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The Effect of Indole-3-butyric acid (IBA) Rooting Hormone on the Rooting of Camphor (*Ocotea usambarensis)* Coppice Stem Cuttings

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Abstract: ***Ocotea usambarensis*** **Engl. is a high value timber tree that is threatened in Kenya due to overexploitation and poor natural regeneration. It seeds once in eight to ten years but most of its fruits are lost pre-maturely due to pest attack. In addition its seeds are recalcitrant. Alternative methods of propagation should be developed to complement the few seed available. A rooting trial was set up to determine the ability of Indole-3-butyric acid (IBA) to promote rooting in *O. usambarensis* coppice stem cuttings. The experiment was laid out in a complete randomised design and cuttings treated with IBA at 0, 60, 90 and 120mg/l concentrations. Each IBA concentration was replicated 3 times and each replicate had 10 cuttings. The experiment was incubated in a non-mist propagator with vermiculite as the rooting medium. Assessment for rooting was done on 84th, 104th and 136th day. Data on rooting, number of roots and the length of the longest root and sprouting was collected and analysis done using Genstat. The highest rooting was 43.5% at 60 mg/l IBA and the least 6.7% in both 90 and 120 mg/l concentrations. However, the difference was not significant (*p=0.264)*. The number of roots and length of the longest root were not significantly influenced by IBA treatment. However, the proportion of cutting that sprouted, the number and growth of sprouts were significantly decreased with increase in IBA concentration. The lack of significant positive increase in the rooting with IBA treatment suggests that *O. usambarensis* may have a relatively high level of endogenous auxins.**

Keywords: **IBA, non-mist propagator, *Ocotea usambarensis,* stem cuttings, Rooting, vegetative propagation, auxins, Vermiculite.**

1. INTRODUCTION

Ocotea usambarensis Engl. (East Africa camphor-wood*)* belongs to family lauraceae. This species occurs in the wet montane forests of Kenya, Tanzania and Uganda. It grows naturally between 1375 and 2600 meters above the sea level (asl) but it is more prevalent between 1830 and 2156 m asl (Beentje, 1994). In Kenya *O. usambarensis* is found on the southern and eastern forests of Mt. Kenya and on the eastern slopes of the Aberdares mountains ranges and in Nyambene hills. *Ocotea usambarensis* tree attain a diameter at breast height (dbh) of 200cm and maximum height of 45m with a clear bole of 9-15m. The tree has a valuable timber that is resistance to fungal decay, woodborers, and moderately resistant to termites. Timber is used for furniture making, flooring and boat building. The roots and bark have some medicinal values and are exploited by the local communities for malaria, backache, whooping cough and measles (FAO, 1993; Orwa et al, 2009)

Ocotea usambarensis is classified as threatened due to high exploitation pressure and difficulties in natural regeneration. It seeds once in eight to ten years (Kigomo, 1987), majority of seeds are, however, dropped pre-maturely due to attack by gall-insects and birds (Bussmann, 2001). The mature seeds of *O. usambarensis* are recalcitrant and remain viable for only few days even under the most favourable storage conditions (Orwa et al, 2009).

In nature, *O. usambarensis* regenerates through suckers growing from trunk bases and old roots especially after harvesting the mother tree. The importance of this mode of regeneration in the *O. usambarensis* natural regeneration dynamics in Kenya has not been investigated. When young trees are cut, coppices develop on the stumps but their subsequent growth and development has not been documented in Kenya but has been reported to be successful in Tanazania. Under natural conditions only a few seedlings manage to establish due heavy browsing by herbivores especially elephants (Bussmann, 2001). Majority of *O usambarensis* populations in Kenya have continued to decline due to lack of adequate natural recruitment and illegal exploitation. In addition, encroachment on and excisions of forest land have exacerbated the problem (Mbugua, 2007). In order to save *O. usambarensis* from extinction other means of propagation should be developed for conservation and domestication. Vegetative propagation through rooted stem cuttings is one of the most viable alternatives. Vegetative propagation is considered desirable alternative to gathering seeds thus eliminating reliance on seasonally available seeds and more so for those tree species with seeding problems (Tchoundjeu et al., 2004). However, a wide variety of factors influence the rooting ability of stem cuttings (Leakey et al., 1990, Mesen et al., 2001; Schiembo, et al, 1997; Dick et al., 2004). Among these factors is the use of commercial rooting hormones. Exogenous application of commercially available auxins like Indole-3-butyric acid (IBA) and α–naphthalene acetic acid (NAA) promote rooting in stem cuttings but the level of success vary from species to species (Tchoundjeu *et al.*, 2002; Takoutsing et al, 2014; Husen and Pal, 2007). No studies on the effect of rooting hormones on the rooting of *O. usambarensis* stem cuttings have been undertaken. This paper gives results of experiment conducted at Muguga research nursery, Kenya to investigate the effect of indole-3-butyric acid (IBA) on the rooting of juvenile stem cuttings from coppice shoots. IBA was chosen for testing since it is more stable compared to other rooting hormones and is generally non-toxic to plants over a wide range of concentration levels (Larsen and Guse, 1997; Kester et al., 1990).

