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Original Article

Vegetative propagation of African black pepper (*Piper guineense*): The role of growth hormones and rooting media

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ABSTRACT

Piper guineense seed is valued for food, medicinal and economic purpose. Therefore, providing alternative means of propagation other than the use of seed becomes necessary. This study was carried out to investigate the effect of growth hormones and media on the rooting, growth, and development of *P. guineense.* Six hundred 5 cm stem cuttings were subjected to four different hormones (Naphthaleneacetic acid [NAA], Indole 3 butyric acid [IBA], NAA+IBA, and control) and three media (river sand, sawdust, and topsoil) in a factorial experiment fitted into completely randomized design in five replications. Data on number of rooted cuttings, callused cutting, and dead cuttings as well as number of roots, root length, number of leaves, and shoot length were subjected to analysis of variance (ANOVA) and turkey HSD at 5% probability level was used to compare significantly means. The results showed that growth media significantly affected number of root, root length, number of leaves as well as shoot length as river sand produced the highest number of roots (6.177) and root length (4.412 cm). Topsoil produced the highest number of leaves (4.096) and shoot length (6.778 cm). The hormones significantly affected all the variables except number of callused cuttings as NAA + IBA had the highest callused cuttings, number of root (6.556), root length (4.568 cm) number of leaves (4.377), and shoot length (7.002 cm). The two way interaction between hormones and media significantly affected number of root, root length, and number of leaves as NAA+IBA rooted in river sand had the highest number of roots (7.730) and root length (5.5549 cm) and NAA+IBA rooted in top soil had the highest number of leaves of 5.145. The findings confirm the possibility of vegetative propagation of *P. guineense* through stem cuttings. NAA+IBA is considered the best growth hormones for *P. guineense.*

Keywords: Indole 3 butyric acid, media, naphthaleneacetic acid, number of root, number of shoot, *Piper guineense*

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INTRODUCTION

Piper guineense is a flowering vine belonging to the family *Piperaceae*. It is commonly known as *African black pepper.* For ages, man has benefitted from African black pepper as a source of food, income generation and medicine. It is mostly valued for its seeds which are usually dried and used as spice for seasoning. The dried seeds are also traded both within Nigeria and outside the country. The leaves are also used as vegetable in making soup in Nigeria. *P. guineense* play important roles in many folklore medicines as every part of it has proven to be useful in pharmacological activities. The roots, seeds, stem bark, and leaves are used in traditional medicine for the treatment of ailments which include vomiting, worms, tonsillitis, rheumatism, and stomach aches.[1-3]

Despite the versatility of this spice, adequate attention has not been paid to its propagation and cultivation. The exploitation of the seeds for commercial and medicinal purposes coupled with unavailability of viable seeds as most of its seeds are either harvested before full maturity or dried for consumption due to high demand militated against its propagation. Since viable seeds of *P. guineense* are not readily available, it therefore become imperative that alternative form of propagation be initiated other than the conventional use of seeds.

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Plants can be propagated sexually by the use of seeds or asexually by the use of plant parts other than seeds. Asexual propagation is the regeneration of new individuals from vegetative organs such as stems, roots, leaves, buds, and even single cells.[4] Plant propagated by seeds result in lots of genetic variability. Vegetative propagation as mean of plant multiplication is noted for producing plants that are true-to-type. It is a means of mass-producing improved plants that are genetically superior. It has also proved to be a better option for plant propagation where seeds collection becomes a problem.[5]

Cutting is a form of vegetative propagation, which can be either stems, roots or leaves that are detached from plants and used to clonally multiply new plants.[6] Successes in cuttings are achieved by root initiation and growth as well as shoot formation. Many scholars had documented several factors affecting adventitious root formation in stem cuttings. This include: Genotypes,[7] physiological age,[8] ontogenetic age of the trees,[9] media used for the cuttings,[10] and the use of exogenous root promoting substances, especially auxins.[11,12]

Auxins can be endogenous in nature, the plant inherent auxins or synthetic auxins, which are mostly applied to plants as root promoting substances. The most commonly applied ones are Indole-acetic acid (IAA), Indole 3 butyric acid (IBA), and naphthalene acetic acid (NAA). These are mostly applied at the basal portions of the stem cuttings. Mixtures of two types of auxins are often used and are sometimes more effective than either component alone.[13,14] However, it is important to note that in some of the vegetative propagated species, adventitious root formation can take place without the application of any growth hormones.[15]

