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Vegetative Propagation of *Picralima nitida* (Stapf.) by Leafy Stem Cuttings: Influence of Cutting Length, Hormone Concentration and Cutting Positions on Rooting Response of Cuttings

A.A. Olaniyi, F.B. Yakubu, M.O. Nola, V.I. Alaje, M.A. Odewale, O.O. Fadulu, and K.K. Adeniyi

Multipurpose Tree Improvement and Multiplication Unit,
Forestry Research Institute of Nigeria (FRIN)
Ibadan, Nigeria

Correspondence: olaniyiaisha@gmail.com

ABSTRACT

Picralima nitida is a valuable tree species in the humid zone of West and Central Africa whose natural regeneration is threatened by seed dormancy and over exploitation. This study investigated the rooting ability of *P. nitida* using mature leafy stem cuttings. Two cutting length (6 and 8 cm), cutting positions (apical and basal) and three concentrations (0, 0.1 and 0.2 mg/l) of Indole-butyric acid (IBA) were evaluated using a split-split plot design. The result showed that cutting length of 8 cm significantly influenced percentage of rooted and callused cuttings than cutting length of 6 cm. Significantly higher number of roots occurred in 8 cm cutting length than 6 cm cutting length. Cutting positions significantly affected percentage of callused cuttings only. Cuttings treated with IBA significantly enhanced rooting percentage, number of leaves and shoot height than untreated cuttings. Interactive effect of cutting positions, cutting length and IBA concentration was significant for only rooting percentage. Mature leafy stem cutting of *P. nitida* can be propagated asexually using cutting length of 8 cm from any position on the shoot to improve the rooting success. Stem cuttings of *P. nitida* should be treated with IBA to enhance the rooting capacity of the species.

Keywords: *Picralima nitida* - auxins- clonal propagation – cutting length – nodal positions.

INTRODUCTION

Forests provide resources that are utilised differently at both household and industrial scale (Suleiman *et al*. 2017). These resources are classified into timber and non-timber forest products (NTFPs). Apart from timber forest products that are largely valued worldwide, the non-timber forest products also play an indispensable role in contributing to household income, health care and food security in developing countries (Jimoh *et al.* 2013). *Picralima nitida* (Stapf.) T. Durand and H. Durand is a valuable NTFPs native to Nigeria and widely distributed in the high forest zones of West Tropical Africa. The species belongs to apocynaceae family and it grows to a height of 4 - 35 m as an understorey tree with a dense crown and bole diameter of about 60 cm.

Picralima nitida is highly valued for its wide medicinal properties throughout its distribution areas. All the plant parts; roots, leaves, fruits, seeds and stem bark are utilised extensively for treatment of pneumonia, malaria, stomach disorder, pain relief and intestinal worms (Burkill 1995). In Ghana and Nigeria, the fruit shell is infused with palm wine and taken as fever remedy while the seed decoction and oral ingestion is used as an enema, pneumonia and gastrointestinal disorder treatment respectively (Irvine 1961). The leaves are utilised as a vermifuge and the leaf sap is dripped in to the ear to treat otitis (Iwu 1993) while the bark is used as a laxative or purgative, anti-inflammatory, antipyretic, anthelmintic and treatment of venereal diseases (Ezeamuzie *et al*. 1994). The seeds are also used as external applications for abscesses treatment in Gabon. Research into pharmacological activities of *P. nitida* has further confirmed its wide range of ethnomedicinal uses (Erharuyi *et al*. 2014). Phytochemical evaluation of the plant parts has revealed significant amount of alkaloids in the seeds (Tane *et al*. 2002).

Despite the various applications of *P. nitida* in African folklore medicine, research into its propagation has been given little attention. The seeds of *P. nitida* are highly traded at premium prices in West African markets and thus hindering the amount of germplasm available for natural regeneration. The development of protocols for its vegetative propagation has significant implications for mass regeneration and sustainable utilization of the species. Hence, it becomes imperative to develop protocols for alternative propagation of *P. nitida* using stem cuttings. Although *P. nitida* have been rooted in-vitro using excised embryo from ripe seeds (Gbadamosi 2013), but the method is capital intensive and may not be affordable by low-income farmers.

