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Full Length Research Paper

Seed germination and *in vitro* propagation of *Piliostigma thonningii* – an important medicinal plant

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***Piliostigma thonningii* is a multipurpose tree of high priority for conservation in Nigeria. Almost all its parts are used in traditional medicine and its seeds are a good source of antioxidant micronutrients, rich in crude protein and carbohydrate. Its seeds are however dormant and the plant is uncultivated. Seed germination and *in vitro* regeneration experiments were carried out on *P. thonningii*. Seed dormancy was successfully broken by physical and chemical scarification using concentrated sulphuric acid for 15 min with 91.7 ± 4.01 and 95.0 ± 2.24% germination, respectively. The seed dormancy was due mainly to the hard seed-coat. Callus production was observed in both cotyledon and hypocotyl explants cultured in auxin-supplemented Murashige and Skoog (MS) medium. Explant orientation on the medium and the position from which the explant is taken affected callus production. Complete plantlet was regenerated from the hypocotyl-derived callus in medium containing auxin only.**

Key words: *Piliostigma thonningii*, scarification, *in vitro* regeneration, explant orientation, explant position.

INTRODUCTION

Piliostigma thonningii (Schum.) Milne-Redh. is a leguminous tree belonging to the family Caesalpiniaceae and is distributed in tropical Africa and Asia. It grows in open woodland and savannah regions that are moist as well as wooded grassland in low to medium altitudes (Jimoh and Oladiji, 2005). It is a multipurpose tree of vast economic importance.

This evergreen species is a good shade tree that fixes nitrogen and plays vital ecological roles in nutrient cycling from deep soil. The wood is used as fuel-wood while salt can be extracted from its ash (Moller and Lerdorf, 2000). The ashes and fresh pods are used in soap making (Moller and Lerdorf, 2000). The seeds, which are a good source of antioxidant micronutrients and rich in crude protein and carbohydrate, are eaten by African antelope and elephant while farmers in the lower savannah region grind the seed as fodder for cattle during winter months (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, diarrhoea, toothache and gingivitis (Akinpelu and Obuotor, 2000; Ogundaini, 2005). These authors established the antibacterial activity of its stem bark. Ibewuike et al. (1997) reported the isolation of compounds with anti-inflammatory and antibacterial activities from its leaves. Novel extracts having antiviral action, useful in treatment of pathologies of viral origin, such as herpetic, influenza and broncho-pulmonary diseases as well as active on HIV virus have also been reported (US. Patent No. 5635185).

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-arid 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 with its attendant 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. *In*

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Abbreviations: MS, Murashige and Skoog; **NAA**, α-naphthalene acetic acid; **2,4-D**, 2,4-dichlorophenoxy acetic acid.

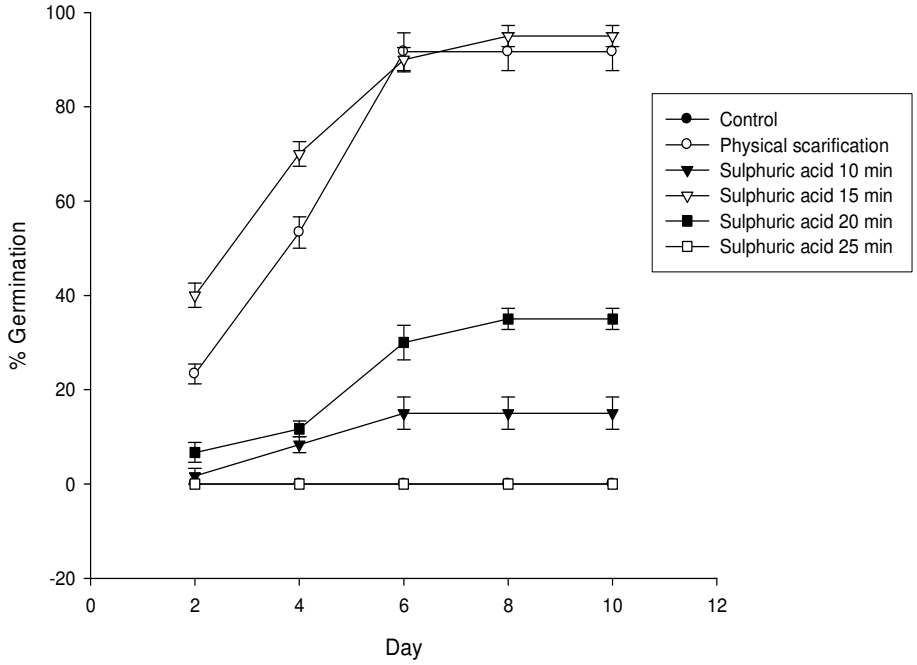

Figure 1. Effect of scarification treatments on seed germination of *P. thonningii*. Bar at each data point represents the standard error of mean.

