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MACROPROPAGATION OF *PYCNANTHUS ANGOLENSIS* (WELW.)WARB.- (AFRICAN FALSE NUTMEG)

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ABSTRACT

Pycnanthus angolensis is an important multi-medicinal tropical tree species that is vulnerable owing to high rate deforestation and exploitation without replacement. A study was carried out to develop a macropropagation protocol using stem cuttings and to investigate the effects of plant growth regulator IBA on rooting of both mature tree and juvenile stem cuttings of *P. angolensis.* Stem cuttings were collected from both the mature tree in situ and young seedlings within the period of May to August 2017. Stem cuttings were treated with Indole Butyric acid (IBA).The control cuttings were dipped in sterile water. Only cuttings from juvenile seedlings responded to treatment with IBA. The result revealed a significant effect of IBA concentration on the rooting performance of the stem cuttings and number of shoots per cutting. The highest rooting percent of cuttings from juvenile seedlings was recorded with 2000ppm IBA which was significantly different ($P< 0.05$) from 2500ppm IBA. After acclimatization, maximum survival rate of plants was 70%.
A successful macro propagation protocol has been developed; therefore, it can be employed for uniform plantation establishment of *P. angolensis* for mass propagation of this multi-medicinal tree species and tree planting programme.

Keywords: Macropropagation, Juvenile Stem cuttings, *P. angolensis*

INTRODUCTION

The tropical forest zone of Africa is rich with invaluable tree species, which have economic potentials meeting both food and medicinal needs of man. Plants are an important universally recognized source of medicines and play a key role in world health (Kala, 2005; Street and Prinsoloo, 2013). The gradual increase in the exploitation of forest trees for timber and non-timber product (food and medicine) have led to impaired regeneration. Deforestation has also been frightening as far back as three decades ago in Nigeria resulting in rapid loss of forest (FORMECU, 1991; Chakravarty *et al.*, 2012). In addition to the changes in land use, existing forests are being degraded by pathogens and pests, fire, atmospheric pollution, extreme weather events, climate change and unsustainable forest management practices (Chakravarty *et al.*, 2012; EPA, 2016). FAO (2006) indicates that the world's forested area is attenuating, predominantly in tropical regions where biodiversity and growth rates are highest. Okafor (1993) estimated that over 90% of the natural forest and natural vegetation in Nigeria is still being lost annually in the country while Ola-Adams and Iyamabo (1975) opined that the possible consequence of deforestation would be extinction of many Nigerian trees and substantial loss of plant diversity.

Pycnanthus angolensis, a tree species native to tropical Africa (GRIN, 2008) is one of such trees that could suffer the consequences of deforestation (Namuene *et al.,* 2014). It is a member of the family Myristicaceae. *Pycnanthus angolensis* has a variety of human uses and is variously called: box board, card board, Kombo butter, wild African nutmeg and commonly African false nutmeg. In Africa, it is widely known as Ilomba (Richter and Dallwitz, 2000) and called Akomu by Yorubas in Nigeria. The family is a rich source of bioactive phytochemicals useful in treating various ailments (Valderrama, 2000; Lopes *et al.,* 2004; Wiart, 2006; Denny *et al.,* 2008; Calliste *et al.,* 2010; La Frankie, 2010).

Pycnanthus angolensis can be used for many purposes including medicinal, nutritional and industrial. The bark, leaves, and seeds are employed for several ailments such as anaemia, stomach and menstrual disorders, tooth ache, emeto-purgative and can act as an antidote to poisoning and as a blood tonic; treatment of leprosy and infertility, gonorrhea and malaria (Zapfact *et al.,* 2001, Mapongmetsem, 2007; Orwa *et al.,* 2009; Ofori *et al.,* 2012). The reddish sap is syptic (arrest bleeding) and it is also used in treatment of thrush, mouth ulcer, gum diseases and bad breath and for treatment of cataracts and filariasis (Onocha and Otunla, 2010). IITA (2012) reported that the plant is used more for medicinal than culinary purposes. It has anthelmintic, anti-fungal and anti-cancer properties (Achel *et al.,* 2012).

The aromatic non edible seed is a source of butter, solid reddish/yellowish vegetable fat (about 70%) called kombo butter which is used for making soap and candles. Seed remnants are used for composting (Mapongmetsem, 2007). The wood is whitish grey or pink tinged in colour. It is suitable for furniture and house construction to make shingles, both for roofing and covering the sides of native houses, and planks for doors and window frames (Zapfact *et al.,* 2001;Orwa *et al.,* 2009).

