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Full Length Research Paper

Influence of rooting media and indole-3-butyric acid (IBA) concentration on rooting and shoot formation of *Warburgia ugandensis* stem cuttings

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This study investigated the influence of different rooting media and indolebutryic acid (IBA) hormone concentration on root and shoot development in stem cuttings of *Warburgia ugandensis*. Stem cuttings were treated with three different levels (0.3, 0.6 and 0.8% w/w) of IBA concentration (including a control-no IBA hormone) and propagated in three rooting media (milled pine bark, top forest soil and sand) under non -misting propagators. Data on root and shoot development, callusing, number and length of roots and shoots were analyzed using analysis of variance (ANOVA) and Chi square test. Callusing, root and shoot development were significantly ($p<0.05$) influenced by rooting media and IBA concentration. Milled pine bark and 0.8% w/w IBA concentration gave the highest percentages of stem cuttings that callused, rooted and shooted (38, 37, 41% and 57, 41, 59%), respectively. Similarly, milled pine bark and 0.8% w/w IBA concentration gave the greatest number and longest roots and shoots per stem cutting. Vegetative propagation of *W. ugandensis* through stem cuttings can be appropriately achieved by treating the cuttings with 0.8% w/w IBA hormone using milled pine bark as a growth medium.

Key words: *Warburgia ugandensis,* indolebutryic acid (IBA), vegetative propagation, callusing, rooting, milled pine bark.

INTRODUCTION

Warburgia ugandensis Sprague commonly referred to as the East African 'Greenheart', is a species of evergreen trees native to Democratic Republic of Congo, Ethiopia, Kenya, Malawi, South Africa, Swaziland, Tanzania and Uganda. This tree species occurs in lowland rainforests, upland dry evergreen forest, as relicts in secondary bush land, grasslands and termitaria in swamp forests. *W. ugandensis* have many uses which include provision of medicine, fodder, shade, manure and timber. It is also used as a remedy for a variety of diseases including stomach-ache, constipation, toothache, cough, fever, muscle pains, weak joints, general body pains and malaria (FAO, 1986; Wamalwa et al., 2006; Olila et al., 2001). *W. ugandensis* has gained a lot of popularity due to the high demand for the medicinal extracts from the bark, roots and leaves for use by traditional healer

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practioners (Wamalwa et al., 2006; Olila et al., 2001). This high demand has resulted into serious population deteroriation and a need for intensive cultivation and conservation programmes. It is anticipated that promoting cultivation and conservation of *W. ugandensis* can easily be carried out if proper vegetative propagation methods have been developed. Thus, to support rapid multiplication of planting materials, propagation of *W. ugandensis* by stem cuttings using different rooting media and IBA rooting hormone concentration can be a viable option.

According to Ofori- Gyamfi (1998), rooting performance depends on the type of medium used in the propagating structure. This is so because the various materials and mixes of materials that can be used in rooting of cuttings provide physical support, oxygen and water (Kester et al., 1990; Larsen and Guse, 1997). Although *W. ugandensis* can be traditionally propagated through stem cuttings without any manipulation, this method can perform better when rooting hormones (auxins) are used to hasten root initiation (Leonardi et al., 2001). Auxins often hasten root initiation, increase the number and percentage of cuttings rooted as well as quality of roots produced per cutting (Newton et al., 1992).

Larsen and Guse (1997) and Kester et al. (1990) reported that the most reliable rooting hormone is indolebutyric acid (IBA) although others such as naphthalene acetic acid (NAA) can also be used. Although there are reports that it may also be toxic to young/ succulent cuttings of certain species, IBA is still probably the best hormone for general use because of being non-toxic to plants over a wide range of concentration levels (Kester et al., 1990). This study therefore aimed at determining the most appropriate rooting medium and IBA hormone concentration for propagating stem cuttings of *W. ugandensis*.

MATERIALS AND METHODS

Experimental design

Stem cuttings of *W. ugandensis* were taken from Mabira Central Forest Reserve in Central Uganda. The experiment was carried out under non misting propagation tunnels at the National Forestry Resources Research Institute headquarters, located 12 km along Mukono- Kayunga Road in Central Uganda. The tunnels were made of 1 m wide and 4 m long wooden boxes constructed from timber boards measuring 30 cm wide by 2 cm thick. The boxes were placed over 0.5 mm thick polythene sheets and then filled with rooting media. Semi -circular aluminium rods were fixed onto the wooden boxes and covered with a transparent 0.5 mm thick polythene sheet to create humid chambers which were then placed under a shade of 70% heat intensity penetration. The experiment was set up in a complete randomized design with a 3 × 4 factorial treatment structure (Jeruto et al., 2008). It involved three types of rooting media (milled pine bark, top forest soil and sand) and four levels of Indolebutyric acid hormone concentration (0, 0.3, 0.6 and 0.8%). Forty five stem cuttings were used in each set of rooting media and level of IBA hormone concentration. Each set of IBA treated cuttings were then inserted into each type of rooting media and replicated three times. In order to avoid bias, each cutting was allocated randomly to each rooting media by use of a table of random digits (Johnson and Bhattacharyya, 2006).