Knowledge of application of auxins is a giant step into achieving success in cuttings through its usage. Auxins have both inhibitory as well as stimulatory effects on cuttings. Application of certain auxins may have stimulatory or inhibitory effect on rooting of some plants.[16-19] Thus, it is essential to determine the right auxin for certain plant in order to ensure optimum rooting in stem cuttings. For example, Yusnita *et al*.[14] reported that NAA was the most effective auxin treatment to promote root initiation but a combination of NAA and IBA produced higher root length, better root morphology, and higher shoot sprouting in Malay apple semi-hardwood cuttings. Kumar and Swarnker[20] reported that rooting and sprouting in *Jathropha curcas* was more with IBA than NAA. Sally[21] also reported the highest shoot height of *Treculia africana* when cuttings are treated with IBA.

Rooting media type used is also another important factor determining the success of root and shoot development in cuttings. There exist a number of interactions of factors within the media such as water, oxygen, and nutrient availability.[22-24] These factors influence the growth and development of the cuttings.

This study was therefore thoughtfully carried out to investigate and document the possibility of propagating *P. guineense* through stem cuttings and also to determine the right growth hormones and growing media that will be necessary for optimum rooting and development of *P. guineense* cuttings with the view of mass producing good quality planting stock.

MATERIALS AND METHOD

Sources of Experimental Materials

The cuttings used in this experiment were collected from young climbing vines of *P. guineense* raised in the nursery with secateurs, into a bucket containing water to prevent transpiration.

The synthetic growth hormones used in this experiment is IBA and NAA. These were used in their powdery form as described by Leakey *et al*.[25]

The media used include: Cure sawdust, river sand, and topsoil. The cured sawdust was collected from forest product and development unit. The river sand was collected from flowing river while the top soil was collected from forest arboretum all at Forestry Research Institute of Nigeria (FRIN).

Experimental Site

The experiment was conducted at tree physiology and breeding nursery of sustainable forest management department of Forestry Research Institute of Nigeria, Ibadan (FRIN). FRIN is located on the longitude 070 23'18''N to 070 23'43''N and latitude 03051'. 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%.[26]

Experimental Procedure

A total of 600 juvenile stem cuttings were obtained from the plants raised in the nursery. The collected cuttings were cut slantly into 5 cm length. The leaves were reduced to half.

The cuttings were dipped into the different hormones in quick successions and set into the different media contained in a 15 cm propagating sieve at 10 cuttings per sieve.

Thereafter, the cuttings were transferred into a propagating chamber constructed following the method described by Howland,[27] modified by Leakey and Longman[28] and further modified and used by Leakey.[25] This was placed in a shade house roofed in transparent plastic sheets with 20% light

intensity. The cuttings and the propagator were sprayed with fungicide. Watering was done once daily with the aid of hand sprayer. The cuttings were monitored daily for freshness and emergence of roots for 12 weeks, after which the plants were assessed.

Experimental Design and Data Analysis

The experiment was laid out in completely randomized design with factorial arrangement.

There were 2 factors. Factor 1 is growth hormones (NAA, IBA, NAA + IBA, and control) and factor 2 is rooting media (river sand, sawdust, and topsoil). Each of the treatment had 10 cuttings with five replications.

Data were collected on rooted cuttings, callused cuttings, dead cuttings, number of roots, root length (mean of three longest roots), number of leaves, and shoot length.

The number of root and leaves per cutting was assessed using physical count. The root length and shoot per cutting were assessed by the use of meter rule. Data collected were subjected t Analysis of Variance (ANOVA) using[29] and significant means were compared using Tukey HSD at 5% level of probability.

RESULTS

High rooted cuttings of over 50% were observed in all the treatment combinations except IBA treated cutting rooted in topsoil. The result gotten from all the treatment combinations shows that the untreated cuttings rooted in topsoil produced the highest percentage rooted cuttings of 77% [Table 1]. Next to this are the cuttings treated with a combination of NAA and IBA rooted in sawdust (73%) and the ones rooted in river sand (71%). The least percentage rooted cuttings of 48% was observed in cutting treated with IBA rooted in topsoil. In terms of callused formation, the cuttings treated with IBA rooted in topsoil had the highest percentage of callused cutting of 28% and the least was seen in NAA treated cuttings rooted in river sand. Low mortality of cuttings was observed in all the treatment combination when compared to the percentage survived (rooted) cuttings. In all, the untreated cuttings had the least mortality percentage.