P. angolensis is propagated conventionally by seeds but the seeds produced are few (60-100 seeds per tree annually) and recalcitrant while trial vegetative propagation by stem cuttings failed to succeed (Mapongmetem, 2007). There is, therefore, the need to introduce a sustainable method of cultivation of this multi-medicinal tree species. The use of macropropagation technologies have been documented and published especially for ornamental plant species specifically by horticulturist. Macropropagation allows for production of large quantity of true clone from the ortet for the purpose of conservation (Leaky, 2004). Early reports on rootability of mature cuttings of *P. angolensis* was not successful according to Onefeli and Akinyele (2014). Rooting success of cuttings have been attributed to a number of factors such as age, early selection of desired characters and the interaction with the environment (Ali and El-tigani, 2003). Difficulty of rooting in hard wood has been characterized to the degree of lignifications in the primary phloem which inhibit root primordial tissue to develop root initials (Ali and El-tigani, 2003; Fadwa and Yahia, 2014). Auxins are plant growth regulators well known for their ability to induce rooting (Eklof *et al.,* 2000, Nordstrom *et al.,* 2004; Fadwa and Yahia, 2014 and Abidin and Metali, 2015) and different auxins have been employed for macropropagation of many medicinal and timber value tropical trees and shrubs (Kipkemoi *et al.,* 2013, Abidin and Metali, 2015). This is as a result of its ability to improve rooting percentages, hasten root initiation, increase the number as well as the quality of roots and promote uniform root developments (Blythe *et al.,* 2007, Boyer *et al.,* 2013 and Abidin and Metali, 2015). However, the optimal concentration of plant hormone as well as responses to different auxins vary considerably in tree species as a result of intraspecific variation (Alejandro *et al.,* 2009, Guo *et al.,*2009). The aim of this research was to develop a reproducible method for the conservation of this tropical tree species as well as its multiplication for forest restoration programmes. Therefore, the specific objective of the study was to investigate the effect of plant growth regulator on the survival and rootability of stem cuttings from mature and juvenile trees of *P. angolensis* with a view to developing a reproducible macropropagation method.

MATERIALS AND METHODS

Plant materials

The propagules (cuttings) were obtained from the upper position on the mother tree and juvenile seedlings (8 months old) of *Pycnanthus angolensis* species within Obafemi Awolowo University, Ile-Ife, Osun State (Lat. 7^0 31^I 8.4 and Long. 4^0 31^1 15.96). Authentication was carried out at Ife Herbarium (IFE-17630). The cuttings were planted in river sand in a humidified environment. The cuttings were of three categories, the woody and herbaceous cuttings from mature tree and cuttings from juvenile plants. The leaves of the cuttings were reduced to half of the original sizes.

Root induction

This was carried out in two forms:

1. Direct Auxin Treatment

Two sets of cuttings were treated differently with auxin (IBA hormone), prepared into seven different concentrations viz :*0ppm, 1000ppm, 1500 ppm, 2000ppm, 2500 ppm, 3000ppm and 3500 ppm* using (A) quick dip method (Gbadamosi and Oni 2005; Onefeli *et al.*,2013, 2014) and (B) soaking in the specified IBA concentration for 18 hours (Fadwa and Yahia, 2014). The cuttings were thereafter planted in moist river sand placed in a humidified environment. Each of the treatments contained 10 cuttings and was replicated once making an aggregate of 140 cuttings per treatment. Watering of the set cuttings was carried out twice a day and monitored for a period of 20 weeks.

2. Auxin Treatment after soaking in water

The cuttings were soaked in water for nine hours before being treated with auxin (IBA hormone-0ppm, 1000ppm, 1500 ppm, 2000ppm, 2500 ppm, 3000ppm and 3500 ppm) using the quick dip method. The cuttings were then planted in moist river sand in a humidified environment. Each of the treatments contained 10 cuttings and was replicated once making an aggregate of 140 cuttings. Watering of the set cuttings was carried out twice a day and monitored for a period of 20 weeks.

Macropropagation Growth Measurement

The number of rooted cuttings, sprouted cuttings, survived cuttings and number of roots per cutting were recorded. Furthermore, the percentage rooting, percentage sprouting, and percentage survival was estimated. After five months of monitoring the cuttings under a humidifier, rooted cuttings were transplanted into pots filled with top soil and assessed every day for another four weeks for acclimatization.

Statistical Analysis

Data collected were subjected to Analysis of Variance using analysis of Variance with SAS Software version 9.1 (SAS Institute, 2004). Treatment means were separated with Duncan‘s Multiple Range Test (DMRT) at 5% probability level to determine the significant differences among the means of the recorded parameters.

RESULTS

Direct Auxin treatment

All cuttings from mature tree (both woody and the herbaceous) and from young seedlings died when treated with IBA concentrations using quick dip methods. Cuttings pretreated by soaking in hormone for 18 hours before they were planted in moist river soil also did not survive for all the ramets (herbaceous and woody stem cuttings of the mature tree as well as stem cuttings from treated young seedlings). Only the cuttings from young seedlings soaked (18 hours) in ordinary water (control) survived (53%) and sprouted (27%) (Table 1).

