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EFFECT OF HORMONE AND NODAL POSITIONS ON STEM CUTTINGS OF *P. ANGOLENSIS* WELW. AND *Z. XANTHOXYLOIDES* LAM

SUMMARY

This study examined the effect of hormone and nodal position on stem cuttings of *P. angolensis Welw.* and *Z. xanthoxyloides Lam*. Forty eight (48) cuttings were collected from each of the three nodal positions (i.e. upper, middle and basal) on the selected mother trees for the study. The leaves of the cuttings were reduced to half of the original sizes and then treated with Indole butyric acid (IBA) hormone, prepared into four different concentrations (0ppm, 1000ppm, 2000ppm and 3000ppm). The cuttings were set in sterilized river sand and replicated two times with 12 cuttings per replicate, and arranged in a 3x4 factorial experiment in completely randomized design (CRD) under a high humidity propagator. Aggregately, there were two hundred and eighty eight (288) cuttings per tree species. The cuttings were monitored for a period of twelve weeks to determine their percentage callusing, percentage rooting, number of root per cutting and length of root per cutting. Data obtained were analyzed with Analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). None of the cuttings from the two species rooted, but 89.6 % and 89.9% of *P. angolensis* and *Z. xanthoxyloides* cuttings respectively treated with 3000ppm had the best percentage callusing. Effect of hormone concentration on callusing was significant ($p<0.05$) while that of nodal position was not significant ($p>0.05$) but cuttings obtained from the upper nodes of the two species proved to callus better than the middle and the basal nodes cuttings. Further research is recommended for macropropagation of *P. angolensis*, and *Z. xanthoxyloides* with higher concentration of hormone.

Keywords: Macropropagation, Stem cuttings, Indole butyric acid, *Pycnanthus angolensis, Zanthoxylum xanthoxyloides*

INTRODUCTION

Pycnanthus angolensis Welw. belongs to the family Myristicaceae. It is an evergreen, monoecious, medium-sized to large tree up to 25–40m tall. The species occurs in upland and wet evergreen forest and semi-deciduous forest with more than 1600mm rainfall (Orwa *et al.*, 2009). It is especially abundant in old fallows and secondary forest as its rate of natural recruitment after disturbance of the forest is high. In southern Africa, it occurs in riverine and swamp forest, but

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in West Africa it does not occur in swamps (Keay, 1989). The bole of the tree is usually straight and cylindrical, branchless for up to 15–25m high, up to 120–150cm in diameter, usually without buttresses. Its outer bark is greyish brown, with orange-brown exudates. The leaves are distichously alternate, simple and entire, without stipules. The fruit is an ellipsoid to oblong or globose drupe, yellowish orange when ripe. Seeds of *P. angolensis* are recalcitrant with duration of germination from 16–36 days after planting (Dike and Aguguom, 2010).

According to Mapongmetsem (2007), a yellow to reddish brown fat, called 'kombo butter' or 'Angola tallow', is extracted from the seed and is important in West and Central Africa for illumination and soap making. It is not edible. The seeds somewhat resemble those of nutmeg (*Myristica fragrans* Houtt.) and are burnt as candles. Traditionally, the wood is highly valued as fuel and is used to make split planks, known as 'calabot' or 'caraboard' in the coastal zone of Cameroon (Orwa *et al.,* 2009). Because it is easy to work, it is used to make shingles, both for roofing and covering the sides of native houses, and planks for doors and window frames. The long straight bole makes it suitable for making canoes. Since the Second World War, the wood has become an important timber for plywood corestock, veneer, mouldings, interior trim, interior joinery, furniture components and paper pulp. Leakey (1999) reported that, in agroforestry *P. angolensis* is planted or retained for shade in coffee and cocoa plantations in the humid lowlands of Cameroon, in Uganda often also in banana plantations.

Z. xanthoxyloides Lam. is a small tree, which grows to a height of 18m high and 13cm girth. It belongs to the family Rutaceae. A major characteristic is that the trunks, branches, branchlets, leaf stalks and inflorescence axes of all these species are covered by prickles or what others describe as spines. This makes the Zanthoxylum species a big nuisance to farmers who always mark them for destruction when sighted on farms and vegetations. It is primarily a plant of the forest vegetation and found frequently in the Southern parts of Nigeria.