Preparation of rooting media

The three types of rooting media (milled pine bark, top forest soil and sand) (Scalabrelli et al., 1983) were prepared by pasteurization and fumigation. Soil and sand were pasteurized when moist for 30 min by heating over an open cast metal plate and cooled (Kester et al., 1990; Larsen and Guse, 1997) while milled pine bark was fumigated with Dithane M45 chemical at 2000 ppm. The different rooting media were spread in the non mist propagators over a layer of stone gravel placed on an impervious polythene sheet base, watered and then covered with a 0.5 mm thick white polythene sheet (Jeruto et al., 2008).

Preparation of the stem cuttings

Softwood stem cuttings of 7.5 cm long consisting of two or more lateral buds were sequentially harvested from tagged *W. ugandensis* plants (Kester et al., 1990). A total of 540 stem cuttings were collected from coppices of selected *W. ugandensis* plant populations in their natural habitat (Mabira Central Forest Reserve). The cuttings were collected only in the morning hours, and kept moist/ cool at all times by placing in cool boxes (Agbo and Obi, 2007). The selected stem cuttings were softwood obtained from young/ succulent stems with new growth (Agbo and Obi, 2007). The bases of all the cuttings were squared by use of a sharp pair of scissor to avoid one- sided rooting. During working, the cut bases were dipped in water up to a length of 1 cm so as to avoid water loss and hence prevent wilting (Kester et al., 1990).

For each softwood stem cutting, the 5 mm basal end was dipped into the different concentrations (0.8, 0.6 and 0.3% w/w) of rooting hormone (IBA) for 5 s (Reinten et al., 2002). Excess powder was then tapped off the cuttings. The softwood stem cuttings were each stuck in different rooting media at depth of 2.5 cm. The rooting media were then compacted around the bases of the cuttings (in order to give support), watered and covered with a thick polythene sheet over the wooden frames. The purpose of the polythene sheet was to maintain 80 to 90% humidity and reduce water loss from the cuttings within the propagation chamber.

Dithane M45 at 2000 ppm was sprayed on all cuttings to control fungal infection (Yeboah and Amoah, 2009). Monitoring was carried out every two days in a week to remove dead leaves and cuttings. The cuttings were also watered twice a week in order to keep the media moist until the cuttings had been rooted.

Data collection

Data collection commenced in the first week and continued until the 12th week of the experiment. Data were collected on number of cuttings alive (callused, rooted and shooted), number and length of budded shoots on the cuttings, number and length of roots developed per rooted cutting for each stem cutting type in the different rooting media following Yeboah and Amoah (2009).

Data analysis

Data were entered in Microsoft Excel spreadsheet and analyzed to obtain mean number and percentages of cuttings that formed callus, developed roots and sprouted shoots for the different stem cutting (Badji et al., 1991). The data were then imported into

Table 1. Callusing, rooting and shooting success of stem cuttings of *W. ugandensis* in the different rooting media and IBA concentration (N = 540).

Rooting media	IBA concentration (%)	Number of stem cutting		
		Rooted	Shooted	Callused
Pine bark	0.0	1	6	5
	0.3	1	6	3
	0.6	6	12	7
	0.8	9	20	6
Top forest soil	0.0	0	0	0
	0.3	0	0	0
	0.6	0	0	0
	0.8	1	2	1
Sand	0.0	2	2	1
	0.3	0	0	0
	0.6	0	0	0
	0.8	1	1	0

MINITAB version 12.22 and analyzed using analysis of variance (ANOVA) to obtain means and standard deviations for number and length of roots and shoots; and the interactions of stem cutting type and rooting media (Moreira et al., 2009).

The significant effect of each factor (stem cutting type, rooting media and their interactions) on number and length of roots and shoots were separated by Tukey's pair wise comparison post hoc (Least Significance Difference) test at 5% level of significance (Moreira et al., 2009). Chi square test was also conducted to test whether there were significant differences in callusing, rooting and shooting success of the different stem cuttings used (Badji et al., 1991).

RESULTS

Softwood stem cuttings treated with 0.8% w/w IBA hormone concentration and propagated in milled pine bark recorded the highest mean number of cuttings that formed callus, developed roots and sprouted shoots. While the stem cuttings treated with 0.3 and 0.6% w/w IBA and propagated in top soil did not record any cutting which is callused, rooted and sprouted shoots (Table 1).

There is significant difference among the different rooting media in relation to rooting, shooting and callusing success (Table 2). This is not the case with IBA concentration. The total effect of IBA levels was only significantly different in shooting success. The interactive effect of rooting media and IBA concentration were not significantly different in rooting, shooting and callusing success (Table 2).