The mean square analysis from the ANOVA revealed that the four different hormones used significantly affected the entire cuttings variable assessed except callused cuttings [Table 2]. The three media used do not have significant effect on the initial rooting variables such as rooted, callused, and dead cuttings but the developmental variables of the cutting (number of roots, root length, number of leaves, and shoot length) were significantly affected by the media used. The two way interaction between growth hormones and rooting media only shows significant effect on three out of the seven variables assessed. These variables are number of root, root length, and number of leaves.

Table 3 presents the effect of hormones on the rooting and growth of *P. guineense*. The followed up test showed that though the untreated (control) cuttings produced the highest rooted cuttings and callused cuttings, the cuttings treated with NAA and IBA had the highest of all the growth developmental variables. The cuttings rooted through this combination were able to grow faster by accumulating higher number of roots, longer roots, higher leaf production as well as longer shoot when compared to other types of hormones. Combination of NAA+IBA had the highest callused cuttings, number of root (6.556), root length (4.568 cm) number of leaves (4.377), and shoot length (7.002 cm). Application of IBA resulted to higher mortality of cuttings of *P. guineense.*

The post test result of the effect of rooting media on rooting and growth of cuttings of *P. guineense* is presented in Table 4. Although the rooting media used had no significant effect on the initial rooting ability of *P. guineense* as all the media produced the same effect on rooted, callused, and dead cuttings [Table 2]. The effect of media was much pronounced on the growth variable of *P. guineense* as the river sand used produced the highest number of roots (6.177) and root length of 4.412 cm [Table 4]. Topsoil produced the highest number of leaves of 4.096 and shoot length of 6.778 cm.

The interaction between hormones and media showed that untreated cuttings rooted in forest soil (topsoil) produced the highest number of rooted cuttings of 15.4 and the least rooted cuttings of was observed IBA treated cuttings rooted in topsoil which was not significantly different from each other [Table 5]. This trend was reversed in the number of callused cuttings

Table 1: Percentage rooting ability of *Pipers guineense* as affect by growth hormones and rooting media

Growth hormone	Rooted cuttings (%)			Callused cuttings (%)			Dead cuttings (%)		
	River sand	Sawdust	Topsoil	River sand	Sawdust	Topsoil	River sand	Sawdust	Topsoil
NAA	60.00	57.00	57.00	13.00	19.00	16.40	28.00	24.00	27.60
IBA	56.00	61.00	48.00	14.00	23.00	28.00	30.00	21.00	24.00
NAA+IBA	71.00	73.00	57.00	17.00	16.00	22.00	16.00	11.00	21.00
Control	66.00	65.00	77.00	21.00	20.00	12.00	13.00	15.00	10.00

NAA: Naphthaleneacetic acid, IBA: Indole 3 butyric acid

Table 2: Mean square analysis for the rooting ability of *Pipers guineense* as affected by growth hormones and rooting media

Sources of variation	DF	Mean square						
		Rooted cuttings	Callused cuttings	Dead cuttings	Number of roots	Root length	Number of leaves	Shoot length
Growth hormones	3	28.667**	3.517^{ns}	28.667**	6.386**	3.096**	2.868**	7.388**
Rooting media	2	4.117^{ns}	3.626^{ns}	3.416^{ns}	8.310**	5.233**	3.698**	9.806**
Growth hormones* Rooting media	6	9.717^{ns}	4.937^{ns}	2.674^{ns}	3.329**	2.129**	1.562**	3.621^{ns}
Error	48	4.592	2.466	2.838	0.078	0.053	0.012	2.265
Total	59							

ns: Non significant, **Significant @ P<0.05

Table 3: Mean effect of growth hormones on rooting and growth of cuttings of *P. gineense*

Hormones	Rooted cuttings	Callused cuttings	Dead cuttings	Number of root	Root length	Number of leaves	Shoot length
NAA	11.6^{bc}	3.16^{a}	5.31^{a}	5.463^{b}	3.807^{b}	3.647^{b}	5.835^{a}
IBA	11.0^{c}	4.33^{a}	5.00^{a}	5.299^{bc}	3.6925^{bc}	3.538^{c}	5.659^{a}
NAA+IBA	13.4^{ab}	3.67^{a}	3.2^{b}	6.556^{a}	4.568^{a}	4.377^{a}	7.002^{a}
Control	13.87^{a}	3.60^{a}	2.53^{b}	5.096^{c}	3.552^{c}	3.395^{d}	5.423^{a}

Means with the same alphabet are statistically the same and means with different alphabet are different according to Tukey HSB means separation at 5% level of probability.