Economically, the timber of *Z. xanthoxyloides* is used in house and boat-building, decorative panelling, joinery, construction of talking drums and in the pulp and paper industry (Adeniyi *et al.*, 2010). The roots, bark and leaves of the species are used in various medicinal preparations for curing (Olatunji, 1983; Oliver-Bever, 1982; Adesina, 2005; Odugbemi, 2006) sickle cell anaemia, stomachache, tooth-ache, coughs, urinary and veneral diseases, leprous ulcerations, rheumatism, lumbago etc. Adeniyi *et al.* (2010) also investigated the antimicrobial activities of *Z. xanthoxyloides* and found that its crude extracts inhibited the growth of fungi and bacteria. They further reported that the microbial growth inhibition property is an indication that the plant can be used as a source for antimicrobial agent in the formulation of toothpaste, thus justifying the use of the plant locally as chewing sticks.

Studies have shown that Zanthoxylum solvent extract is a good repellant of stored grains insect pests. For instance, Owusu *et al.* (2007) and Udo (2011), revealed that extract of this species gave an hundred percent protection to maize

and cowpea against damage by *Sitophilus zeamais* and *Callosobruchus maculatus* respectively, while causing complete inhibition of F1 progeny production and egg development within grains.

Despite all the benefits derived from these tree species, little efforts are being placed upon their domestication, which had been thwarted and resulted to disappointment. This has occurred as a result of many reasons, for instance, lack of knowledge in their reproductive biology (Leakey and Newton, 1994; Akinyele, 2010). In addition, if the trees are to be domesticated through sexual reproduction, there is also a problem of seed availability. This is because the fruiting period of these trees is basically concentrated within few weeks (Xiao-Xia *et al.,* 2008). Within the short period available, maximum fruits must be collected by the fruit collectors, where there are competitions from wild animals, cattle and humans, which are also utilizing the fruits. Over the years, this mode of reproduction has not been successful for a large scale domestication as a result of these problems. Rema *et al.* (2007) opined that the large scale propagation of *P. angolensis* and *Z. xanthoxyloides* from seed is not ideal as a result of their recalcitrant nature. Consequently, this however calls for a substitute means of propagation using stem cuttings. This study therefore examined the effect of hormone concentration (i.e. IBA at 0ppm, 1000ppm, 2000ppm and 300ppm) and nodal positions (upper, middle and base) on stem cuttings of *P. angolensis* and *Z. xanthoxyloides*.

MATERIAL AND METHODS

Stem cuttings used for this study were collected on mother trees in University of Ibadan campus, while the experiment was conducted at the Department of Forest Resources Management, University of Ibadan, Nigeria. The University of Ibadan campus is endowed with diverse indigenous economic tree species among which are *Terminalia superba, Holarhena floribunda, Pycnanthus angolensis, Zanthoxylum xanthoxyloides,* etc. (Onefeli *et al.*, 2012). It is located north of Ibadan along Oyo road at approximately latitude $7^0$28'N and longitude $30^0$52'N. It is at an altitude of 277m above sea level (Akinyele, 2010). The climate is the West Africa monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold wind of hamattan (Akinyele, 2010). The wet season usually starts from April to October with occasional strong winds and thunderstorms (Akinyele, 2010).

The cuttings were obtained at upper, middle and basal position on each of the tree species. The leaves of the cuttings were reduced to half of the original sizes and then treated with Indole butyric acid (IBA) hormone, prepared into four different concentrations (0ppm, 1000ppm, 2000ppm and 3000ppm) using quick deep method (Oni, 1987; Gbadamosi and Oni, 2005; Akinyele, 2010). Subsequently, the cuttings were set in seed plastic trays containing sterilized riversand. Each of the treatment contained twelve (12) cuttings. This was replicated twice making an aggregate of two hundred and eighty eight (288) cuttings per tree species. The experiment was arranged in a 3x4 factorial

experiment in completely randomized design (CRD) under a high humidity propagator. Watering of the propagules was carried out twice daily with the use of knapsack sprayer. The cuttings were monitored for a period of twelve weeks to determine their percentage callusing, percentage rooting, number of root per cutting and length of root per cutting. Data obtained were analyzed with Analysis of variance (ANOVA) and mean separation was achieved with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

At the end of the study, none of the tree species' cuttings rooted, but they both callused, which is a definite sign of rooting.

