EFFECTS OF GROWTH MEDIA AND HORMONES ON THE SPROUTING AND ROOTING ABILITY OF *Massularia acuminata* (G. Don) Bullock ex Hoyl.

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ABSTRACT

Massularia acuminata is both a medicinal and economic tree species. Despite its usefulness, adequate attention has not been given to its propagation and cultivation. Therefore, this study investigated the effect of growth media and hormonal concentration on the sprouting and rooting of M. acuminata stem cuttings using Indole Butyric Acid (IBA), Naphathelene Acetic Acid (NAA) and Coconut water at four levels (0, 500/25, 1000/50 and 1500/100ppm/%).

Six hundred and forty eight (648) single node stem cuttings were collected from Forestry Research Institute of Nigeria. Using a 3 x 3 x 4 factorial experiment with three replicates, the cuttings were planted in three growth media (Sawdust, Riversand + Sawdust and Riversand) under a non mist propagator. Data on percentage sprouting, callusing, number of leaves, rooting, number of root, length of root and shoot length were analysed using Analysis of variance (ANOVA) and LSD at 5% probability level was used to compare significantly different means.

The results showed that growth media and hormonal concentration significantly affect percentage sprouting, shoot length, callusing, number of leaves, rooting, number of roots and length of root per cutting. Highest percentage sprouting (66.67%) and callusing (0.19) was recorded in riversand. Percentage sprouting (71.30%) and callusing (0.23) was greatly affected by coconut water. Highest number of roots (2.67), rooting (0.59cm), shoot length (1.11cm) and root length (0.59cm) were significantly affected by Level 2 (500ppm/25%). The untreated cuttings did better in terms of rooted cutting (0.71) and number of leaves (5.32). The highest number of roots (3.18) and root length (3.66cm) was recorded under NAA (1500ppm). IBA at 1000ppm had the highest shoot length (1.63cm).

The findings confirm the possibility of vegetative propagation for mass production of this species in afforestation programmes.

Key words: *Massularia acuminata*, growth media, hormonal concentration, single node stem cuttings.

INTRODUCTION

The forest, besides timber, contains many useful goods and services of subsistence and commercial value called Non-Timber Forest Products (NTFPs), which sustain rural people and rural economies. Today 75% of poor people in the world living in rural forest areas depend on NTFPs for their subsistence and 80% of forest people in the developing countries use NTFPs daily (Noubissie *et al.,* 2008). NTFPs include such diverse products like animal parts, leaves, sticks, local building materials, edible fungi, medicinal plants, forest foods, sponges, chewing sticks, fibres, gums and rattan canes, shrubs, among others. *Massularia*

acuminata (G.Don) Bullock ex Hoyl (Rubiaceae) species as part of the NTFPs family are the stems of a perennial evergreen shrub that are harvested from the forests ecosystem and processed into chewing stick for the traditional brushing of teeth in Southern Nigeria. *Massularia acuminata* known as *pako ijebu* or *orin ijebu* (Yoruba-Western Nigeria), is a tree growing up to 5 m high. Keay (1964) described the habit as a borderline case between a shrub and a tree. The plant has large oblanceolate. The large leaves are practically stalkless, elliptic, acuminata and almost glaborious. The flower which is 5cm long, narrowly ovoid beaked, borne in short auxillary lymes is seen in January of every year. Socio economically, the species provide employment for the peasants in both the Urban and Rural areas of West Africa through the exploitation and processing of the stem. The juice from the fruits is used as eye drops in Sierra Leone (Gill,1992). The stem is used as chewing stick for oral hygeine in Nigeria (Ndukwe *et a*l., 2004).This product constitutes rural industrial raw materials for the native chewing sticks industry, and cultural symbol for the Yoruba traditional medicine (FAO, 1996; Nkwatoh, 2000).The stem is also claimed to be used as an aphrodisiac and anticarcinogenic by making a decoction or an infusion (Gill, 1992). Despite the versatility of this species, adequate attention has not been paid to its propagation and cultivation. The exploitation of the stem of plant for commercial and medicinal purposes always prevents most of them from attaining reproductive age. With increasing socioeconomic development, population growth and continued exploitation of the tropical high forests of the West Africa, the stock of the species has been badly depleted. Thus, the species is now becoming seriously endangered (Oni and Ojo, 2002).Vegetative propagation technique by stem cutting has been recognized as a method of mass propagating exact coppices of desirable plants for clonal plantation, reforestation and for commercial purposes (Follosco- Edminston, 2002). Therefore, this study was carried out to investigate the potential of vegetatively propagating *Massularia acuminata* through stem cuttings with the aid of artificial stimulation with hormones.

