Greener Journal of Biological Sciences
Vol. 3 (1), pp. 013-020, January, 2013
ISSN: 2276-7762; ICV: 5.99

DOI Link: http://doi.org/10.15580/GJBS.2013.1.110912247
http://gjournals.org/GJBS

Germination Biology of *Picralima nitida* (Stapf) under Pretreatments

GBADAMOSI Alaba E.

Department of Plant Science & Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

ARTICLE INFO

Article No.: *110912247*
Type**:** *Research*
DOI: *10.15580/GJBS.2013.1.110912247*

Submitted: *09/11/2012*
Published: *28/01/2013*

Corresponding Author
Gbadamosi Alaba E.
E-mail: *emmanuelreal @yahoo.com*
Phone: *+2348063567002; +23480596364424*

Keywords:
Pretreatments, medicinal plant, germination, seed coat, sowing, durian-epigeal, Picralima nitida

ABSTRACT

Viable seed sometimes fails to germinate under favourable conditions. The study assessed the germination of *Picralima nitida* (Stapf) seeds following pretreatments. Fruits were collected from Benin, Southern Nigeria (lat. 6^0 23^1N and long. 5^0 13^1E). The seeds were extracted manually and processed for sowing. Seeds were pretreated using ten pretreatment methods: control; soaking in water at 90^0C and allowing to cool overnight; soaking in water at room temperature; seeds which coats were manually removed; soaking in concentrated sulphuric acid (H_2SO_4) for 1min; 2mins; and 5mins; and soaking in primarily recycled H_2SO_4 for 2mins, 5mins and 10mins. One hundred seeds were assigned to each treatment and replicated in two locations of Akungba (lat. 7^0 28^1N and long. $5^0$$46^1$E) and Ibadan (lat. 7^0 24^1N and long. 3^0 49^1E). Seeds were sown in germination trays containing washed-sterilized river sand in the screen house. Germination counts were taken daily. *P. nitida* exhibited "durian-epigeal" germination, seeds which coats were manually removed were the first batch to germinate 17 and 22 days after sowing (DAS) in Ibadan and Akungba respectively. The highest germination percentage of 100 in Ibadan and 84 in Akungba were obtained among this batch. Seeds soaked in water at room temperature gave 86% germination in Akungba and 63% in Ibadan. Seeds soaked in water at 90^0C, and primarily recycled H_2SO_4 acid for 10mins did not germinate for 106days. Analysis of variance revealed significant differences at P≤0.05 in treatment and location effects. Mean germination percentage of seeds which coats were removed was significantly different from other treatments. The inherent dormancy in *P. nitida* seed can be treated by manually removing the seed coat before sowing.

INTRODUCTION

Seeds are genetic materials that carry heritable traits across generational boundaries. Evans and Turnbull (2004) submitted that seeds are expensive, scarce and precious. Often, seeds of tropical forest species are objects of commerce applicable to various end-uses. The regeneration of such species is usually hampered by this practice; yet, some seeds do not readily germinate even under optimum environmental conditions. Gbadamosi (2005) opined that there appeared to be a natural time-clock in seeds which regulate the germination process. The interplay of seed dormancy and multiple end-uses constitutes a great obstacle in the regeneration efforts of multi-purpose species. These species, according to Leakey (1999), have traditionally provided communities and households with their everyday needs for food and medicinal products.

Picralima nitida (Stapf) is a medicinal plant with diverse end-uses (Keay, 1989); extracts from its seeds, fruit rind and stem bark demonstrated anti-malarial activity (Iwu and Klayman, 1992); antimicrobial effect (Fakeye *et al*., 2002), anti-inflammatory and analgesic actions (Duwiejuna, 2002). It is used in traditional medicines in the treatment of inflammation, otitis, pulmonary bronchitis and venereal diseases. Five industrially important indole alkaloids namely picratidine, akuammine, pseudoakuammine, and akuammicine and akuammidine; and recently, picranitine have been isolated from the seed of *P. nitida* (spearson-trustpass.alibaba.com/product/12300525-1137302).