2. MATERIALS AND METHODS

Fresh juvenile stem cuttings of *O. usambarensis* coppices were harvested from Kereita forest. Kereita forest occupies the southeastern block of the kikuyu escarpment forest in Kiambu County, Kenya. It is situated approximately 0°56'S, 36°40'E at an altitudinal range between 1800 to 2700 metres above the sea level (asl). The mean annual rainfall is 1500 mm and temperature ranges from 16 to 18^0C. The coppicing stumps were remnants of trees that had been illegally cut by wood poachers. Healthy coppice stems of diameter range 4-10 mm were harvested and transported in cool boxes to the Kenya Forestry Research Institute's (KEFRI) Muguga research nursery. At the nursery, the bigger cuttings were further subdivided into smaller multiple-nodal cuttings of 12 cm mean length and 50 cm^2 leaf surface areas per cutting. Cuttings were then surface sterilized in 1% sodium hypochlorite for 10 minutes and rinsed twice in distilled water before applying indole-3-butyric acid (IBA) rooting hormone. Indole-3-butyric acid was applied to the cuttings at 0, 60, 90 and 120mg/l by dipping overnight. The cuttings under 0 mg/l IBA treatment served as control and were dipped in distilled water overnight. The treated cuttings and the control were then inserted in sterilized vermiculite for rooting. The experiment was laid out in a complete randomised design (CRD) with four IBA hormone concentrations; each replicated 3 times and consisting of 10 cuttings per replicate. The experiment was incubated in a non-mist propagator (Leakey et al., (1990) located at Muguga research nursery. Muguga research nursery lies within the KEFRI forest estate that lies within an altitude range of 2040 to 2100 meters asl and 1° 12′ S, 36° 37′E. The soils are classified as nitisols that are derived from volcanic rock. The mean annual temperature is 18 ^{0}C (minimum12^{0}C and maximum 25 ^{0}C) and the mean annual rainfall is 1100mm distributed bimodaly with peaks in April and December. Humidity in the propagator varied between 70 and 95 ± 2 % and maximum and minimum day-night temperature at 31± 1 ^{0}C to 26 ± 1 ^{0}C respectively. Whenever the propagator was opened for inspection, mist spraying was applied to raise the relative humidity inside the propagator. Assessment for rooting was done on 84^{th}, 104^{th} and 136^{th} day. Cuttings that rooted were potted and removed from the experiment.

Data was collected on the; number of cuttings with roots, number of roots and the length of the longest root (centimetres) per rooted cutting and the point of origin of the root (whether from callus or ordinary tissue). The data on the proportion of the cuttings forming new shoots (sprouts), number of sprouts formed and the total height of the tallest sprout per every sprouting cutting was also captured. The whole set of data was organized using ms-excel 2010 and analysed using Genstat version 16. Analysis of variance (ANOVA) on mean rooting, sprouting percentage and height of the tallest sprout was used to determine significant differences between IBA concentrations. The data was arcsine transformed before analysis. The data on number of roots and number of sprouts was analysed using log linear regression modelling. The data on the root length was analysed directly by ANOVA since it met the assumptions of a normal distribution. Significance differences were declared at 5% for treatment effects. Chi square test was performed to determine whether there was any relationship between callusing and root formation.

3. RESULTS

Rooting:

The cuttings treated with 60mg/l IBA recorded the earliest rooting (16.7 %) by 84th day, whereas no cuttings in the other IBA concentrations had rooted at this date. However, in the subsequent assessments, cuttings in all the IBA concentrations and even in the control recorded some level of rooting success (Figure 1). It should, however, be noted that the recorded rooting successes in different IBA treatments and control were not significant at both 104th days (*p=0.189*) and at 136th days (*p=0.192*). Cumulatively, the cuttings rooted best at 60 mg/l IBA concentration and least at both 90 and 120 mg/l IBA concentrations (Figure 1). However, the difference was not significant (*p=0.264*.)