MATERIALS AND METHODS

Study site: The experiment was carried out at the nursery of National Centre for Genetic Resources Conservation and Biotechnology (NACGRAB) Ibadan. It has a mean annual rainfall of 788-1884mm geographical location, altitude of 209m above sea level. It lies between latitude $7^0$22'N and longitude $3^0$50'E.

Preparation of leafy stem cuttings and planting medium

A total of 648 juvenile stem cuttings were obtained from *M. acuminata* plants raised in the West African Hardwood Improvement Project (WAHIP) Nursery of the Federal Research Institute of Nigeria (FRIN), Government Reservation Area Jericho, Ibadan, Oyo State. Shoots were collected early in the morning from stock plants .The cut shoots were kept moist after collection. The shoots were severed into cuttings of 3-6cm long; all leaflets were removed leaving only one at the top node.

The rooting hormone Indole-3-butyric acid (IBA), Naphthalene acetic acid (NAA) and coconut water were used and different concentrations were prepared. The powdery form of Indole butyric acid (IBA) and Naphthalene acetic acid (NAA) were prepared as prescribed by Leakey *et al.* (1990).They were prepared by weighing 0.5g, 1g and 1.5g each using electric weighing balance at NACGRAB laboratory, the weighed powder was diluted with 6 drops of Sodium hydroxide (NAOH) using a micro pipette. Thereafter, the resulting solution was diluted with 75ml of distilled water. The required concentrations (500ppm,1000ppm and 1500ppm) were obtained from the resulting solution. Coconut fruits were purchased from Bodija Market, Ibadan and the water was extracted in the laboratory. The water was then filtered with Whatman filter paper and diluted with distilled water to obtain different concentrations (25%, 50% and 100%) (Agele *et al*., 2013).

The cuttings were dipped into: indolebutyric acid (IBA), Naphthalene acetic acid (NAA) using quick method (Oni 1987; Gbadamosi and Oni, 2005; Akinyele, 2010) and coconut water. After the treatments, the cuttings were set in three layers containing river sand, sawdust and riversand +sawdust at a rate of 6 cuttings per tray in the propagator which were replicated thrice per treatment /levels. Thereafter, the propagating chamber was covered with clear transparent plastic and watered twice daily with a knapsack sprayer.

Non mist propagator used was constructed out of a wooden frame enclosed in clear polythene to make base water tight. The propagator was covered with tight fitting hinged lids sealed with clear polythene to maintain high humidity around the cuttings and allow the penetration of light into propagator. The base chambers were filled with sand about 5cm. Successive layers of large stones to a depth of 10-15cm, small stones and gravel to a depth of 20cm and then sprayed with water to a depth of about 5cm. To prevent insect attack and other micro organisms, the propagator was sprayed with Gladiator insecticide and left for some days.

The experiment was laid out in completely randomized design with factorial arrangement. There were 3 treatments, each treatment having 6 cuttings with 3 replications. Data were collected after sixty days for percentage sprouting, rooting, callusing, number of leaves, shoot length, number of roots and length of longest root per cuttings. The number of root and leaves per cutting were assessed using physical count. The length of longest root and shoot per cutting were assessed by the use of meter rule. Data collected were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Least Square Difference (LSD) test.

RESULTS

Percentage sprouting: Percentage sprouting was significantly influenced by growth media and auxin but not significantly affected by interaction between growth media and other factors (Table 1). Among the growth media, the highest percentage sprouting (66.67%) was recorded under River sand (Table 2). Highest percentage sprouting (71.30%) was observed under coconut water (Table 3). The interaction between hormone and concentration shows that coconut water at 0% gave the highest sprouting percentage (87.04%) while NAA at 1000ppm (27.78%) (Table 5).

Table 1: Summary of the analysis of variance (ANOVA) of the different parameters assessed in the study

Treatments	Sprouting %	Number of leaves	Shoot length	Callusing	Rooting	Number of roots	Root length
GM	0.00*	0.34^{ns}	0.01*	0.01*	0.78^{ns}	0.55^{ns}	0.07^{ns}
HT	0.00*	0.01*	0.10^{ns}	0.00*	0.37^{ns}	0.14^{ns}	0.02*
HC	0.00*	0.00*	0.04*	0.65^{ns}	0.00*	0.04*	0.16^{ns}
GM* HT	0.18^{ns}	0.86^{ns}	0.16^{ns}	0.00*	0.58^{ns}	0.05*	0.73^{ns}
GM * HC	0.08^{ns}	0.59^{ns}	0.44^{ns}	0.21^{ns}	0.78^{ns}	0.69^{ns}	0.86^{ns}
HT * HC	0.00*	0.00*	0.00*	0.07^{ns}	0.00*	0.03*	0.04*
GM * HT * HC	0.06^{ns}	0.09^{ns}	0.20*	0.21^{ns}	0.58^{ns}	0.08^{ns}	0.83^{ns}

*=Significant ($\alpha=_{0.05}$), GM=Growth media, HT=Hormone type, HC=Hormone concentration,
Ns= not Significant ($\alpha=_{0.05}$)

Table 2: Effects of growth media on *M. acuminata* stem cuttings.