Table 1: Effect of soaking stem cuttings of *P. angolensis* for 18 hours in different concentrations of IBA on root and shoot formation after 3 months

Concentration of IBA (PPM)	% Survival	% sprouted cutting	% Rooted cuttings	No of Roots/ cuttings	No of shoots/ cuttings	Shoot height(cm)	Degree of basal callus
0	53.33±0.00[a]	26.67±0.00[a]	-	-	0.00±0.00[a]	2.53±0.04[a]	++
1000	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	-
1500	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	+
2000	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	+
2500	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	+
3000	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	-
3500	0.00±0.00 [b]	0.00±0.00[b]	-	-	0.00±0.00 [a]	0.00±0.00 [b]	-

Auxin Treatment after soaking in water

The above result led to another trial using cuttings from the young seedlings of *Pycnanthus angolensis*. The cuttings from young seedlings were soaked in water for 9hrs prior to their treatment with all the IBA concentrations (0 ppm, 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm, 3000 ppm, 3500 ppm) using quick dip method. All the stem cuttings survived (Table 2). Cuttings treated different (P≤0.05) from one another with respect to all the treatments (Table 2). Water soaked cuttings treated with IBA concentrations of 0-3500 ppm induced single shoot buds except 3000 ppm IBA with 1500 ppm IBA had the highest percentage survival (66.67) which was not significantly different (P ≤0.05) from the control (untreated) with 46.70% (0ppm). However, the cuttings did not form roots.

Cuttings from young seedlings soaked in water prior to quick dipping in IBA concentrations induced shoots after four weeks of planting. The percentage sprouted cuttings were not significantly which formed multiple shoots per explants (Plates 1a&b) but they were not significantly different from one another in percentage number of sprouted cuttings.

Table 2: Effect of soaking in water for nine hours prior to quick dip in IBA on root and shoot formation on stem cuttings from seedlings of *P. angolensis* after 4 months.

Concentration of IBA(PPM)	% SURVIVAL	% sprout cutting	% Rooted cuttings	No. of Roots/cutting	No. of Shoots/ Cuttings	Mean Shoot height(cm)	Degree of basal callus
0	46.67±6.67[a]	40.00±11.55[a]	0.00±0.00[c]	0.00±0.00[c]	1.00±0.00[b]	3.50±0.29[ab]	++
1000	53.33±6.67[a]	26.67±6.67[a]	0.00±0.00[c]	0.00±0.00[c]	1.00±0.00[b]	4.70±0.40[a]	+++
1500	65.00±6.67[a]	60.00±0.00[a]	0.00±0.00[c]	0.00±0.00[c]	1.00±0.00[b]	4.43±0.64[a]	++
2000	53.33±17.63[a]	26.67±17.64[a]	40.00±0.00[a]	1.00±0.00[b]	0.67±0.00[c]	4.07±0.29[a]	++
2500	53.33±17.63[a]	46.67±13.33[a]	20.00±0.00[b]	2.00±0.00[a]	1.33±0.33[ab]	4.97±0.32[a]	++
3000	60.00±20.00[a]	40.00±11.55[a]	0.00±0.00[c]	0.00±0.00[c]	1.75±0.33[a]	3.90±0.26[a]	++
3500	66.67±24.04[a]	60.00±20.00[a]	0.00±0.00[c]	0.00±0.00[c]	2.00±0.00[a]	1.00±0.00[b]	++

Values with the same superscript in a column are not significantly different from one another at P< 0.05.

Keys: IBA: Indole Butyric Acid; + Slight callus formation; ++ Moderate callus formation.

+++ Massive callus formation

The shoot height ranged from 4.3 cm to 5.9 cm at the end of five months of study. The cuttings treated with 2000 ppm and 2500 ppm IBA were the only ones that formed roots (Plates 2 a & b) and Plates 3 (a & b) were the only ones that survived. The roots were short, thick and yellowish similar to the radicle on emergence from a germinating seed. All the cuttings induced moderate callus except the control (0ppm) with massive basal callus. Plantlets derived from cuttings were potted on topsoil. After four weeks of acclimatization, 70% of the plantlets survived.

Plate 1(a & b): The effect of 3500 ppm IBA (a) and 3000ppm IBA (b) (after soaking in water for 9 hours) on stem cuttings of *Pycnanthus angolensis* after 5 weeks of planting

Plate 2(a & b): Sprouted cuttings from young seedlings of *Pycnanthus angolensis* after soaking in water for 9 hours prior to treatment with 2000ppm IBA (a) showing sprouted cuttings and (b) short thick single root after 4 months.