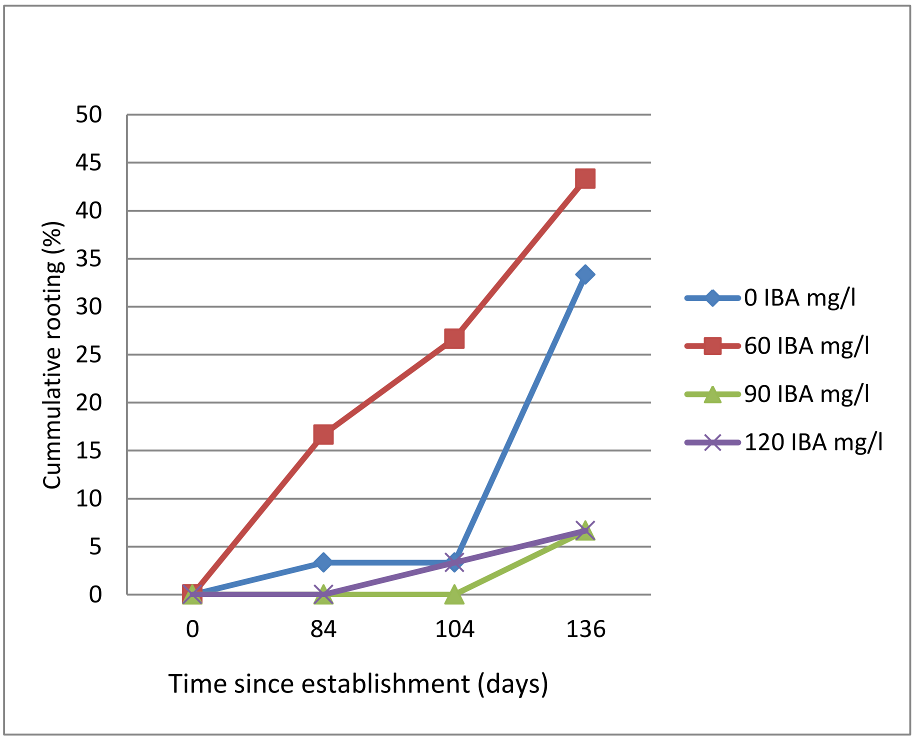

Figure 1. Trends in the rooting of the coppice stem cuttings of *Ocotea usambarensis* at different IBA concentrations, Muguga nursery

Number of roots:

There was no significant difference in the mean number of roots per rooted cutting at 84th, 104th and 136th days between different IBA concentrations. There was also no apparent trend with increasing IBA concentrations (Table I) at 84th and 104 days. However, at 136th day the mean number of roots increased with increase in IBA concentrations to a climax at 60 mg/l IBA concentration then dropped to its lowest at 120mg/l IBA concentration (Table I).

Table I. Effect of different IBA concentrations on root number and the length of the longest root in *O. usambarensis* coppice stem cuttings

IBA Concentrations (Mg/l)	Number of roots per rooted cutting			Length of the longest root (cm)		
	Assessment dates (days)					
	84	104	136	84	104	136
0	2	0	2.2	3	0	2.17
60	2	2.33	4.4	3.6	1.37	2.74
90	0	0	3.5	0	0	1.8
120	0	1	2	0	0.5	6.3
chi p	chi p= 0.917	chi p= 0.717	chi p= 0.256	*P=0.978*	*P=0.313*	*P=0.067*

Root length:

The mean length of the longest root per rooted cutting at 84th days was not significantly influenced by different IBA concentrations. Similar results were obtained at 104th and 136th Days(Table I).

Sprouting:

The proportion of cutting that sprouted was significantly different at 84th day (*p<0.001)* and 104th day (*p=0.015*) but not at 136th day (*p=0.15*) (table II), the mean number of sprouts also followed a similar trend being significantly different at 84th day (Chi *p=0.001*) and at 104 days (chi *p*=0.029) but not at 136th day (Chi *p*= 0.095) between different IBA concentrations The height growth of the sprouts was significantly influenced by IBA concentrations at all the assessment dates (84th day: *p=0.010*, 104th day: *p=0.001*, 136th day: *p= 0.016).* In all the treatments, the higher the IBA concentration the lower the proportion of cuttings that sprouted and the lower the number of sprouts formed and also the lower the mean height of the tallest sprout. In summary the application of IBA suppressed formation of new shoots.