With rapid forest plantation depletion in the tropics, the supply regime of these useful forest produce is severely threatened with attendant dire consequences on the well-being and welfare of local people dependent on these produces, Hence the need for reforestation. However, dormancy in seeds results in staggered germination in the nursery and ultimately affects seedlings production for reforestation and afforestation activities of these species.

Various methods of hastening germination, including mechanical and chemical scarification, have been proffered for different species; therefore, this study was carried out to recommend the appropriate method of breaking the inherent dormancy problem in *P. nitida* seeds.

MATERIALS AND METHODS

Study Area

Mature fruits of *Picralima nitida* were collected from National Institute for Oil Palm Research, Benin, Southern Nigeria (lat. 6^0 23^1N and long. 5^0 13^1E). Seeds were extracted from the fermented fruits manually. One thousand seeds were randomly selected and these were divided into ten parts of one hundred seeds each. The experiment was set up at the National Centre for Genetic Resources and Biotechnology, Moor plantation Ibadan, Nigeria (lat. 7^0 24^1N and long. 3^0 49^1E) and Adekunle Ajasin University, Akungba-Akoko, Nigeria (lat. 7^0 28^1N and long. 5^0 46^1E).

Seed Pretreatment and Sowing

There were ten treatments in all: control, seed coat manually removed, soaking in concentrated H_2SO_4 for 1min, soaking in concentrated H_2SO_4 for 2mins, soaking in concentrated H_2SO_4 for 5mins, soaking in hot water at 90^0C and allowed to cool-down overnight, soaking in cold water for 24hours, soaking in primarily recycled H_2SO_4 acid for 2mins, soaking in primarily recycled H_2SO_4 acid for 5mins and soaking in primarily recycled H_2SO_4 acid for 10mins. One hundred seeds per treatment were sown. Seeds soaked in hot water at 90^0C and water at room temperature air-dried in the laboratory for one hour before sowing while the ones in acid were thoroughly rinsed with water to remove any trace of acid and later air-dried for about one hour before sowing.

The seeds from each treatment were then sown in well-labeled germination trays containing sterilized-washed river sand and set under high humidity propagator. Watering was done daily in the morning using a fine-meshed watering can. Germination was said to have taken place when the plumule emerges above the soil surface and daily observation of germination was done until no further germination could be observed for about a week. The entire experiment was replicated in two locations of Akungba and Ibadan.

RESULTS

Germination of *P. nitida* seeds varied significantly at 5% due to treatment and location effects (Table 1).

Table 1: Summary of analysis of variance for the Arc-Sine transformed percentage germination of pretreated seeds of *P. nitida* in two locations in Nigeria

Source of variation	Degree of freedom	Ibadan		Akungba	
		Mean square	*F*	Mean square	*F*
Treatment	9	1439.4	17.5*	2358.1	24.1*
Location	1	708.7	8.6*	897.8	9.2*
Error	9	82.4		97.8	
Total	19				

In Akungba, seeds which coats were manually removed were the first set to germinate at 22 days after sowing (DAS), this was followed by seeds soaked in conc. H_2SO_4 for 5mins at 31DAS, and seeds under control treatment were the last to germinate at 46DAS. Seeds soaked in hot water and those treated with primarily recycled $H2SO_4$ acid for 10mins did not germinate throughout the experimental period. (Figure 4)