NAA: Naphthaleneacetic acid, IBA: Indole 3 butyric acid

Table 4: Mean effect of rooting media on rooting and growth of cuttings of *P. guineense*

Rooting media	Number of root	Root length	Number of leaves	Shoot length
River sand	6.177^{a}	4.412^{a}	3.262^{c}	5.694^{ab}
Sawdust	4.906^{c}	3.390^{c}	3.859^{b}	5.467^{ab}
Forest soil (Top soil)	5.729^{b}	3.912^{b}	4.096^{a}	6.778^{a}

Means with the same alphabet are statistically the same and means with different alphabet are different according to Tukey B means separation at 5% level of probability.

produced. In terms of cutting mortality, the highest number of dead cuttings was observed in IBA treated cuttings rooted in river sand which was not significantly different from other treatment combinations. Cutting treated with the combination of NAA and IBA rooted river sand had the highest number of roots of 7.730 and root length of 5.5549 cm which was significantly different from others. This combination treated cuttings rooted in top soil had the highest number of leaves of 5.145 and shoot length of 8.268 cm.

DISCUSSION

Effect of Rooting Media on Rooting and Survival of *P. guineense* Cuttings

The results demonstrated that *P. guineense* can root successfully on any of the three media used as indicated by non-significant different of rooting media on rooted, callused as well as dead cuttings. However, in terms of cutting growth and development, the media used had significant effect on number of root, root length, number of leaves as well as shoot length. River sand performed better than the other two media in terms of the root component (number of roots and root length). The better performance of river sand for higher number of roots and root length could be attributed to the properties of river sand. River sand has better aeration potential and drainage which enhance development and spreading of roots. The large pores of river sand also allows for easy movement of air as well as water, this will aid in the proliferation of fine roots in the soil with little or no obstruction. In plant propagated through cuttings, the roots formed are tender and delicate and when faced with obstruction in the soil can hinders its development and thereby affecting the plant development. This findings collaborate collaborates with the work of Florence *et al*.,[30] and Yakubu *et al*.,[31]

The top soil used performed better than the other two media in terms of the shot component of the cuttings as topsoil had the highest number of leaves as well as the highest shot length. Top soil contained reasonable amount of nutrient which aided in producing higher number of leaves and shoot length.

Effect of Growth Hormones on Rooting and Survival of *P. guineense* Cuttings

The success of cuttings is evident by root and shoot formation and growth which is affected growth hormones used.[10,14,19,32,33]

Table 5: Mean interactive effect of growth hormones and rooting media on rooting and survival of cuttings of *P. guineense*

Growth hormones	Rooted	Callused	Dead	Number of root	Root length	Number of leaves	Shoot length
NAA							
River sand	12.00^{a}	2.400^{a}	5.600^{a}	5.298^{cd}	3.550^{de}	3.030^{e}	5.718^{a}
sawdust	11.400^{a}	3.800^{a}	4.800^{a}	4.650^{cfg}	3.246^{ef}	4.288^{b}	6.890^{a}
Forest soil	11.400^{a}	3.28^{a}	5.52^{a}	6.442^{b}	4.624^{b}	3.624^{d}	4.896^{a}
IBA							
River sand	11.200^{a}	2.800^{a}	6.000^{a}	5.139^{cde}	3.443^{ef}	2.939^{ef}	5.546^{a}
sawdust	12.200^{a}	4.600^{a}	4.200^{a}	4.51^{fg}	3.148^{ef}	4.159^{b}	6.683^{a}
Forest soil	9.600^{a}	5.600^{a}	4.800^{a}	6.248^{b}	4.393^{b}	3.515^{d}	4.749^{a}
NAA+IBA							
River sand	14.2000^{a}	3.400^{a}	3.200^{a}	7.730^{a}	5.549^{a}	3.442	4.651^{a}
sawdust	14.6000^{a}	3.200^{a}	2.200^{a}	4.882^{def}	3.271^{ef}	3.951^{c}	5.269^{a}
Forest soil	11.400^{a}	4.400^{a}	4.200^{a}	4.285^{g}	2.991^{f}	5.1456^{a}	8.268^{a}
Control							
River sand	13.200^{a}	4.200^{a}	2.600^{a}	6.357^{b}	4.260^{c}	3.636^{d}	6.862^{a}
Sawdust	13.00^{a}	4.000^{a}	3.00^{a}	5.580^{c}	4.260^{bc}	2.792^{f}	6.349^{a}
Forest soil	15.400^{a}	2.600^{b}	2.000^{a}	6.119^{b}	4.485^{b}	4.348^{b}	5.875^{a}

Means with the same alphabet are statistically the same and means with different alphabet are different according to Tukey HSB means separation at 5% level of probability.