Hormone Concentrations (HC) and Callusing of P. angolensis, and Z. xanthoxyloides

Cuttings treated with 3000ppm hormone had the highest percentage callusing, 89.6 % (Table 1). This was followed by 2000ppm (57.4%), 1000ppm (32.1%) and 0ppm (2.1%). Analysis of variance showed that effect of HC was significant ($p<0.005$) on the percentage callused *P. angolensis* cuttings.

The result of callused *Z. xanthoxyloides* (Table 1) was similar to what was obtained in *P. angolensis.* Cuttings treated with 3000ppm Indole butyric acid (IBA) had the highest callused cuttings (89.9%) while the least was observed in cuttings without hormone treatment (5.0%). Effect of hormone concentrations was significant ($p<0.05$) on percentage callusing in *Z. xanthoxyloides.*

Table 1: Callused *P. angolensis* and *Z. xanthoxyloides* from different hormone concentrations

HC	*P. angolensis* (%)	*Z. xanthoxyloides* (%)
0ppm	2.01a	5.0a
1000ppm	32.1b	16.0b
2000ppm	57.4c	49.3c
3000ppm	89.6d	89.9d
p-value	0.000*	0.000*

*=significant ($p<0.05$)
HC = Hormone concentration

Nodal Positions (NP) and Callusing of P. angolensis, and Z. xanthoxyloides

In *P. angolensis*, there was no significant difference ($p>0.05$) in the percentage callused cuttings from the three nodal positions. Although, cuttings from the upper position had the highest number of callused cuttings (49.5%) while those obtained from middle and basal positions had 43.4% and 42.9% callusing respectively.

Cuttings of *Z. xanthoxyloides* obtained from the upper NP (42.1%) callused well than those from the middle (39.3%) and at the basal parts (38.8%), hence, there was no significant difference ($p>0.05$) among them.

Table 2: Callused *P. angolensis* and *Z. xanthoxyloides* from different nodal positions

NP	*P. angolensis* (%)	*Z. xanthoxyloides* (%)
U	49.47	42.1
M	43.43	39.3
B	42.91	38.8
p-value	0.057ns	0.379ns

ns= not significant (p>0.05)
NP = Nodal position, U= upper, M = Middle and B = Base

Table 3: Interaction effect of Hormone concentrations (HC) and Nodal positions (NP) on sprouting of *P. angolensis* and *Z. xanthoxyloides*

		Tree species	
NP	HC	*P. angolensis*	*Z. xanthoxyloides*
U	0ppm	6.3	4.2
	1000ppm	37.1	22.9
	2000ppm	62.9	51.3
	3000ppm	91.7	90.0
M	0ppm	0.0	5.0
	1000ppm	30.0	14.6
	2000ppm	55.8	47.9
	3000ppm	87.9	89.6
B	0ppm	0.0	5.8
	1000ppm	29.2	10.4
	2000ppm	53.3	48.8
	3000ppm	89.2	90.0
	p-value	0.982ns	0.551ns

ns= not significant (p>0.05)
HC = Hormone concentration
NP = Nodal position, U= upper, M = Middle and B = Base

Interaction effect of HC and NP (Table 3) was also not significant (p>0.05) on the callusing of the cuttings of the two tree species. For the *P. angolensis*, cuttings collected at the upper node, treated with 3000ppm hormone had the highest callusing (91.7%) while the lowest was observed on the cuttings obtained at the middle and basal positions, received no hormone treatments (0.0%). In the case of Z. *xanthoxyloides*, the highest callused cuttings were observed on those collected from the upper and basal position, treated with 3000ppm hormone. The cuttings from the upper node with no hormonal treatment happened to have the smallest callusing.