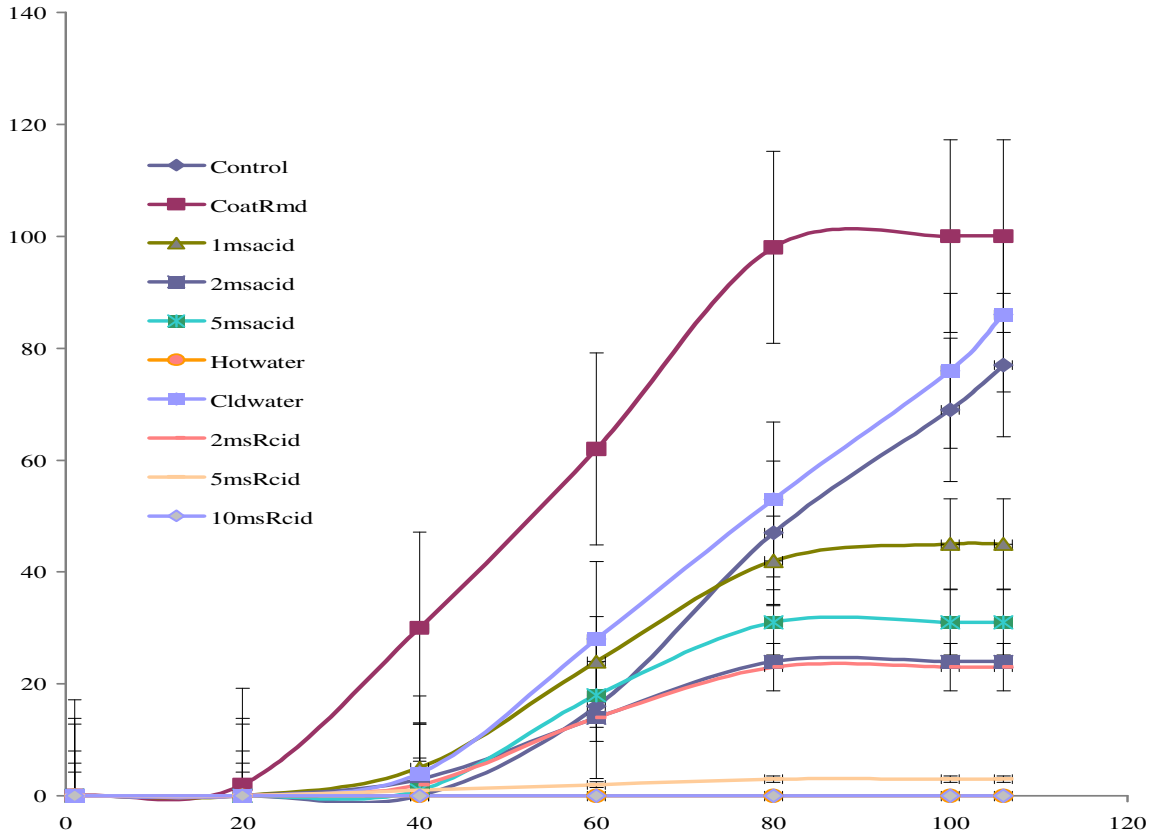

Figure 4: Cumulative germination percentage of *P. nitida* seeds under different pretreatments in Akungba

In Ibadan, seeds which coats were manually removed germinated 17DAS; this was followed closely by seeds soaked in conc. H_2SO_4 for 2mins at 19DAS. Seeds under control treatment were the last batch to germinate at 28DAS. Seeds soaked in hot water, conc. H_2SO_4 for 5mins and primarily recycled conc. H_2SO_4 for 10mins did not germinate for over 100days. The highest germination percentage of 100 days (Figure 3) was recorded in seeds which seed coats were removed manually in Akungba; this treatment also gave the highest germination percentage of 84 in Ibadan (Table 2).

