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Effects of Chemical Pre-Treatment and Media Composition on *In-Vitro* Propagation of *Piliostigma Thonningii* (Schumach) Milne-Redh

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Abstract

Camel's foot, (*Piliostigma thonningii*), is a tree of high priority for conservation in Nigeria. However, its seeds are dormant and the plant is uncultivated. Efficient protocols were developed for three different media composition and chemical pre-treatment of *P. thonningii* seeds. 10 seeds each were soaked in concentrated H_2SO_4 for 0, 5, 10, 15, 20 and 25 mins respectively. Each treatment and the control (un-scarified seeds) had 5 replicates and the experiment was repeated three times. Germination was recorded at 2 days interval over a period of 20 days. Seeds were inoculated into Murashige and Skoog media (MS), Woody Plant Media and Preece media respectively. Data were collected on germination count and shoot length. The data were subjected to ANOVA in CRD at 5% probability level. Result reveals that the seeds chemically scarified for 15 minutes gave the highest percentage germination of 95%. At the end of 12 weeks of inoculation, the highest shoot length, 10.30cm was observed in MS media while least shoot length, 6.26cm was observed in Preece media. There was no significant difference in the three media used. In-vitro propagation of *P. thonningii* seeds using concentrated H_2SO_4 pretreatment for 15 minutes and any of the three media will allow development of a mass production system to meet the increasing demand for this species.

Keywords: *Piliostigma thonningii*, scarification, micro-propagation, germination test.

Introduction

Piliostigma thonningii is a leguminous plant belonging to the family Caesalpiniacea, a family that comprises of trees and shrubs. It is commonly known as Monkey bread or Camel's foot (Schmidth and McCleland, 2002). It is a multipurpose tree, gathered from the wild and providing food, medicines and a range of commodities for the local population. It has potential for use as a pioneer species. It is a good shade tree that fixes nitrogen and plays a vital ecological role in nutrient cycling from deep soil. This might be a useful species to for restoring woodland or setting up a woodland garden. The wood is used as fire wood while salt can be extracted from its ash. The ashes and fresh pods are used in soap making (Moller and Lerdof 2000). The seeds are good source of anti-oxidant micro nutrient and rich in crude protein and carbohydrate. The seeds are also eaten by African Antelopes and Elephants, while famers grind the seed as fodder for cattle during winter month (Jimoh and Oladiji, 2005).
Traditionally, the bark, root and leaves are used in treating leprosy, smallpox, yellow fever, chest pain, cough, bronchitis, wounds, chronic ulcers, diarrhea, toothache and gingivitis (Akinpelu and Obuofor, 2000). Novel extracts from the plant has antiviral action and is useful in the treatment of pathogen of viral origin such as hepatic, influenza and Broncho-pulmonary diseases as well as active on HIV virus

Tender leaves are used to treat stomach-ache, coughs and snakebites (Ruffo *et al.,* 2002). The roots are used to treat prolonged menstruation, haemorrhage and miscarriage in women and also for the treatment of STDs (Ruffo *et al.,* 2002). An infusion of the root, combined with the root of the wild cow pea (*Vigna sp*.), is said to be a contraceptive.

A fiber from the inner bark is used to make string, ropes and cloth (Ruffo *et al.,* 2002). A red-brown dye can be obtained from the macerated bark. A blue dye is obtained from the roasted seed. The bark contains up to 18% tannins (von Maydell, 1990). The inner bark contains a gum that swells in water and so can be used for caulking boats. The wood is straight-grained. It is used for poles, grain mortars, tool handles, spoons and bedsteads (Ruffo *et al.,* 2002).

P. thonningii has a hard seed coat which result in its poor imbibitions and germination potential and this can be broken either by physical or chemical pretreatment (Ibiang *et al.,* 2012). Ayisire *et al;*(2009), reported that the ease and convenience of chemical scarification could make it a better choice. The use of concentrated H_2SO_4 for more than 15 minutes especially for 25 minutes has a lethal effect on the embryos of the seeds. Chemical pretreatment has been reported effective in breaking seed dormancy and improving germination in different forest seeds (Isikhuemen and Kalu, 2006).

Ayisire *et al.* (2009), recommended tissue culture (*in-vitro*) propagation as a powerful tool in the regeneration and mass propagation of difficult to propagate species such as *P. thonningii. In-vitro* plant regeneration has been reported in other leguminous species such as *Parkia biglobosa*, *Tetrapleura tetraptera, Acasia* spp. (Amoo and Ayisire, 2005).