Growth media	Sprouting %	Shoot length(cm)	Callusing	Rooting
Riversand	66.67[a]	0.78[b]	0.19[a]	0.53[a]
Sawdust	55.09[b]	0.99[ab]	0.13[ab]	0.50[a]
Riversand +Sawdust	55.09[b]	1.16[a]	0.09[b]	0.49[a]

Means with the same alphabet are not significantly different from each other.

Table 3: Effect of growth regulators on *M. acuminata* stem cuttings

Growth regulators	Sprouting%	Number of leaves	Callusing	Root length(cm)
IBA	58.33[b]	3.38[b]	0.13[b]	2.08[b]
NAA	56.94[b]	3.24[b]	0.05[c]	2.89[a]
Coconut water	76.39[a]	4.30[a]	0.23[a]	2.25[b]

Means with the same alphabet are not significantly different from each other
IBA=Indolebutyric acid, NAA =Naphthalene acetic acid

Number of leaves: Number of leaves was not affected by growth media, growth media and hormone type, growth media and hormone concentration and interaction between the three factors. Hormone type, hormone concentration and interaction between hormone type and hormone concentration had significant effect on number of leaves (Table 1).

Coconut water produced the highest mean number of leaves (4.30) while NAA had the least (3.24). Interaction between hormone type and concentration shows that coconut water at control (0%) had the highest number of leaves (5.32) while NAA at Level 3 (1000ppm) had the least (1.43) number of leaves (Table 5).

Shoot length: Shoot length was not affected by hormone type, growth media and hormone type and growth media and hormone concentration. Growth media, hormone concentration, hormone t type and hormone concentration and the interaction between the three factors had significant effect on shoot length. The result shows that Riversand+ Sawdust had the highest mean shoot length (1.16cm) while Riversand alone had the least (0.78) shoot length.

Interaction between hormone type and concentration(Table 4) shows that IBA at (1000ppm) had the highest mean shoot length (1.63cm).while NAA at (1000ppm) had the least (0.33cm).

Callused cuttings: Growth media, hormone type and interaction between growth media and hormone type had significant effect on callusing (Table 1). Hormone concentration and the interaction between the three factors had no significant effect on callusing. Cuttings treated in river sand gave the highest callusing (0.19) followed by Sawdust (0.13) (Table 2). The highest mean value of callused cutting was obtained under Coconut water (0.23) while the lowest was obtained under NAA (0.05).

Table 4: Effects of hormone concentration on *M. acuminata* stem cuttings.

Hormone concentration	% sprouting	Number of leaves	Shoot length(cm)	Rooting	Number of roots	Root length (cm)
Level 1	68.53^{a}	4.49^{a}	1.04^{a}	0.58^{a}	2.23^{ab}	2.63^{ab}
Level 2	60.49^{a}	4.43^{a}	1.11^{a}	0.59^{a}	2.67^{a}	2.68^{a}
Level 3	59.26^{b}	3.19^{b}	1.03^{a}	0.45^{b}	1.78^{b}	1.97^{b}
Level 4	47.53^{c}	2.46^{b}	0.73^{b}	0.42^{b}	2.14^{ab}	2.33^{ab}

Means with the same alphabet are not significantly different from each other.
Note: L1=control (0), L2=25/500ppm/%, L3=50/1000ppm/% and L4=100/1500ppm/%

Table 5: Effect of hormone type and concentration on *M. acuminata* stem cuttings