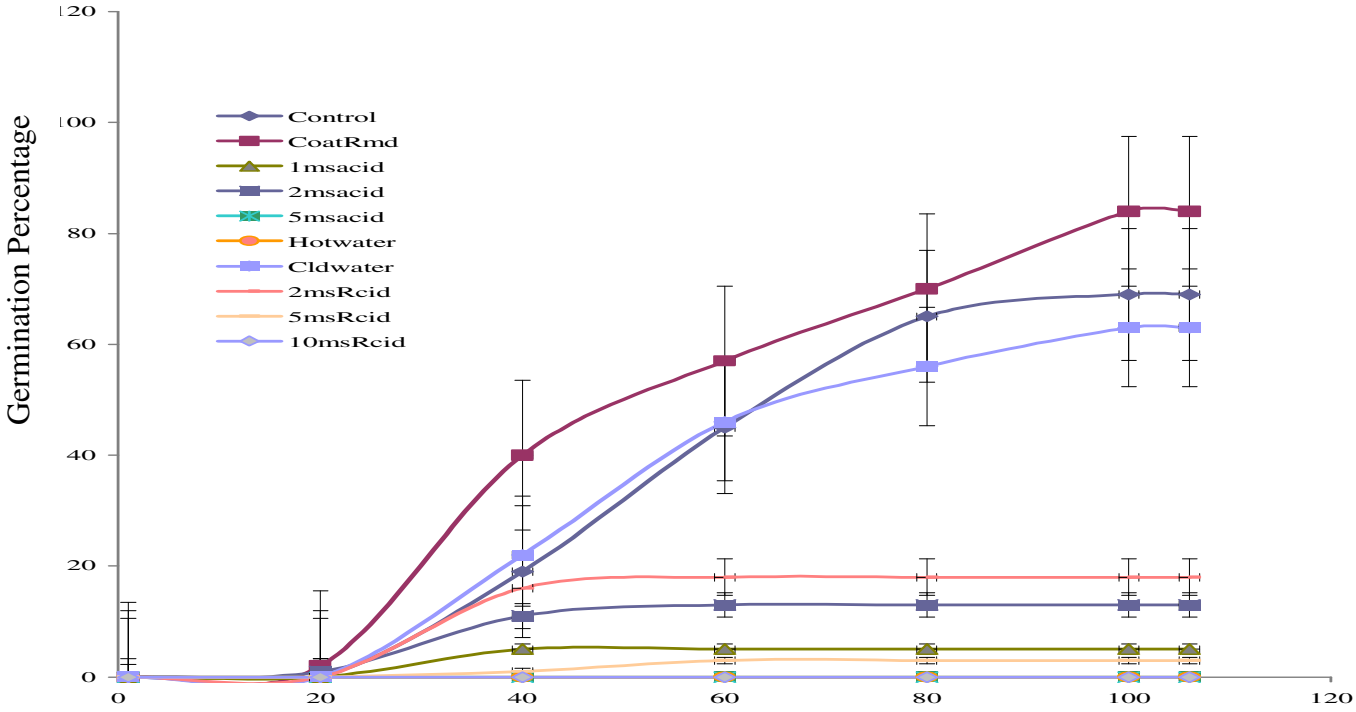

Figure 3: Cumulative germination percentage of *P. nitida* seeds under different pretreatments in Ibadan

Table 2: Germination percentage of pretreated seeds of *P. nitida* in two locations in Nigeria

Pretreatment method	Ibadan	Akungba
Soaking in hot water	0	0
10mins in recycled H_2SO_4	0	0
5mins in recycled H_2SO_4	3	3
2mins in recycled H_2SO_4	18	23
5mins in conc H_2SO_4	0	31
2mins in conc H_2SO_4	13	24
1min in conc H_2SO_4	5	45
Control	69	77
Soaking in ordinary water	63	86
Manual seed coat removal	84	100

The seeds pretreated with hot water had 0% germination in both locations while cold water pretreated seeds gave 86% and 63% germination in Akungba and Ibadan respectively. Among seeds treated with concentrated H_2SO_4, duration of treatment affected germination largely, seeds soaked in conc. H_2SO_4 for 1min, 2mins and 5mins had germination percentages of 45, 24 and 31 respectively in Akungba. Seeds soaked in conc. H_2SO_4 for 2mins and 1min had 13% and 5% respectively, while those treated with conc. H_2SO_4 for 5mins failed to germinate in Ibadan.

Seeds of *P. nitida* soaked in primarily recycled H_2SO_4 acid for 10mins failed to germinate in both locations, also, 3% germination was recorded in both locations for seeds soaked in 5mins of recycled H_2SO_4 acid. At lower duration of 2mins, seeds soaked in recycled acid had 23% and 18% germination in Akungba and Ibadan respectively.

Seeds under Control treatment had germination of 77% in Akungba and 69% in Ibadan. There were significant differences in mean percentage germination of *P. nitida* under different pretreatments. The mean germination of *P. nitida* seeds which coats were manually removed was significantly different from seeds under other treatments. The mean percentage germination of seeds soaked in cold water and control were significantly different from those in other treatments (Table 3).

