuts made on the seed which makes it easier for absorption of water and exchange of gases resulting in enzymatic hydrolysis and thus transforming the embryo into seedlings. The highest germination percentage observed in nicked seeds is in agreement with the study carried out by Asinwa *et al.* [21] who observed increased germination percentage of *Calophylum inophylum* seeds treated by scarification and the findings of Fredrick *et al.* [22] who noted that mechanically scarified seeds had the highest germination percentage in *Faidherbia albida* seeds when compared to other treatments used in the study. Boltsheleng *et al.* [23] also observed the highest germination percentage in *Afzelia quanzensis* and *Baikiaea plurijuga* seeds treated by scarification. Poor germination parameters observed in seeds treated with sulphuric acid could be attributed to damage to embryo thus impairing germination. This may also be due to the fragility of the seeds which was probably damaged in this process thus giving poor percentage germination. This observation is similar to the result of Asif *et al.* [24] who observed that subjecting *Prosopis juliflora* and *Dalbergia sissoo to* 95% sulphuric acid and hot water treatment failed to germinate. Some seeds took a very long time to germinate maybe due to impermeable seed coat which prevented water and gases from getting into it to stimulate germination. Thus, soaking seeds in hot water or 98% concentrated H_2SO_4 caused serious damage and deleterious effects on the embryo. In addition, water and gas impermeability of seeds is caused by physical and biochemical obstacles of the seed coat [25]. Poor germination parameters observed in the control treatment are an indication that seeds of this species need to be pre-treated to enhance germination. Amusa, [26] and Falemara *et al.* [27] noted that the control exhibited longer germination commencement period in *Afzelia africana* and *Adansonia digitata*, respectively when compared with other treatments used in their studies. This also conforms to the report by Iroko *et al.* [28] on the germination of *Vitellaria paradoxa* which showed that the seeds of the species needed to be pre-treated to enhance germination. The result of this study is in conformity with the statement by Luna *et al.* [29] that "the conditions necessary to allow seeds to break dormancy and germinate can be highly variable among species, within species or among seed sources of the same species" with respect to the parameters measured. Extended latent period is an impediment to nursery establishment and forest restoration practices as it may delay crop establishment, growth and seedling vigor and ecological competitiveness [30].

Pre-treatments did not significantly affect early growth performance (seedling height, collar diameter and leaf number). Mechanically scarified seeds produced the highest performance for all growth parameters studied (seedling height, collar diameter and number of leaves growths) followed by the control when compared to the other pre-treatment methods. This is the best method that could be used to enhance germination and early growth of *Carapa* seeds. According to Missanjo *et al.* [7], fast growth of *Acacia polyacantha* seedlings from nicked seeds occurred because seedlings from nicked seeds had an advantage of absorbing much water and started the photosynthetic process much faster than others. Seed germination is the most important stage that affects earlier seedling growth and establishment [31]. Missanjo *et al.* [7] also who observed that nicking produced the highest performance for all growth parameters studied in seeds of *Lupinus varius and Acacia polyacantha* respectively.

On the other hand, Sobola [12] had contrary results following pre-germination treatment of *Afzelia africana*. In addition, Ambursa *et al.* [32] observed a better performance by soaking seeds of *Faidhaerbia albida* in concentrated sulphuric acid for 10 minutes. Seed dormancy is known to occur in many tropical tree species [25]. Seed pre-treatment is species specific and that no one type of treatment has been reported to be universally effective. Therefore breaking the seed dormancy by softening the seed coat to allow water imbibition is crucial for any afforestation programs [33].

5. Conclusion

Carapa grandiflora seeds had a good germination rate influenced by pre-treatments. Seeds that were nicked (T_1) had a better latent period and germination percentage compared to the control. These pre-germination treatment enhanced water imbibition, activating food reserves in the cotyledon for embryo growth. However, early growth parameters were not influenced by pre-treatments. Finally, this study on the agronomic evaluation of *C. grandiflora* highlighted pretreatment for a better growth performance, conservation and sustainable management of the species.

Authors' Contributions

Francoline Jong Nkemnkeng, Walter Ndam Tacham and Christiana Ngyete Nyikop Mbogue carried out the field exercise and produced the first draft of manuscript while Mendi Grace Anjah and Jane Iyinji Anyi edited and fine-tuned the first draft manuscript. All authors read and approved the final manuscript.

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Conflicts of Interest

The authors declare that they have no competing interests.

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