Earlier studies on vegetative propagation of tropical tree species via stem cuttings have identified varying interacting factors such as cutting length, cutting positions, concentration of auxins, leaf area and sowing media that can be optimized to enhance rooting rate in tropical trees (Leakey 2014). It is important to note that cutting length will affect depth of insertion into the rooting medium and this may also affect the capacity of cuttings to root (Leakey 2004). Position of cutting within a shoot is another variable that influence successful rooting in tropical trees (Swarts *et al*. 2018). This variation is a function of concentration of some factors that run from base to the apex of stem tissue in relation to the chronological age like leaf water potential, leaf carbon balance, stem lignifications, carbohydrate content and respiration (Leakey 2014). Previous studies have reported the beneficiary effect of auxins in stimulating cell differentiations, starch hydrolysis and facilitation of carbohydrates and nutrients to the base of stem cuttings in some plants (Hartmann *et al*. 2002). Exogenous application of auxin may have stimulatory or inhibitory effect on rooting of some stem cuttings (Ofori *et al*. 1996, Mialoundama *et al*. 2002). Thus, it is essential to determine the appropriate concentration of auxin in order to ensure optimum rooting in stem cuttings.

Preliminary research on rooting of juvenile stem cuttings of *P. nitida* have reported the effect of different type of hormone, sowing medium and leaf area on its rooting capacity (Gbadamosi 2014). Optimum rooting in juvenile stem cuttings of *P. nitida* was achieved in mixture of sand and saw dust, IBA auxin and a leaf area of 50cm^2. However, information on the influence of cutting length, cutting positions and concentration of auxins on rooting of matured stem cuttings of *P. nitida* is still lacking given the role of these variables as it affect rooting success in tropical trees. Generally, rooting of stem cuttings using reproductively matured ortet or stockplant offers the advantage of reducing the juvenility period the plant takes to maturity in addition to the genetic gain it offers (Hartmann *et al*. 2002). Hence, this study investigated the effect of (i) cutting length (ii) cutting positions and (iii) concentration of IBA on rooting of matured leafy stem cutting of *P. nitida*.

MATERIALS AND METHODS

Study area

The study was conducted in the multipurpose tree improvement unit nursery (latitude 7°26'58″N and longitude 3°53'49″E) of Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria between September 2019 and December 2019. The rainfall distribution pattern is bimodal with peak in June and July. The dry season occurs between November and March while the wet season is usually between April and September. The mean minimum and maximum temperatures are 19.5°C and 34.9°C while relative humidity was between 50% and 86.7%.

Preparation of cuttings

Stem cuttings were obtained from a reproductively matured tree of eight years old grown in the botanical garden of FRIN. This operation was done early in the morning and cuttings were kept moist inside water. Two cutting lengths (6 and 8 cm) were prepared from the apical and basal region of the shoots and labeled accordingly. The lengths of the cuttings were chosen based on nodes arrangement and also to obtain maximum number of cuttings per cutting position on the shoot. Each leafy stem cutting consisted of double nodes with full internode underneath and two leaves. The leaf area was trimmed to 50 cm^2 using a paper template as recommended by Gbadamosi (2014). High humidity propagator was constructed following the method described by Leakey *et al*. (1990) and placed in a shade house roofed in transparent plastic sheets with 20 % light intensity.

Experimental design

A total of one hundred and ninety two (192) cuttings were set as a split-split plot experimental design with two replicates. Each main block contain two cutting positions (apical and basal), while two cutting length (6 and 8 cm) were tested at sub plot level and three hormone concentration (0, 0.1 and 0.2 mg/l) at the sub-sub plot level. Treatment are randomly applied at each level to experimental units containing 2 cutting positions x 2 cutting lengths x 3 hormone concentration x 8 cuttings x 2 replicates (n=192). Three concentrations (0 (control), 0.1and 0.2 mg/l) of Indole-3- butyric acid (IBA) were prepared and the cutting base was treated with the resultant solution using quick dip method (Usman and Akinyele 2015). Control treatment was 10µl alcohol only. The cuttings were inserted into sterilized washed river sand media and a total of eight cuttings were allocated per treatment and replicated twice.