Plate 3(a & b): Rooted cutting from young seedling of *Pycnanthus angolensis* after soaking in water for 9 hours prior to treatment with 2500ppm IBA (a) showing sprouted cutting and (b) short thick single root after 4 months.

DISCUSSION

The results of this study showed that propagation of *Pycnanthus angolensis* by cuttings can yield a high multiplication rate and produce plants with their own root systems when treated with appropriate type and concentration of Plant growth regulators. Sandhu *et al.* (1989) reported that root induction is more effective by auxins than any other plant growth regulators and exogenous application of plant growth regulator produce a significant effect on growth as a result of the usual sub-optimal level of endogenous hormone(Pessarakli, 2002 and Abidin and Metali, 2015). All hormone concentrations with respect to either quick dip method or dipping in IBA for 18 hrs before planting did not induce root development. Onefeli and Akinyele (2013) reported similar result using 1000 ppm, 2000 ppm and 3000 ppm IBA concentration with mature stem cuttings of *Pycnanthus angolensis*. However, instead of root formation, callus was formed. Hydration appears to affect the survival of the cuttings thereby catalyzing root formation. Cuttings of *P. angolensis* treated by soaking in water prior to quick dip in IBA concentrations sprouted, developed roots and survived. Philippis (1966) found that rooting success of field planted cuttings of *Populus deltoids* could be improved by presoaking them in water for at least 48 hours. McKnight and Biesterfeldt (1968) also stored cotton wood cuttings in water-filled trenches prior to planting and reported that rooting and sprouting after planting was excellent. Puri and Thompson (2003) reported that water stressed cuttings took a longer period to root and had fewer roots but presoaking of the cutting in water stimulated rooting. Since percentage survival was not significantly different between the control and the treated cuttings; it appears the cuttings are sensitive to dehydration. Rehydrating the cutting helps to keep the cells alive and improves ability to respond to the applied hormone. In this study, the concentrations of IBA significantly affected the rooting ability of the stem cuttings. Eganathan *et al.* (2000) reported that stem cuttings of *Intsia bijuga* responded best with IBA (2,500 ppm); while *Excoecaria agallocha* produced highest rooting response (68%) and number of roots (7.1) with 2,000 ppm IBA. In this study also, rooting was only obtained in water soaked cuttings treated with 2000 and 2500ppm IBA. Sabah *et al.* (1991) also stimulated root formation in stem cuttings of *Citrus sp* using the higher concentrations of Auxins and observed concentrations of 1000 NAA ppm and 3000 IBA ppm combination yielded the maximum rooting percentage (75%) and produced higher number of roots that were longer and thicker. Stem cuttings from mature trees did not root at all. Compared with stem cuttings from mature trees, stem cuttings from juvenile plants contain negligible amounts of rooting inhibitors and have less differentiated cells and more actively dividing cells (Hartmann *et al*., 1990; Lakshmanan *et al*., 1995; Abidin and Metali, 2015). Also, several studies have reported that juvenile cuttings of tropical plants such as *Dalbergia megaloxylon* (Amri *et al.*, 2010), *Dilleniasu ffruticosa* (Abidin and Metali, 2015) and *Garcinia cola* (Kouakou *et al.,* 2016) formed new roots more readily than did cuttings from mature plants. High degree of lignifications in the primary phloem of some forest trees have been reported to affect rooting ability of cuttings from trees by hindering root primordial tissue to develop root initials (Ali and El-Tigani, 2003 and Fadwa and Yahia, 2014). The success of techniques used is dependent on appropriate physiological conditions of propagated plant cuttings. Rooting of cuttings have been reported to be affected by a number of factors such as; age, early selection of desired characters and interaction with the environment (Ali and El-Tigani, 2003; Brown, 2008 and Fadwa and Yahia, 2014). In the present study, adventitious root formation in stem cuttings of *Pycnanthus angolensis* was induced after four months of planting. Similar result was observed in stem cuttings of *Ginkgo biloba* where rooting occurred 12 weeks (three months) after treatment (Purohit *et al.*, 2009). Onefeli and Akinyele (2014) also concluded that rooting of most of the indigenous tree species occur within the range of 6-12 weeks after planting. In conclusion, successful propagation of *P. angolensis* by stem cutting is dependent on collecting cuttings from juvenile plants (i.e 8 - 9 months old seedlings) and adequate hydration of cuttings. Rooted cuttings can be successfully obtained from juvenile stem cuttings of *P. angolensis* by soaking in water and pre-treating with 2000 - 2500 ppm IBA. This study provides a protocol which can be used for propagation of *Pycnanthus angolensis* in agroforestry systems.

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