The increasing rate of over exploitation for fuel wood energy, the demand for poles and small timber, coupled with shifting cultivation are considered, threats to many woody tree species, including *P. thonningii* grown in the northern semi and region of Nigeria (Oni, 2001). *P. thonningii* has therefore been reported as one of the national priority species in Nigeria for conservation and sustainable utilization (Oni, 2001). It is uncultivated due to its seed dormancy problem. *In-vitro* propagation methods are powerful tools for germplasm conservation and rapid multiplication of such threatened and difficult to propagate species within a small space.

Therefore the objective of this study was to develop a protocol for the *in-vitro* propagation of *Piliostigma thonningii* with a view to mass propagates it for the purpose of plantation establishment.

MATERIALS AND METHODS

Seed collection and pre-treatment:

One hundred seeds were collected from the Seed Section of the Sustainable Forest Management Department, Forestry Research Institute of Nigeria (FRIN) Jericho Ibadan. The seeds were scarified chemically by soaking in concentrated H_2SO_4 for 0, 5, 10, 20 and 25 minutes respectively followed by several rinsing in water. Each treatment and the control (involving un-scarified seeds), had 5 replicates and the experiment was repeated three times. Germination, defined as the emergence of radicle, was recorded at 2 days interval over a period of 20days. The scarified seeds were then surfaced disinfected in 70% alcohol for 5 minutes, and 10% NaOCl together with Tween 20 for 20 minutes and then rinsed with sterile distilled water 3 times. The seeds were then soaked in the sterile distilled water to allow imbibitions. The media used for the propagation of the seeds were Murashige and Skoog, (MS) (1962), Wood Plant Media [WPM] (Lioyd and McCown, 1981) and Preece (Preece *et al.*, 1989). These were prepared accordingly and autoclaved at 121^{o}C for 15 minutes after which they were allowed to cool before inoculated with the seeds. The seeds were aseptically inoculated into the test tubes containing the media before transfer into the Growth room.

Data Collection

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The rate of germination, percentage germination, leave emergence, root initiation and shoot height were considered. The experimental layout was a completely randomized design and the obtained data were subjected to analysis of variance.

RESULT AND DISCUSSION

Germination was observed after four days of inoculation; about 95% of germination was recorded in WPM, 80% in MS and 50% in Preece while there were no germination in the control i.e. un-scarified, seeds as well as those scarified for 25minutes in concentrated H_2SO_4. Germination increased in other treatments until the 10th day. The shoot growth were measured at four weeks interval and recorded as follows:

Table 1: Mean of different media types on the germination of *P. thonningii*.

Treatments	Weeks after inoculation		
	4	8	12
MS	4.16	7.20	10.30
WPM	3.10	5.56	7.88
Preece	2.72	4.98	6.26

Table 2: Effects of media on germination of *P. thonningii* explants

Height	Source of variation	df	SS	MS	F	Sig
	Media	13	8	0.615	0.308	0.905^{ns}
	Error	1	2	2		
	Total	14	10			

ns- not significant (p>0.05)