Data collection and analysis

Rooting success and variables such as percentage of rooted cuttings, callused cuttings, number of roots, length of root, shoot length, number of shoot and leaves, were assessed at 16 weeks after setting. Data collected were subjected to analysis of variance (ANOVA), and mean values were separated using Duncan's multiple range test (DMRT) using SPSS. Percentage of rooted and callused cuttings data were subjected to Arcsin transformation using square root transformation prior to analysis while mean and standard deviation (sd) were calculated using descriptive statistics.

RESULTS

Percentage of rooted cuttings

The effect of cutting length and hormone concentration were significant ($P < 0.05$) on the percentage of rooted cuttings (Table 1). Interaction effect of cutting position and hormone concentration and interaction effect of the three factors were also significant ($P < 0.05$).

Percentage of rooted cutting was significantly higher in stem cutting length of 8 cm (24.3 %) compared to 6 cm cutting length (13.5 %) (Table 2). Similarly, control treatment (0 mg/l) had the lowest value of 10.4 % and this was significantly different from 0.1mg/l (23.5 %) and 0.2 mg/l (22.8 %) treated cuttings (Table 2). However, cutting positions had no significant effect, although, percentage of rooted cuttings was higher in apical position (20.8 %) than basal positions (16.9 %). In general, the percentage of rooted cuttings was relatively low across all the investigated factors.

Percentage of callused cuttings

Cutting length and cutting positions had significant ($P < 0.05$) effect on percentage of callused cuttings while hormone concentration and interaction between hormone concentration and other factors were not significant (Table 1). Percentage of callused cuttings was significantly increased in apical cuttings (50 %) compared to basal cuttings that recorded 22.6 % (Table 2).

Cutting length of 8cm had a significantly lower mean value of 30.4 % compared to cutting length of 6 cm that had 42.2 % (Table 2). The highest percentage (37.8 %) of callused cuttings was observed in cuttings treated with 0.2 mg/l of IBA, while 0.1 and 0 mg/l (control) treated cuttings had 37.0 % and 34.1 % respectively (Table 2).

Number of roots

The effect of cutting length was significant ($P < 0.05$) on the number of roots produced but stem's cutting position, hormone concentration and interaction between the factors were not significant (Table 1). The mean number of root was significantly higher (0.5) in stem cutting length of 8cm than 6 cm cutting length (0.2) (Table 2). Apical and basal cuttings produced comparable mean number of roots which are 0.4 and 0.3 respectively (Table 2). Mean number of roots were highest (0.5) in cuttings treated with 0.1 mg/l of IBA and lowest (0.1) in 0 mg/l (Table 2).

Table 1: Analysis of variance for the effect of IBA concentrations, cutting length, cutting positions and their interactions on rooting variables of *Picralima nitida*.

Source of variation	Df	Mean square					
		Rooted cuttings (%)	Callused cuttings (%)	Number of roots	Root length (cm)	Number of leaves	Shoot height
Cutting length	1	702.82**	842.31*	5.005*	0.113^{ns}	0.333^{ns}	0.253^{ns}
IBA concentration	2	871.01**	30.96^{ns}	2.333^{ns}	0.748^{ns}	1.130*	1.749*
Cutting positions	1	92.26^{ns}	4519.96**	0.005^{ns}	0.014^{ns}	0.000^{ns}	0.002^{ns}
Cutting position*cutting length	1	92.26^{ns}	390.22^{ns}	0.13^{ns}	0.002^{ns}	0.187^{ns}	0.582^{ns}
Cutting position*IBA concentration	2	882.04**	157.50^{ns}	1.08^{ns}	1.362^{ns}	0.141^{ns}	1.750*
Cutting length*IBA concentration	2	139.06^{ns}	98.27^{ns}	0.896^{ns}	0.306^{ns}	0.474^{ns}	0.484^{ns}
Cutting position*cutting length*IBA concentration	2	535.27*	118.27^{ns}	0.146^{ns}	0.412^{ns}	0.047^{ns}	0.003^{ns}
Error	180	673.15	162.13ns	0.886	0.489	0.324	0.562ns
Total	192						

*significant (p<0.05), ** highly significant (p<0.01); ns- not significant (p>0.05)

Table 2: Growth variables of *Picralima nitida* from different cutting length, IBA concentration and cutting positions (mean±sd).