NAA: Naphthaleneacetic acid, IBA: Indole 3 butyric acid

IBA, NAA, or a mixture of the two auxins as root promoting substance are often used in cuttings.[7,34,35] From this study, untreated cutting o*f P. guineense* performed better in terms of rooting ability than the treated cuttings as the untreated cutting had the highest percentage rooted cuttings of 77% [Table 1], but performed poorly in terms of number of roots, root length, number of leaves and shot length [Table 3]. Although higher percentage of rooted cuttings was recorded in untreated cutting when compared to the auxin treated cutting, significant and higher number of roots, root length, number of leaves, and shoot length was achieved in stem cutting propagation of *P. guineense* by a combination of NAA+IBA application. This is an indication that these untreated cuttings of *P. guineense* responded to a certain degree which means that the inherent hormone in this species is only enough to initiate rooting but inadequate for optimum growth and development of the cuttings suggesting that a combination of NAA+IBA is needed to boost the process.

The results of this study indicated that NAA+IBA application favors number of root, root length, number of leaves and shoot length [Table 3]. This result can be explained by the auxins ability to enhance adventitious root development of stem cuttings by facilitating the transportation of nutrient to the base of the cuttings.[13,36,37] When IBA is applied, it acts by converting the IAA into internal free IBA to enhance tissue sensitivity to IAA and also enhances the endogenous IAA synthesis or the action of IAA synergistically. In the same vein when NAA is applied, it act by producing stimulatory effect to induce root formation which is probably associated with inhibition of IAA-oxidase activity, thus preventing IAA degradation and increase its activity.[14] This is further simplified by Hartman *et al.*,[13] who stated that the formation and division of the first root initial cells need the existence of either endogenous or applied auxins.

These findings explained the fact that combination NAA and IBA are effectively used as root promoting substance for cuttings commercial plants, and this has proven to be the back bone of cutting propagation success.[14,33]

Interactive Effect of Growth Hormones and Rooting Media on Rooting and Survival of *P. guineense* Cuttings

Success in vegetative propagation through stem cutting is rated by high percentage of rooted cuttings, root and shoots growth. This success is achieved by treating stem cuttings with suitable hormone before planting in a suitable rooting medium.[12,38,39] The interactive effect of growth hormones and rooting media showed significant effect on number of roots, root length, and number of leaves [Table 2]. Cuttings treated with a combination of NAA and IBA rooted in river sand gave the highest number of root and root length when compared to the other treatment combination [Table 5]. This combination of hormones in topsoil also gave the highest number of leaves and shoot length.

Hormones combination of NAA and IBA in river sand was the most effective treatment combination to promote root initiation in *P. guineense* cutting while the combination in topsoil favors the leaves formation. This could be explained by the fact that NAA+IBA which resulted in root growth and development needs well aerated and porous media for the delicate root to penetrate though. In the same vein, the topsoil used contain considerable amount of nutrient and this is needed by the young and tender leaves to tap from for the entire growth and development of the plant.

CONCLUSION

The study had revealed that *P. guineense* could be propagated vegetative through stem cuttings. Thus, rooting of stem cuttings of *P. guineense* presents a viable propagation system to be used in mass production of good quality materials for conservation and planting program.

The study has demonstrated that the *P. guineense* can be rooted without treating cuttings with hormones on any of the media used. However, these untreated cuttings of *P. guineense* responded to a certain degree, which is an indication that the inherent hormone in the species studied is only enough to initiate rooting but for optimum growth and development of the cuttings, a combination of NAA+IBA is needed to boost the process. The cuttings from the combination of NAA and IBA thrives well in river sand to produce higher number of roots and root lengths but in sawdust to produces higher number of leaves.

Further research is recommended to determine the effect of hormonal concentration on stem cuttings of *P. guineense*.

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