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Effects of Pre-germination Treatments on the Seeds of *Piliostigma thonningii* Schum

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ABSTRACT

Seeds of *Piliostigma thonningii* have hard seed coats which inhibit its germination. This study therefore investigated the effects of different pre-germination treatments on the seed of the species with a view to enhancing its germination towards seedling production for afforestation programmes. *Piliostigma thonningii* seeds were obtained from the seed store of Forestry Research Institutes of Nigeria. The study comprised of mechanical scarification, acid scarification, hot water, cold water scarifications at different levels and a control replicated 3 times with 20 seeds per replicate. The experiment was laid in Completely Randomized Design (CRD). The seeds were placed in between Whatman No1 (9cm) filter paper and set inside Copenhagen germination tank in the seed laboratory. Seeds were daily observed for 28 days to check for germination. Parameters assessed included total germination count and Percentage germination. Germination data collected were analyzed using analysis of variance (ANOVA)
Results showed that mechanical scarification gave 100 % germination. Soaking in HCl for 8mins gave 46.66%, this was followed by 6 minutes that gave 33.33%, and other acid treatments did not germinate at all. The statistical analysis showed that seed soaked in HCl for 8 minutes gave mean germination that was significantly higher than all other. Seeds that were soaked for 6 hours gave the best germination percentage of 40%. The germination decreases with increase in soaking duration. Germination of 73% was obtained when seeds were soaked in hot water for 10 minutes; this was followed by 20minutess that gave 60%. Control seeds did not germinate at all. As a result of this study, it is recommended that mechanical scarification of *Piliostigma thonningii* at the helium and distal end can be adopted. Alternatively the seed can be soaked in hot water for 10 or 20 minutes.

Keywords: *Piliostigma thonningii*, seed dormancy, germination, scarification methods

Introduction

Piliostigma thonningii is an under-explored leguminous plant that belongs to the family, *Fabaceae* and belongs to the subfamily *Caesalpinioideae*. The tree is perennial in nature and it is known across Africa and other sub-Sahara countries as camel's foot. In Nigeria it bears such local names as *Abafe* in Yoruba; *Kalgo* in Hausa and *Okpoatu* in Igbo (Dasofunjo *et al*., 2012). *P. thonningii* is common in open woodland and wooded grasslands of sub-humid Africa at medium to low altitudes. It is found throughout tropical Africa (Bekele, 2007). Its native range includes Botswana, Kenya, Namibia, Nigeria, Senegal, South Africa, Sudan, Tanzania, Uganda, Zambia and Zimbabwe (Aderogba *et al.,* 2004).

Different parts of *P. thonningii* are useful medicinally; a warm infusion of the leaves is used traditionally as antipyretic and analgesic to relieve fever, toothache, treatment of malaria and for management of diarrhoea (Salawu *et al.,* 2007). The roots and twigs have been used locally in the treatment of dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections in Eastern Nigeria (Madara *et al.,* 2010). The leaves are nutritious as they contain proteins, calcium, phosphorous, and amino acids (Missanjo *et al*., 2013; Madara *et al*., 2012; Aderogba *et al*., 2006).

Bark of *P. thonningii* is commonly used for making fence and bridge building. The leaves are also used for wrapping food, and twigs and root serves as toothbrush (Orwal *et al.,* 2009). *P. thonningii* is also an agroforestry tree, suitable for intercropping with agricultural crops. It provides good shade and shelterbelt in homesteads when in full foliage. It is used in live fences around fields and as a live support for vines of weaker plants. Honeybees forage for abundant pollen and nectar in dry land areas and the tree is recommended for planting to increase honey production (Arbonnier, 2004).

The tree of *P. thonningii* also provides poles and timber for local house construction. The wood is also used for kitchen utensils, tool handles, furniture, bedposts, wheel-work and carpentry. The wood is also used as firewood and for making charcoal (Bekele, 2007).

Germination is the process by which a plant grows from a seed (Raven *et al*., 2005). It is the process of reactivation of metabolic machinery of the seed resulting in the emergence of radicle and plumule (Raven *et al*., 2005). For some seeds, their germination is affected by environmental condition chemical inhibitions all this resulted in seed dormancy while some are affected by seed coat and chemical inhibition (Raven *et al*., 2005).