Variables	Rooted cuttings (%)	Callused cuttings (%)	No. of roots/cutting	Root length (cm)	Number of leaves	Shoot height (cm)
Cutting length (cm)						
6	13.5^a±14.4	42.2^a±23.8	0.19^a ±0.6	0.21^a±0.8	0.1^a ± 0.5	0.2^a±0.7
8	24.3^b ± 9.5	30.4^b±11.3	0.51^b ±1.2	0.26^a±0.6	0.2^a ± 0.6	0.3^a±0.8
IBA concentration (mg/l)						
0	10.4^a±11.1	34.1^a±26.6	0.14^a ± 0.5	0.11^a±0.4	0.0^a±0.0	0.1^a±0.2
0.1	23.4^b±14.7	37.1^a±15.5	0.52^b ±1.2	0.32^a±0.8	0.1^a±0.6	0.4^b±0.9
0.2	22.8^b±10.3	37.8^a±15.9	0.39^b ±1.0	0.28^a±0.8	0.3^b±0.8	0.3^b±0.9
Cutting position						
Apical	20.8^a±13.5	50.0^a±16.3	0.35^a ±0.9	0.25^a±0.7	0.14^a±0.6	0.2^a±0.8
Basal	16.9^a±13.1	22.6^b±9.8	0.34^a ±0.9	0.23^a±0.4	0.14^a±0.5	0.2^a±0.8

*values in the same column with similar letters are not significantly different (p<0.05)

Length of root

Cutting length, stem's cutting position and hormone concentration had no significant influence on the length of root (Table 1). The mean length of root produced in 6cm and 8 cm cutting length were 0.2 cm and 0.3 cm respectively while that of apical and basal cuttings were 0.3 cm and 0.2 cm respectively (Table 2). Cuttings treated with 0.1 mg/l and 0.2 mg/l of IBA recorded a mean value of 0.3 cm which was higher than control (0mg/l)) cuttings that had 0.1 cm (Table 2).

Height of shoot

Hormone concentration and interaction effect of cutting position and hormone concentration was significant on the height of shoot (Table 1). Cuttings treated with 0.1 mg/l of IBA gave significantly higher (0.4 cm) mean value than control (0.1 cm), although similar to 0.2 mg/l treated cuttings (0.3 cm) (Table 2). In contrast, cutting length and cutting position did not have significant effect on the height of shoot (Table 1). Stem's cutting length of 6 and 8 cm had same mean value of 0.2 cm while stem cuttings from apical and basal positions had comparable mean value of 0.2 cm and 0.3 cm respectively (Table 2).

Number of leaves

Hormone concentration significantly affected the number of leaves produced by the sprouted cuttings while cutting length, cutting positions and their interactions had no significant effect (Table 1). Cuttings treated with 0.2mg/l of IBA had significantly higher (0.3) mean number of leaves than control (0 mg/l) and 0.1 mg/l treated cuttings (Table 2).

DISCUSSION

Effect of cutting length

Cutting length is an exogenous factor that strongly affects rooting activity in this study. Number of roots, percentage of rooted and callused *P. nitida* stem cuttings was enhanced in stem cutting length of 8 cm in comparison to 6 cm cutting length. This may be explained by greater food (carbohydrate) reserves in the longer cuttings compared to shorter cuttings (6 cm). Similar results were reported in *Khaya ivorensis* (Tchoundjeu and Leakey 1996), *Tinospora crispa* (Aminah *et al*. 2015) and *Picea abies* (OuYang *et al*. 2015) where long cuttings rooted significantly better than short cuttings. These findings were also in agreement with Naidu and Jones (2009) who attributed large storage reserve and photosynthetic area of longer cuttings to rooting success of *Eucalyptus* hybrid clones. In contrast, cutting length had no significant effect on rooting percentage of *Allanblackia floribunda* stem cuttings (Atangana and Khasa 2008) and *Milicia excelsa* (Ofori *et al*. 1996). Generally, capacity of stem cuttings for carbohydrate reserve is an important criterion for rooting stem cuttings of *Eucalyptus grandis* (Hoad and Leakey 1996).