Although the cuttings of *P. angolensis,* and *Z. xanthoxyloides* did not root at the end of the experiment, the trend in percentage callused cuttings based on the hormone concentration is an indication of root formation. This is because callus formation is a prerequisite for root initiation in any hardwood cuttings (Carey, 2008). In other words, callus must be produced before root tissue is formed. The results therefore imply that endogenous hormone concentration in *P. angolensis* and *Z. xanthoxyloides* is too small to be able to activate root initiation. The species therefore require application of more exogenous to boost the level of endogenous hormone in order to promote rooting through cell division (Yeboah *et al.,* 2010). These results are in consonance with (Laubscher and Ndakidemi, 2008; Yeboah *et al.,* 2010 and Akwatulira *et al.,* 2011). These authors equally discovered that the percentage callused cuttings increased with an increase in hormone concentrations.

The more callused cuttings observed at the upper position of the nodes than the other positions may be attributed to the level of auxin present in apical meristem, which is more than that which are found in other parts of the plants (Kurakawa *et al.,* 2007). It is therefore advisable to collect cuttings of *P. angolensis* and *Z. xanthoxyloides* from the upper nodal position.

CONCLUSIONS

This study has been able to unveil the effect of nodal position and hormone concentration on the stem cuttings of *P. angolensis,* and *Z. xanthoxyloides*. It was revealed that the endogenous auxin content of the selected tree species is too small to activate the physiological processes responsible for the root production (Yeboah *et al.,* 2010). Therefore, there is need for an extra application of the Indole butyric acid above the level of 3000ppm. Hence, macropropagation would be a promising method through which the two tree species will be produced inexpensively.

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UTICAJ HORMONA I POZICIJE KOLENACA NA REZNICAMA *P. angolensis Welw.* I *Z. xanthoxyloides Lam*

SAŽETAK

Ovaj rad se bavi ispitivanjem uticaja hormona i pozicije kolenaca na reznicama P. angolensis Welw. i Z. xanthoxyloides Lam. Za ovaj rad je sakupljeno četrdeset osam reznica (48) sa svake od tri pozicije kolenaca (tj. gornje, srednje i bazalne pozicije) na odabranim matičnim stablima. Listovi reznica su smanjeni na polovinu svoje originalne veličine, a potom tretirani hormonom indol-buterne kiseline (IBA), pripremljene u četiri različite koncentracije (0ppm, 1000ppm, 2000ppm i 3000ppm). Reznice su smještene u sterilizovan riječni pijesak i dva puta replicirane sa 12 reznica po replici, te zatim raspoređene u 3x4 faktorski eksperiment potpuno slučajnog dizajna (CRD) pod propagator velike vlažnosti. Sveukupno je bilo dvije stotine osmadeset i osam (288) reznica po vrsti drveta. Vršen je monitoring rezinca u periodu od dvanaest sedmica u cilju utvrđivanja procenta njihovog zadebljanja, procenta zakorjenjivanja, broja korijena po reznici i dužine korjena po reznici. Dobijeni podaci su zatim analizirani Analizom varijanse (ANOVA) i Dunkanovim višestrukim testom intervala (DMRT). Nijedna reznica od ove dvije vrste nije pustila korijen, ali je 89,6 % i 89,9% reznica P. angolensis i Z. Xanthoxyloides, odnosno tretiranih sa 3000ppm, imalo najbolji procenat zadebljavanja. Uticaj koncentracije hormona na zadebljanje je bio značajan ($p<0.05$), dok položaj kolenaca nije bio od značaja ($p>0.05$), ali su reznice dobijene of gornjih kolenaca ove dvije vrste pokazale bolje zadebljanje u odnosu na reznice srednjih i bazalnih kolenaca. Preporučuju se dalja istraživanja vezano za makropropagaciju P. angolensis i Z. Xanthoxyloides uz veću koncentraciju hormona.

Ključne riječi: Makropropagacija, reznica, indol-buterna kiselina, *Pycnanthus angolensis, Zanthoxylum xanthoxyloides*