HT	HC(ppm/%)	Sprouting %	Number of leaves	Shoot length (cm)	Rooting	Number of roots	Length of roots (cm)
1BA	**500**	**59.26^{cd}**	**3.60^{cdef}**	**0.97^{bc}**	**0.50^{bcd}**	**2.50^{bcde}**	**2.45^{abc}**
	1000	**75.92^{fg}**	**4.11^{defg}**	**1.63^{d}**	**0.57^{bde}**	**1.94^{abcd}**	**2.20^{ab}**
	1500	**48.14^{cb}**	**2.41^{abc}**	**0.83^{bc}**	**0.35^{ac}**	**1.20^{a}**	**1.54^{a}**
Untreated	**0**	**61.11^{cde}**	**3.41^{bcde}**	**0.90^{bc}**	**0.46^{abc}**	**2.02^{abcd}**	**2.16^{ab}**
NAA	**500**	**51.85^{cb}**	**4.72^{efg}**	**1.26^{cd}**	**0.69^{de}**	**2.61^{bcd}**	**2.89^{bcd}**
	1000	**27.78^{a}**	**1.43^{a}**	**0.33^{a}**	**0.30^{a}**	**1.56^{ab}**	**1.60^{a}**
	1500	**40.74^{ab}**	**2.04^{ab}**	**0.69^{ab}**	**0.52^{bcde}**	**3.35^{c}**	**3.66^{d}**
Untreated	**0**	**57.41^{cd}**	**4.74^{efg}**	**1.01^{bc}**	**0.57^{bde}**	**2.28^{bcd}**	**3.39^{cd}**
CW	**25**	**70.37^{def}**	**4.96^{fg}**	**1.10^{bc}**	**0.57^{bde}**	**2.89^{de}**	**2.71^{abcd}**
	50	**74.08^{efgf}**	**3.98^{defg}**	**1.12^{bc}**	**0.48^{abc}**	**1.83^{abc}**	**2.13^{ab}**
	100	**53.70^{cb}**	**2.93^{bcd}**	**0.66^{ab}**	**0.39^{abc}**	**1.85^{abcd}**	**1.79^{ab}**
Untreated	**0**	**87.04^{g}**	**5.32^{g}**	**1.22^{cd}**	**0.71^{e}**	**2.39^{bcde}**	**2.35^{abc}**

Means with the same alphabet are not significantly different from each other

Rooted cuttings: Rooting of cuttings is most influenced by hormone concentrations rather than the other treatments (Table 2). This is evidenced by the result of this study in which growth media, hormone type, interaction between hormone concentration and type, growth media and hormone type and concentration and also interaction between the three factors showed no significant difference on rooting.. However, among the growth media, river sand gave the best (0.53) in rooted cuttings (Table 2).

L2(500ppm/25%) had the highest rooted cutting (0.59) while the lowest was at L4 (1500ppm/100%) (0.42)(Table 4).

The interaction between hormone type and hormone concentration (Table 5) indicates that coconut water at control (0%) had the highest rooted cuttings (0.71) while the lowest was obtained under NAA at 1500ppm (0.30).

Number of roots: Both hormone concentration, interaction between growth media and hormone type, hormone type and hormone concentration had significant effect on number of roots.

Growth media, hormone type and interaction between the three factors had no significant difference on number of roots per cutting (Table 1).The results shows that L2 (500ppm/25%) had the highest mean number of roots (2.67) while L3 (1000ppm/50%) had the least (1.78) (Table 4). The interaction between hormone type and concentration indicates that NAA at 1500ppm had the highest (3.35) number of roots while IBA at 1500ppm had the lowest (1.20) number of roots (Table 5).

Length of root: Hormone type, interaction between hormone type and concentration had significant effect on length of root of *M. acuminata.* Length of root is not affected by growth media, hormone concentration, and interaction between growth media and hormone type, growth media and hormone concentration and also between the three factors (Table 1).

LSD test (Table 3) revealed that NAA had the highest (2.89cm) on length of root while the least is IBA (2.09cm) on length of root. The interaction between hormone type and concentration shows that NAA at 1500ppm (3.66cm) had the highest mean length of root while IBA at 1500ppm had the least (1.54cm) length of root (Table 5).

Discussions

Among the growth media, the highest percentage sprouting was recorded under River sand. This collaborates with the work of Florence *et al* (2013) who reported the best sprouting in river sand. This could be due to the fact that river sand has better aeration potential and drainage which enhance development and spreading of roots. Highest percentage sprouting was observed under coconut water followed by IBA. This agrees with Trevisan *et al.* (2005) who demonstrated the advantage of coconut water for stem elongation and plant development in fruit. It is evident from the finding that coconut water contains sugar, amino acid, myo-inositol, and microconstituents of phenyl urea for tree development (Acha *et al*., 2004; Agele *et al*., 2010). Untreated (control) gave the highest sprouting percentage. A similar work was reported by Adekola and Akpan (2012) who stated that untreated cuttings performed better than any other hormones on survival and sprouting of *J. curcas*. This may be due to the fact that juvenile tissues of certain plants tend to have higher phenols than their mature forms. Coconut water produced the highest mean number of leaves while NAA had the least .This indicates that treating stem cuttings with auxins before planting is essential for rooting. Untreated cuttings gave the highest number of leaves.