Effect of IBA

Significant rooting success can be achieved in stem cutting propagation of *Picralima nitida* by optimizing auxin application. The results of this study indicated that percentage of rooted cuttings, shoot height and number of leaves was significantly affected by IBA application although the rooting percentage was low. Number of roots and length of root was not significantly affected by IBA treatment (Table 1). In this study, the significantly high rooting percentage recorded in *P. nitida* stem cuttings treated with hormone (IBA) further confirms the role of IBA in promoting adventitious root growth in stem cuttings of tropical trees (Atangana *et al*. 2011). This result is in conformity with the findings of earlier studies that exogenous application of IBA enhanced rooting in *Switennia macrophylla* (Hossain *et al*. 2004), and *Pentaclethra macrophylla* (Tsobeng *et al*. 2013).

The role of IBA in enhancing rooting percentage and sprouting in *P. nitida* stem cuttings can be explained by its stimulatory function in cutting's physiology which may facilitate transportation of nutrients and/or

Tanzania Journal of Forestry and Nature Conservation, Vol 90, No. 3 (2021) Special Issue*: Embracing Science and Technology in Nature Conservation. pp 84-92*

carbohydrate to the base of the cutting, thus, inducing rooting activity in the cuttings. In this study, it was evident that percentage of stem cuttings that formed callus was more than those rooted and this variation and the relatively low percentage of rooted cuttings might be attributed to the use of mature cutting which may suggest decreased sensitivity of aging tissues. Studies have found that the capacity of stem cuttings to form adventitious roots declines with increasing age of stockplant (Husen and Pal 2006, Amri *et al*. 2010). Moreover, some of the *P. nitida* cuttings that formed callus differentiated into roots, thus, it seems essential to have increased callus formation in order to enhance rooting because callus and root formation are associated with each other (Amri *et al*. 2010). Callus formation was observed in this study as a pre-cursor to adventitious rooting (Plate 1). Although, concentration of hormone had no significant effect on the rooting variables, however, treated cuttings performed significantly better than untreated ones. Researchers have shown that broader leaved cuttings require application of higher concentration of auxins in order to significantly improve their rooting ability (Tchoundjeu 1989).

Plate 1: Rooted matured leafy stem cutting of *Picralima nitida* 16 weeks after setting.

Effect of cutting position

Lack of significant effect on percentage of rooted cuttings between apical and basal cutting positions in this study may suggest similarity in cutting morphology, although apical cutting position recorded significantly higher percentage of callused cuttings than basal cuttings. Generally, comparable results were recorded between apical and basal cuttings for other rooting variables.

The result of this study contrast with other studies for some tree species like *Dalbergia melanoxylon* (Amri *et al*. 2010) and *Lobostemon fruticosus* (Swarts *et al*. 2018) where basal cutting positions gave optimum rooting and callusing percentage. It is possible that the lower callusing percentage observed in the basal cuttings might be due to decreased sensitivity of tissues in the basal cuttings. Ontogenetic aging due to maturation of tissues have been reported to reduce rooting success in physiological older shoots (Wendling *et al*. 2014). On the contrary, greater rooting percentages were recorded in apical cuttings compared to basal cuttings in *Lovoa trichilioides* (Tchoundjeu and Leakey 2001) and *Santalum austrocaledonicum* (Tate 2018). The highly significant interaction between cutting position and IBA concentration suggests that further work be carried out in this regard.

CONCLUSION

This study revealed that mature leafy stem cuttings of *P. nitida* can be propagated asexually. Exogenous application of IBA and cutting length of 8 cm enhanced rooting percentage of *P. nitida*. Cutting positions did not influence the rooting percentage although stem cuttings obtained from the apical position on the shoots had the greatest tendency to improve rooting capacity in the species. The relatively low rooting percentage observed across all the investigated factors might be attributed to age of the stockplant and/or genetic make-up of the species.

RECOMMENDATIONS

It is recommended that vegetative propagation of matured stem cuttings of *P. nitida* can be optimized when treated with IBA auxin using cutting length of 8 cm obtained from any position on the shoot. Further study may be necessary using higher concentrations of IBA to determine the possibility of enhancing rooting capacity in mature cuttings of *P. nitida.*

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