vitro plant regeneration has been reported in other leguminous species such as *Parkia biglobosa, Tetrapleura tetraptera*, *Acacia* sp., and *Caesalpinia pulcherima* (Rahman et al., 1993; Xie and Hong, 2001; Ayisire and Amoo, 2004; Amoo and Ayisire, 2005). In this paper, we report the results of preliminary experiments carried out to improve on seed germination of *P. thonningii* and develop a protocol for its *in vitro* regeneration for conservation purpose.

MATERIALS AND METHODS

Seed collection and germination study

Dried, mature fruits of *P. thonningii* were collected along Ilorin - Offa road in Kwara State, Nigeria. The fruits were cracked and the seeds extracted. The seeds were scarified physically and chemically. Physical scarification was done by rubbing the seeds against cement wall while chemical scarification involved soaking the seeds in concentrated sulphuric acid for 10, 15, 20 and 25 min followed by several rinsing in water. The scarified seeds were then surface-decontaminated in 2% NaOCl for 10 min followed by rinsing in distilled water. The seeds were soaked in distilled water for 20 min to allow imbibition. Ten seeds were then placed in each Petri dish lined with filter paper moistened with distilled water and the Petri dishes were incubated in the dark at 25 ± 2°C. Each treatment and the control (involving unscarified seeds) had three 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 10 days.

In vitro propagation

Cotyledon and hypocotyl explants obtained from 10 day old seedling germinated in Petri dish were decontaminated in 2% NaOCl containing 2 drops of Tween 20 per 100 ml for 15 min followed by rinsing in three changes of sterile distilled water. Leaf explants collected from potted 4-week old seedlings were washed thoroughly under running tap water, decontaminated in 2% NaOCl containing 2 drops of Tween 20 per 100 ml for 25 min and rinsed in three changes of sterile distilled water. The edges of the explants were trimmed off after the decontamination treatments and the cotyledon, hypocotyl and leaf explants were then cut to approximately 5 x 5 mm^2, 5 mm and 10 x 10 mm^2 sizes, respectively. The cotyledon and leaf explants were cultured separately with either the adaxial surface face down or up. Hypocotyl explant was taken from either the distal or proximal section to the radicle. The culture medium consisted of full strength Murashige and Skoog (MS) (1962) macro- and microelements, 3% sucrose and 0.1 gl^{-1} myo-inositol supplemented with 0, 0.27, 0.54, 0.81, 1.07 µM naphthalene-acetic acid (NAA) or 0.90 µM 2, 4-dichlorophenoxyacetic acid (2, 4-D). The pH of the medium was adjusted to 5.7, gelled with 0.7% agar before autoclaving at 121°C and 103 KPa for 15 min. The cultures were incubated in the dark at 25 ± 2°C. The experiment was repeated two times.

RESULTS AND DISCUSSION

Seed germination

The percentage germination of the scarified seeds as well as the control over time is shown in Figure 1. There was no germination observed in the unscarified seeds (the control) as well as those scarified for 25 min in concentrated H_2SO_4. Germination increased in other treatments until the eighth day. The seeds chemically scarified for 15 min gave the highest germination of 95%. While this is not significantly different from those physical-