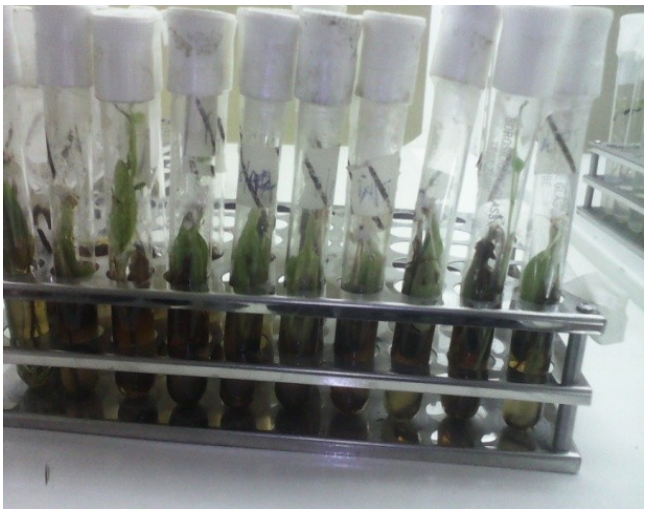

Plate 1: Plantlets of *P. thonningii* in WPM medium

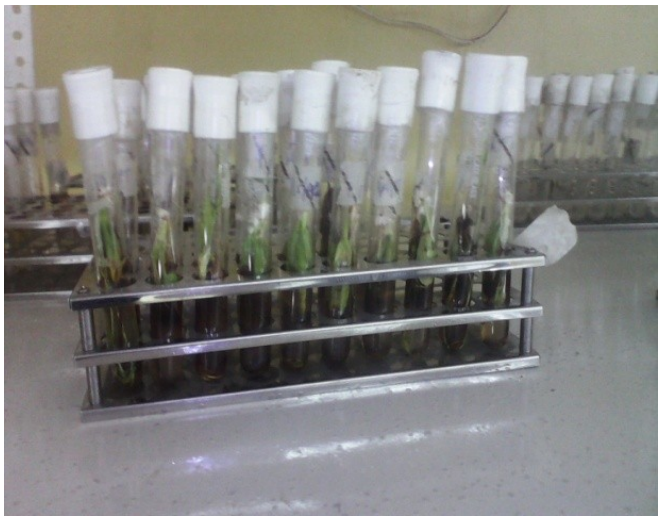

Plate 2: *P. thonningii* plantlets growing in MS medium

Table 2: Percentage Germination of *P. thonningii* seeds soaked in Conc.H_2SO_4

Time (minutes)	% Germination
0	10
5	30
10	60
15	95
20	20
25	0

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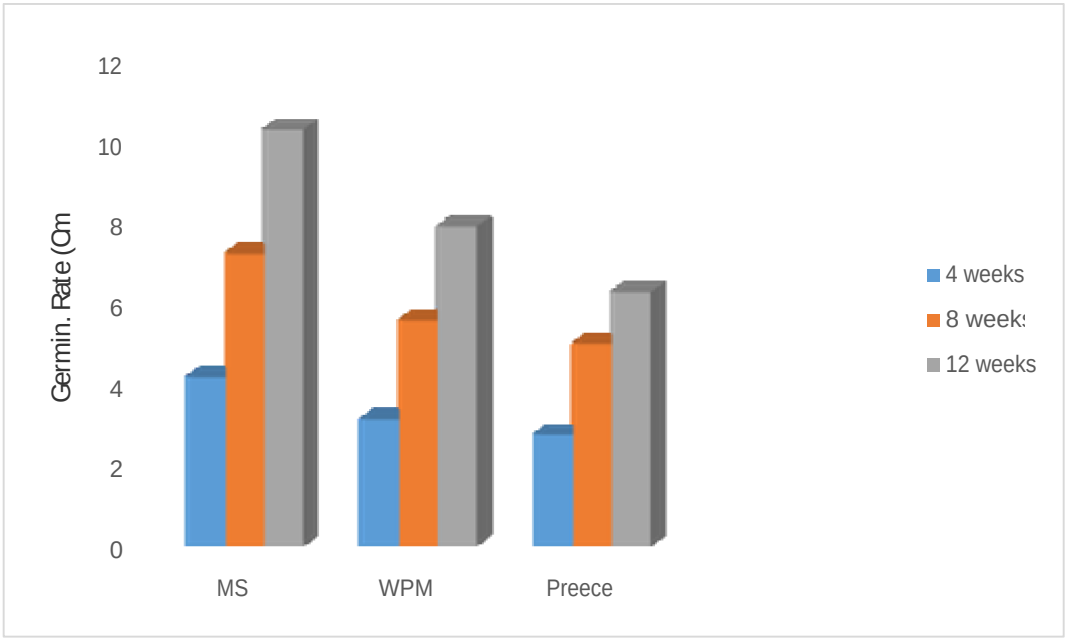
Fig. 1: Percentage germination of *P.thonningii* seeds soaked in conc. H_2SO_4



MS – Murashigee & Skoog; WPM – Woody Plant Media

Fig. 2: Germination rate of *P.thonningii* seeds soaked in conc. H_2SO_4

The result revealed that seed dormancy in *P. thonningii* is mainly due to the hard seed coat which can be broken by chemical scarification when soaked in concentrated H_2SO_4 for 15 minutes. The highest germination of *P. thonningii* was found on the seed cultured in WPM, but later the germinated seedlings in MS outgrew the ones cultured in WPM and Preece. The reason could be as a result of the nutritional composition of MS medium when compared with WPM and Preece. It was observed that MS media contains KI, KNO_3 and $CoCl_2$ which were absent in both WPM and Preece Media. These compounds are responsible for making plants to grow faster by enhancing effective shoot and root regeneration (Frit Industries, 2010).

CONCLUSION

It can be deduced from the above experiment that the chemical components of media play an important role in both the rate of germination and percentage germination of *P. thonningii in-vitro*. It is pertinent therefore to consider the type of media and media composition before embarking on the process of the tissue culture of this species. Further work is recommended for the *in-vitro* regeneration of *P. thonningii* for mass propagation and conservation purposes using MS media or optimization of the chemical composition of other media through the inclusion of the chemical constituent that is present in the MS media.

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