Table 3: Means of germination percentage of pretreated seeds of *P. nitida* in two locations in Nigeria

Pretreatment method	Ibadan	Akungba
Soaking in hot water	0 [a]	0[a]
10mins in recycled H_2SO_4	0 [a]	0 [a]
5mins in recycled H_2SO_4	9.98 [ab]	3 [ab]
5mins in conc. H_2SO_4	16.91 [ab]	15.5 [ab]
2mins in conc. H_2SO_4	25.23 [ab]	18.5 [ab]
2mins in recycled H_2SO_4	26.88 [b]	20.5 [ab]
1min in conc. H_2SO_4	27.53 [b]	25.0 [b]
Control	58.76 [c]	73.0 [c]
Soaking in ordinary water	60.28 [c]	74.5 [c]
Manual seed coat removal	78.21 [c]	92.0 [d]

Figure 1: Mature seeds of *P. nitida* in the pod

Figure 2: Germinating seeds of *P. nitida* encapsulated by the seed coat

DISCUSSION

Breaking of seed dormancy through appropriate, cheap and easily handled methods of pretreatment remain a very important tool for rapid domestication of endangered useful species by local people. Most tropical forest tree species have recalcitrant seeds which do not germinate readily even under favourable conditions (Chin *et al*., 1989); hence the need for improved methods of pre-treating seeds (Olayode and Gbadamosi, 2009).

Seeds of *P. nitida* soaked in hot water and allowed to cool down did not germinate as well as those soaked in primarily recycled H_2SO_4 for 10mins in both locations. Although, *P. nitida* exhibited seed coat dormancy, it was clear that the seed coat could not withstand a long duration of treatment. Treatment with concentrated and primarily recycled acid gave lower percentage germination with increased duration of treatment. This is contrary to the findings of Olayode and Gbadamosi (2009) where the soaking of the seeds of *Dialium guineense* in conc. H_2SO_4 for longer periods was more effective in hastening germination in the species.

Devising appropriate technique that can be easily adopted by local farmers remains a veritable means for rapid domestication of useful tropical species (Leakey, 2004). Manual removal of the inhibiting seed coat in *P. nitida* gave the highest germination and rate of germination in both locations of this study; Oni and Gbadamosi (1998) submitted that these factors are important in nursery and field establishment of forest crops; also, this method will be easier to demonstrate to peasant farmers than treatment with concentrated H_2SO_4 which may be dangerous and cumbersome for them to practice. This method of pre-sowing treatment also enhanced the early growth of the sprouting seeds. Eyob (2009) submitted that enhancement of seed germination is important in propagation and breeding programme as well as for testing and using germplasms.

P. nitida seeds exhibited "durian-epigeal" germination as observed by Gbadamosi and Oni (2004) in *Enantia chlorantha* where the seed coat encapsulated the emerging seed leaves for a long time, thus limiting the development of the seedlings until the seed coats were shed. With seed coat manually removed, this problem has been solved and seedlings can establish faster.

A higher percentage germination of untreated seeds than acid-treated batches put paid on these pretreatment methods for this species. Therefore, *P. nitida* can be hastened to germinate and establish quickly by manually removing the seed coat of the seeds before sowing. The average seed size of the species favoured the adoption of this pre-sowing method.

CONCLUSION

Picralima nitida seeds demonstrated inherent seed coat induced dormancy. Manual removal of seed coat is recommended to promote the germination process and improve the early establishment of seedlings in the nursery. This method is equally desirable since it is cheap and easily adaptable by local peasant farmers to accelerate the domestication of the species.

ACKNOWLEDGMENT

This research was funded with grants provided by the International Foundation for Science (IFS) - D/4670-1.

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Cite this Article: Gbadamosi AE (2013). Germination Biology of *Picralima nitida* (Stapf) under Pretreatments**.** Greener Journal of Biological Sciences, 3(1): 013-020, http://doi.org/10.15580/GJBS.2013.1.110912247**.**