Seed dormancy has been defined as the incapacity of a viable seed to germinate under favorable conditions (Finch-Savage and Leubner–Metzger, 2006). Dormancy is regulated at different developmental phases, in interaction with environmental factors, and this makes it difficult to detect when the genetic and physiological differences are established (Kucera *et al.,* 2006). This

difficulty arises because all dormancy assays are based on seed germination, which is the result of the balance between the degree of dormancy and the capacity of the embryo to overcome dormancy (Baskin and Baskin, 2004). The seed of *Piliostigma thonningii* is reported to have a hard seed coat and should be scarified before sowing in order to enhance and improve germination (Orwa, *et al*., 2009).

Despite the numerous economic and industrial importance of *Piliostigma thonningii*, deliberate efforts at large scale cultivation in form of plantation is limited. This may be due to the hardness of the seed-coat which makes the seeds to remain dormant, and the seeds cannot germinate until the dormancy is broken (Azad *et al*., 2006; Ayisire *et al.*, 2013). Also, afforestation programme needs availability of viable and healthy seedlings in required quantity, and calls for the large production of the seedlings in the nursery (Lars, 2000). This study therefore investigated the effects of different pre-germination treatments on the seed of the species with a view to enhancing its germination towards seedling production for afforestation programmes.

MATERIALS AND METHODS

Seeds of *Piliostigma thonningii* were collected from the Seed Store of Forestry Research Institutes of Nigeria, Ibadan. The seeds were subjected to different pre-germination treatments which include: mechanical scarification, acid scarification, hot water (100^{o}C), cold water at different levels and no pre-germination treatments (control). The control and treatments were replicated 3 times with 20 seeds per treatment. Mechanical scarification was carried out by filing at the helium and distal after which scarified seeds were sown. For acid scarification, the seed were immersed in HCL acid for periods of 2, 4, 6 and 8 minute respectively during which they were frequently stirred and thereafter washed with several changes of distilled water before being sown. Cold water treatment was done by soaking the seed in cold water for periods of 6, 8, 10, and 12 hours respectively. For hot water treatment, the seeds were immersed in hot water for periods of 5, 10, 15, and 20 minute respectively. Treated seeds were allowed to cool at room temperature before they were sown.

The experiment was laid in Completely Randomized Design (CRD). The seeds were placed in between Whatman No1 (9 cm) filter paper and set inside Copenhagen germination tank in the Seed Laboratory of Forestry Research Institute of Nigeria, Ibadan where they were being observed.

Seeds were daily observed for 28 days to check for germination. The parameters assessed include total germination count and Percentage germination. Germination data collected were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests was used to test level of differences among means that significantly different from each other.

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RESULTS AND DISCUSSION

Effects of mechanical scarification on germination of *Piliostigma thonningii*

The two mechanical scarifications (Scarification at the helium and scarification at the distal) gave 100% germination at the end of the experiment. This is in line with the work of Daguma *et al*., (1988) who stated that mechanical seed scarification is the most effective way of improving seed coat permeability. This implies that hard coat of the species seed prevent penetration of moisture and gases into the seed and inhibits development of embryo (Orinos and Mitrakos, 1991). The analysis of variance showed no significant difference in the mean as shown in the Table1 and 2 indicates that mechanical scarification on any part of seeds breakdown hard layer which leads to penetration of water and activation of embryos. Optimum germination of the species under the treatments validate the fact that scarification treatments soften and increase permeability of the endocarp layer of *Piliostigma thonningii* seeds (Rostami and Shasavar, 2009)

Table 1: Analysis of variance for effect of Mechanical Scarification on germination of *Piliostigma thonningii*

SV	df	SS	MS	F-cal	F-tab
Treatment	2	2000	1000	0.00^{ns}	4.46
Error	6	000	0		
Total	8	2000			

Table 2 Effect of mechanical scarification on germination percentage of *Piliostigma thonningii*

Treatment	Germination (%)
Control	0
Seed scarified at helium	100
Seed scarified at distal	100

Effects of HCL on germination of *Piliostigma thonningii*

Seeds of *Piliostigma thonningii* soaked in HCL for 8 minutes gave 46.66%, this was followed by the ones soaked for 6 minutes that gave 33.33%, and other treatments i.e. the seeds soaked for 4 and 2 minutes did not germinate at all (Table 5). This could be attributed to short period of treatment with chemical scarification as Asinwa *et al*., (2008) and Ailero, (2004) reported higher germination percentages when seeds of *Canavalia ensiformis* (10 minutes) and *Parkia biglobosa* (15 minutes) were subjected to chemical scarification. The ANOVA showed that there was significant difference among HCl scarification treatment (Table 3). The seeds soaked in HCl for 8 minutes gave mean that was significantly higher than all other treatment at 5% level of

probability (Table 5). This is in line with the findings of Asinwa *et al*., (2008) who reported significant difference among duration of chemical treatments.