Table II. Effect of IBA on development and growth of new shoots in *O. usambarensis* coppice stem cuttings

IBA concentrations (mg/l)	Mean sprout (%)			Mean height of the tallest sprout (cm)		
	Assessment dates (days)					
	84	104	136	84	104	136
0	50b	43.3b	46.7a	5.2b	5.8b	5.2b
60	50b	36.7b	30.0a	3.5ab	4.5b	4.5ab
90	6.7a	10.0a	16.7a	1.2a	0.8a	1.2a
120	3.3a	16.7ab	16.7a	0.5a	0.1a	2.1ab

The numbers with different letters are significantly different at 0.05 % significant level

Mortality:

At the time of closing the experiment on 136th day, the highest mortality was 36.7 % recorded in cuttings treated with 90 and 120mg/l IBA concentrations. Control and cuttings treated with 60mg/l had the least mortality (16.7%). However, the difference in mortality levels was not significant (*P=0.274*).

Callussing:

Overall, the number of the rooted cuttings whose roots developed directly from callus tissue was significantly higher (chi pr< 0.001) compared to those with roots originating from non-calluss tissues (Figure 2)

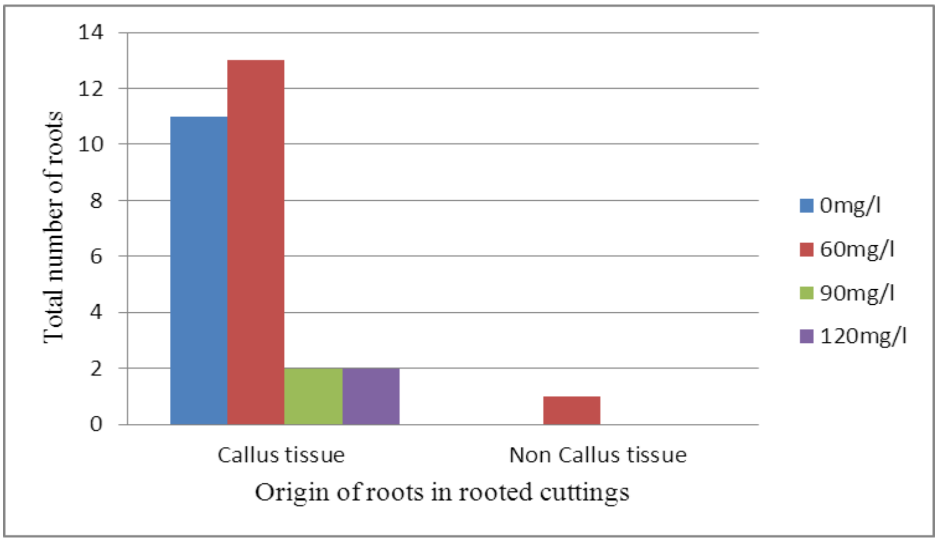

Figure 2. The number of rooted cuttings whose root developed from callus and non-callus tissue in *O. usambarensis* coppice stem cuttings at 136 days

4. DISCUSSIONS

The results from this study revealed that *O. usambarensis* can be successfully propagated by juvenile leafy stem cuttings from coppices in a non-mist propagator system. The earliest recorded rooting at eighty fourth day in cuttings treated with IBA at 60mg/l could be attributed to IBA rooting hormone. The apparent hastening of rooting in these cuttings is line with observations of Stromquist and Hansen, (1980) and Newton et al., (1992) that applications of auxins to cuttings often hasten root initiation and improve quality of roots formed. However, this initial superior rooting was short lived since results from the subsequent assessments and even the cumulative rooting success at the close of experiment showed no significant differences between IBA concentrations. There was no significant positive increase in the rooting percentage with application of IBA rooting hormone. The lack of significant positive increase in the rooting of juvenile coppices stem cuttings of *O. usambarensis* may suggest that this species is relatively insensitive to IBA treatment. Similar results have been reported on other tropical tree species such as *Irvingia Gabonesis* (Shiembo et al., 1996), *Nauclea diderrichii* (Leakey, 1990), and *Irvingia wombolu (*Dolor et al., 2010). According to Hartmann et al., (1990) the exogenous application of auxins may be promotive, ineffective or even inhibitory to the rooting of cuttings. This depends on the endogenous level of growth regulating substances. The application of IBA to *O. usambarenesis* cuttings may be described as ineffective. According to Shiembo *et al*., (1996), tree species that are generally insensitive to exogenous auxins are usually well supplied with endogenous auxins, *O. usambarensis* may be among such group of trees. The length of the longest root was also insensitive to IBA treatment. This may be due to the fact that once the rooting process has been induced either by endogenous or exogenous auxins other hormones and growth regulators become more important in the subsequent root growth and elongation (Hartmann et al., 1990). However, in some cases supra-optimal concentration of auxins inhibit root elongation through enhancement of ethylene biosynthesis (Ali et al, 2009). Ethylene is root growth inhibitor. Such inhibition was not evident in this study and we may assume that the levels of exogenous IBA applied were not injurious to the rooting formation processes in *O. usambarensis* stem cuttings.