The results therefore imply that the species might not really require application of more exogenous hormone to produce leaves. The result shows that Riversand+ Sawdust had the highest mean shoot length while Riversand alone had the least. This agrees with the statement that treating stem cuttings with different hormone concentrations before planting in a suitable rooting medium is required for effective rooting (Kelen and Ozkan, 2003, Coutessa and Valentini, 2011).This indicates that the species require little amount of exogenous hormone for shoot formation. Interaction between hormone type and concentration shows that IBA at (1000ppm) had the highest mean shoot length while NAA at (1000ppm) had the least. This is in conformity with the work of Sally (2012) who reported the highest shoot height in IBA.

Cuttings treated in river sand gave the highest callusing followed by Sawdust. Ofodile *et al* (2013) confirms river sand as the most suitable medium for propagation of of *Grongronema latifolia* using stem cuttings. This could be due to the fact that river sand has better aeration potential and drainage which enhance development and spreading of roots. The highest mean value of callused cutting was obtained under Coconut water while the lowest was obtained under NAA. This is similar to the work of Gbadamosi and Oni (2005) who reported that the least mean

value for callusing was obtained at NAA. The rooting of cuttings was increased by auxin treatment, especially treatment by Coconut water. This in collaboration with the work of Komamine *et al.*, (1990), Agele *et al*., (2010) who reported that coconut water promote root Formation and shoot initiation. Untreated cuttings had the highest rooted cuttings while the lowest was obtained under NAA at 1500ppm. This is in conformity with the work of Mudge and Brenna (1999) who reported better survival and rooting under control treatment. This finding is also in agreement with Oni and Ojo (2002) who reported similar work on *Massularia acuminata* that without treatment is an added advantage. This may be due to the juvenility factor of the plant or different type of auxins used. Juvenile tissues of woody plants tend to have higher levels of endogenous auxins and are less differentiated and therefore more prone to dedifferentiated (Hartmann *et al.,* 2000).This suggests that *M. acuminata* leafy stem cutting had high levels of endogenous auxins to initiate rooting.

The findings indicate that the species need little amount of hormone concentration to develop roots. This could be due to difference in the amount of polysaccharides hydrolyzed.

The interaction between hormone type and concentration indicates that NAA at 1500ppm had the highest number of roots while IBA at 1500ppm had the lowest. This is similar to the work carried by Memon *et al* (2013) who reported the higher concentration of NAA had a pronounced inhibitory effect on number of roots at NAA. Similarly NAA treatment was found to have inhibitory effect on *Tectona grandis* stem cuttings (Husen and Pa; 2007a).The findings indicate that the species require higher application of auxins to boost the level of exogenous hormone in order to promote rooting. The results from the interaction between hormone type and concentration indicate NAA at 1500ppm gave the best for root length. This indicates a decrease in hormone concentration may reduce length of the root. This is in agreement with the work of Memon *et a*l (2013) who stated the length of roots increased with the increasing concentration of NAA. Tiwari and Das (2010) also confirmed that NAA had significant effect on the cuttings of *Embelia tsjariam and Caesalpinia bonduc.*

The increase in length of roots in cuttings treated with growth regulators may be due to the accumulation of metabolites at the site of application of auxins, cell enlargement, enhanced hydrolysis of carbohydrates, synthesis of new proteins, and cell division (Strydem and Hartman, 1960).

Plate 1: Rooted cuttings showing root length under NAA, Coconut water and IBA at different concentration.

Conclusion

Rooting and sprouting potential of stem cuttings is one of the critical steps in plant propagation of tree species. Due to limited rates of success in sprouting and rooting, many researchers tried various auxins for initiation and development of cuttings in various tree species. Propagation by vegetative means is often the best way to preserve selected traits in tree species. Auxins are known to promote adventitious root development of stem cuttings, through their ability to promote the initiation of lateral root primodia and enhance transport of carbohydrates to cuttings' bases (Hartmann *et al.,* 1990)..Cuttings treated with no hormone (control) responded to a certain degree, which is an indication that the endogenous hormone in the species studied is enough to initiate rooting but for optimum result, a certain concentration of exogenous hormone is needed to boost the process.This shows that with or without auxins treatment have the potential for enhancing leafy stem cuttings of *M. acuminata*. Thus, rooting of stem cuttings of *M. acuminata* presents a viable propagation system to be used in enrichment planting programme.

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