Table 3: Analysis of variance for the effect of HCI on germination of *Piliostigma thonningii*

SV	Df	SS	MS	F-cal	F-tab
Treatment	4	6026.66	1506.6	28.26*	3.11
Error	10	533.33	53.3		
Total	14	6560.22			

Table 4: Follow up test for HCl scarification

Treatment	Mean
Control	0.00[a]
2minutes	0.00[a]
4minutes	0.00[a]
6minutes	33.33[b]
8minutes	46.66[c]

Means with the same superscript alphabet in a column are not significantly different at 5% probability level

Table 5: Effects of HCL on the germination percentage of *Piliostigma thonningii*

Treatment	Germination %
Control	0.00
2 min	0.00
4 min	0.00
6 min	33.33
8 min	46.66

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Effects of Cold water treatment on the germination of *Piliostigma thonningii*.

The result showed that seeds that were soaked for 6 hours gave germination percentage of 40%. The germination decreases as the duration of soaking increases (Table 8). ANOVA showed significant difference among cold water treatments (Table 6 and 7). This observation conforms to the finding of Asinwa *et al.,* (2008) on the seed of *canavalia ensiformis* and Otegbeye and Momodu (2002) on *Parkia biglobosa* who reported decrease in germination of seeds with increase in hours of soaking. This implies that soaking of seeds in water for longer period of time reduces germination; probably as a result of oxygen deficiency which is one of important factors that enhances seed germination. In contrary to the findings, Owonubi *et al*., (2005) reported that seeds of *Azardirachta indica* germination increases with increase in hours of soaking in cold water. This is an indication that different species have varying rates at which their seeds coat is permeable to water and gases (Asinwa *et al.,* 2008)

Table 6*:* Analysis of variance for the effect of cold water on germination of *P. thonningii*

SV	Df	SS	Ms	F-cal	F-tab
Treatment	4	3040	760	14.25*	3.11
Error	10	533.33	53.33		
Total	14	5573.33			

Table7: Follow up test for cold water scarification

Treatment	Mean
Control	0.00[a]
12hrs	33.33[bc]
10hrs	20.00[b]
8hrs	33.33[bc]
6hrs	40.00[c]

Means with the same superscript alphabet in a column are not significantly different at 5% probability level

Table 8: Effects of cold water on germination percentage of *Piliostigma thonningii*

Treatment	Germination %
Control	0.00
6hrs	40.00
8hrs	33.33
10hrs	20.00
12hrs	33.33

Effects of hot water treatment on the germination of *Piliostigma thonningii*

Result showed that highest germination of 73% was obtained when seed were soaked in hot water for 10 minutes; this was followed by the seed soaked in hot water for 20 minutes that gave 60%. Other treatments gave percentages that were lesser than 50% (Table 11). Table 9 shows that there were significant difference among hot water treatments at 5% level of probability Seeds that were soaked for 10mins gave germination percentage which was significantly higher than other treatments (Table 10). Germination of seeds at different durations of soaking in the hot water depicts that hot water has influence on the hard coat of the seeds which allow permeability water and gases most especially as control treatments has 0% germination. This observation contradicts the report of Gill *et al*., (1992) on seeds of *Calliandra portoricensis* which did not germinate at varying duration of soaking in hot water. This implies that different seeds of different species react differently to hot water treatment.

Table 9: Analysis of variance for hot water scarification

Sv	df	SS	Ms	F-cal	F-tab
Treatment	4	9866.67	2466.667	5.441*	3.11
Error	10	4533.3	453.33		
Total	15	14400.00			

Table 10: Follow up test for hot water treatment

Treatment	mean
Control	0.00[a]
20minutes	60.00[bc]
15minutes	40.00[abc]
10minutes	31[ab]
5minutes	26.66[ab]

Means with the same superscript alphabet in a column are not significantly different at 5% probability level

Table 11: Effect of hot water on the germination percentage of *Piliostigma thonningii*

Treatment	Germination %
Control	0
5min	26.66
10min	73.33
15min	40.00
20min	60.00

CONCLUSION

Seed germination of *Piliostigma thonningii* was enhanced with pre-germination treatments. Comparing the effect of each treatment, the analysis of variance carried out showed that there was significant difference at 5% level of probability among treatments. Therefore, pre-germination treatment are needed to break physical dormancy caused by hard seed coat as it is known that seed coat prevent the imbibitions of water and sometimes exchanges of gases. As a result of this study, mechanical scarification of *Piliostigma thonningii* at the helium and distal end can be adopted. Alternatively the seed can be soaked in hot water for 10 or 20 minutes.

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