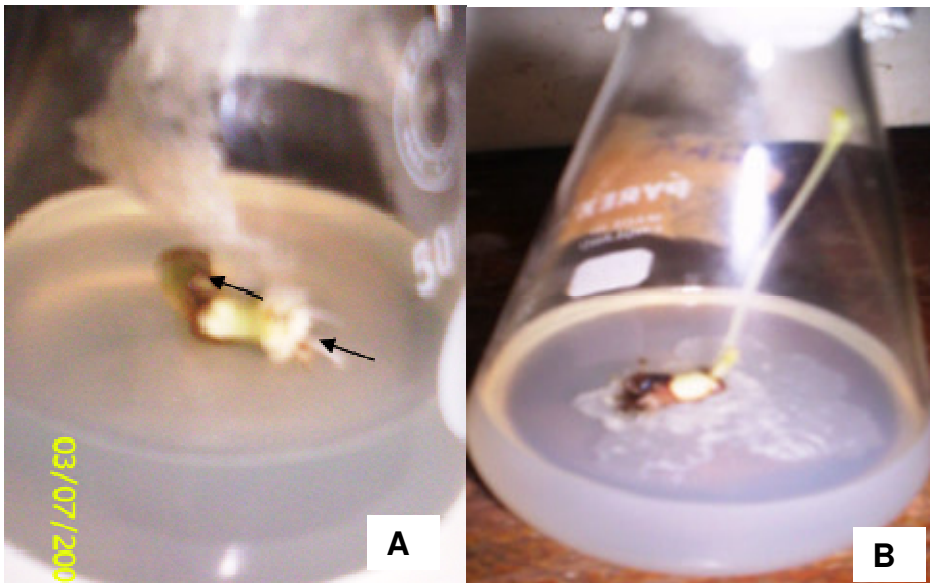

Figure 2. Hypocotyl segment cultured on Murashige and Skoog (MS) medium supplemented with 1.07 µM NAA. (A) Arrows show root and shoot buds (B) regenerated plantlet.

ly scarified (91.7%), both were however significantly different (p = 0.05) from all other treatments. The ease and convenience of chemical scarification could make it a better choice though. The use of concentrated H_2SO_4 for more than 15 min, especially for 25 min, has a lethal effect on the embryos of the seeds. Razanamandranto et al. (2005) reported zero germination when *P. thonningii* seeds were treated with smoke water, 2% in buried-smoked seeds and 3% when the seeds were exposed to aerosol smoke for 60 min. Gashaw and Michelsen (2002) reported less than 30% germination when *P. thonningii* seeds were exposed to heat treatment for either 1 or 5 min. The increased percentage germination recorded in this study with both the physical and chemical treatments indicates that the seed dormancy in this plant is mainly due to its relatively hard seed coat, a common feature of many leguminous plants. The hard seed coat renders the seeds impermeable to water and oxygen needed for germination process (Baskin and Baskin, 1998).

In vitro regeneration

A little swelling of the explants was observed in all the treatments and control, which is presumably a wound response due to limited cell division and a rapid increase in metabolic activity (Street, 1977). Neither callus formation nor organogenesis was observed in cultures containing MS basal medium only as well as MS basal medium supplemented with 0.27 µM NAA.

Cotyledon explants in cultures containing MS medium supplemented with 0.54, 0.81, 1.07 µM NAA or 0.90 µM 2,4-D concentration showed callus production. Callus proliferation was relatively more pronounced when the adaxial surface of the cotyledon was placed face down on the medium (data not shown). The effect of cotyledon/leaf explants orientation on callus production has been reported (Morini et al., 2001; Amoo and Ayisire, 2005). In the medium supplemented with 1.07 µM NAA however, protrusions later found to be roots were observed during the third week in cultures with adaxial surface facing up the medium.

Callus production was also observed in hypocotyl explants cultured in 0.90 µM 2,4-D or 1.07 µM NAA after a week of culture. Callus proliferation from the distal hypocotyl segment was relatively more than those from proximal part (data not shown). Nhut et al. (2001) reported a high regenerative capacity of *Lilium longiflorum* explants taken from the proximal end of the stem to the shoot tip. They observed shoot formation efficiency to decrease with an increase in distance from the proximal end of the shoot tip. Generally, high cytokinin to auxin ratio is known to induce adventitious shoot regeneration in tissue cultures. However, root and shoot buds were observed after the second week of culture in medium containing 1.07 µM NAA (Figure 2A) which later produced a complete plantlet (Figure 2B). This could be due to the level of endogenous growth regulator (particularly cytokinin) in the explant at the time of excision. Similarly, Özcan et al. (1992) reported a high regeneration response in pea cotyledons cultured on MS medium with a high auxin to cytokinin ratio. The leaf explant showed no regenerative response in all the treatments used.

Conclusion

Our results revealed that seed dormancy in *P. thonningii*

is mainly due to the hard seed coat, which can be broken by either physical or chemical scarification. The regenerative capacity of the cotyledon and hypocotyl explants in micropropagation is established. However, further work is needed to optimise the *in vitro* regeneration protocol of this plant for mass propagation and conservation purposes.

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