There was no significant positive or negative change in the number of roots formed with increasing IBA concentration. This trend of results was therefore similar to that recorded in the rooting. However, this was a mere coincidence rather than a rule since different authors have reported different scenario exhibited by different tree species; Shiembo (1996) in his studies on the rooting of leafy stem cutting of *Gnetum africanum* reported that rooting was insensitive to IBA treatment but the number of roots per rooted cutting was positively related to IBA treatment. Similar results were obtained with *Ricinodendron Heudelotii (*Newton and Leakey, 1997). In another rooting study conducted on *P. africana* stem cuttings by Tchoundjeu, (2002), IBA significantly increased rooting percentage but not mean number of roots. Yet in other tropical tree species such as *Albizia guachepele* and *Cordia alliodora* (Mesen, 1993*)*, *Nauclea diderrichii* (Leakey, 1990) and *Bobgunnia madagascariensis* (Amri, 2011), application of IBA resulted to sharply increase in number of roots formed.

The application of IBA had an inhibitory response on the initiation and development of sprouts compared to the control. The higher the concentration of IBA applied the severe the inhibitory effect on the number of sprouts formed and their height growth and also on the proportional of cuttings forming new sprouts. This may be due presence of adequate amount of endogenous growth regulating factors that control the initiation and development of new sprouts in *O. usambarensis* coppice stem cuttings. The exogenous IBA applied may have exceeded the critical level beyond which its action was antagonistic thus inhibiting new shoot formation and development but no corresponding positive effect on rooting. In other tropical tree species such as D*albergia sericea* application of IBA at lower concentration (100ppm) increased sprouting but completely inhibited sprouting at 500 ppm (Uniyal, 1993). In *Warburgia ugandensis*, application of IBA greatly increased the number and length of shoots per sprouted stem cutting (Akwatulira, et al, 2011). Shoot production can be positively or negatively related to rooting. Mesen et al., (1997) reported that new shoot was a strong competing sink for assimilates that inhibited root production in *Cordia alliodora*. However, in *Triplochiton scleroxylon*, the length of the newly emerged shoot was positively associated with root production (Dick *et al*., 2004).

The current study indicates that root formation in *O. usambarensis* coppice cuttings requires formation of callus tissue first. After a cutting is wounded, callus tissue forms at the base, primarily from the vascular tissue. In easy-to-root plant species, callus formation and root formation are independent processes that occur at the same time because of similar environmental triggers (Luna and Haase, 2014). In difficult-to-root species, adventitious roots arise from the callus mass (Luna and Haase, 2014). *O. usambarensis* can therefore be classified as a difficult to root species that require formation of callus first prior to rooting. However, more trials are required to confirm that callus formation is essential in rooting all types of *O. usambarensis* stem cuttings.

5. RECOMMENDATION AND CONCLUSIONS

The authors experienced a serious challenge in getting adequate quality stem cuttings for research. Reliable source of vegetative propagules should therefore be established to supply quality cuttings for both experimental and production purpose. The current experiment was established with material collected directly from the wild. The age of coppices from different stumps could not be established. Establishment of cut-hedge will help to overcome these hitches. The effect of season of harvesting the cuttings was not taken into account but it should be included in future research. Tentatively, *O. usambarensis* seedlings can be raised through rooted coppice cuttings using 60mg/l IBA in non-mist propagator using vermiculite. It was also found that callus formation was essential prerequisite for root formation in *O. usambarensis* coppice stem cuttings. However, more research work based on different *O. usambarensis* provenances and cutting types is needed to confirm this observation.

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