The type of rooting media and hormone concentration levels used in the experiment influenced the percentage of cuttings that callused, rooted and sprouted shoots (Figures 1 and 2). Softwood stem cuttings propagated in milled pine bark significantly ($p < 0.05$) resulted into the highest percentage of cuttings (Figure 1a to c) which developed callus (57%), roots (41%) and sprouted shoots (59%). As presented in Figures 2a to c, stem cuttings treated with 0.8% w/w IBA hormone concentration recorded the greatest percentages of cuttings which callused (38%), rooted (37%) and shooted (41%).

The number and length of roots that developed from the stem cuttings propagated in the different rooting media were not significantly ($p > 0.05$) influenced by the various IBA concentrations (Table 3). However, the number and length of shoots per sprouted stem cutting were greatly influenced ($p < 0.001$) by rooting media and IBA concentration (Table 3). The number of sprouted shoots propagated in milled pine bark increased with increase in IBA concentration. Stem cuttings that were treated with 0.8% IBA and propagated in milled pine bark recorded the greatest number of sprouted shoots compared to those treated with 0.6, 0.3 and 0% (control) IBA. Increase in IBA concentration did not affect the sprouting in top and sandy soils (Table 3). The length of shoots developed per sprouted stem cutting propagated in the different rooting media were also significantly ($P < 0.001$) influenced by different IBA concentrations (Table 3). In milled pine bark, shoot length increased with increased IBA concentration. The longest shoots per sprouted stem cutting were produced from 0.8% IBA treatment in milled pine bark.

Stem cuttings that were propagated in milled pine bark recorded the most number of roots, shoots and longer roots and shoots than cuttings propagated in top and sandy soil rooting media (Table 4).

Different IBA concentrations influenced the number and length of roots and shoot that developed. Stem cuttings that were treated with 0.8% IBA concentration produced

Table 2. ANOVA table showing interactive effects of IBA hormone and rooting media.

Source	Dependent variable	SS	df	MS	F	p-value
Corrected model	Rooted	0.9920	11	0.0902	2.0837	0.0199
	Shooted	5.2054	11	0.4732	5.1916	0.0000
	Callused	1.3593	11	0.1236	2.6860	0.0023
Intercept	Rooted	0.6322	1	0.6322	14.6089	0.0001
	Shooted	2.6130	1	2.6130	28.6667	0.0000
	Callused	0.5937	1	0.5937	12.9045	0.0004
Rooting media	Rooted	0.3172	2	0.1586	3.6651	0.0263
	Shooted	3.1059	2	1.5530	17.0373	0.0000
	Callused	0.7847	2	0.3924	8.5286	0.0002
IBA concentration	Rooted	0.3093	3	0.1031	2.3819	0.0686
	Shooted	0.8293	3	0.2764	3.0328	0.0289
	Callused	0.2231	3	0.0744	1.6163	0.1846
Rooting media * IBA concentration	Rooted	0.1473	6	0.0245	0.5671	0.7566
	Shooted	0.4923	6	0.0820	0.9001	0.4944
	Callused	0.2294	6	0.0382	0.8309	0.5462
Error	Rooted	22.8506	528	0.0433		
	Shooted	48.1279	528	0.0912		
	Callused	24.2907	528	0.0460		
Total	Rooted	25	540			
	Shooted	60	540			
	Callused	27	540			

the longest and highest number of roots and shoots compared to cuttings that were treated with 0.6, 0.3 and 0.0% (control) IBA (Table 5).

DISCUSSION

Milled pine bark supported the highest number of cuttings that developed roots and sprouted shoots. The production of the highest number of roots from stem cuttings propagated in milled pine bark could be due to the high level of moisture content and aeration given the physical properties of pine bark (Scalabrelli et al., 1983). Compared to soil and sand, pine bark is loose in texture hence allowing for more aeration and water flow. Aeration and water holding capacity of the media are often negatively correlated and therefore a balance between these must be achieved to ensure optimal rooting (Ofori et al., 1996). The significant high rooting in milled pine bark may therefore be attributed to better aeration and water drainage because high aeration and porosity are responsible for promoting root development (Olabunde and Fawusi, 2003; Puri and Thompson, 2003). Therefore, the type of rooting media used can have a major influence on the rooting capacity of cuttings. A study of rooting performance in *Vitellaria paradoxa* by Yeboah and Amoah (2009) showed that high aeration in rooting media is responsible for promoting metabolic activities and enhancing root initiation.

An appropriate rooting medium generally has to have an optimal volume of gas filled pore space and oxygen diffusion rate adequate for the needs of respiration (Fonteno and Nelson, 1990). According to Caron et al. (2000), media physical properties should not be constrained to just measurements of air- filled porosity, water holding capacity and bulk density, but also gas exchange characteristics. The highest number of shoots and length of shoots recorded from milled pine bark may also be due to easy translocation of water and mineral nutrients to the above ground parts of the cuttings, leading to their rapid growth and multiplication.

Stem cuttings propagated in sand produced the least percentage and number of roots per rooted stem cutting. This result was interesting given that high rooting percentages have been obtained from *Cordia alliodora* stem cuttings propagated in sand media by Leakey et al.

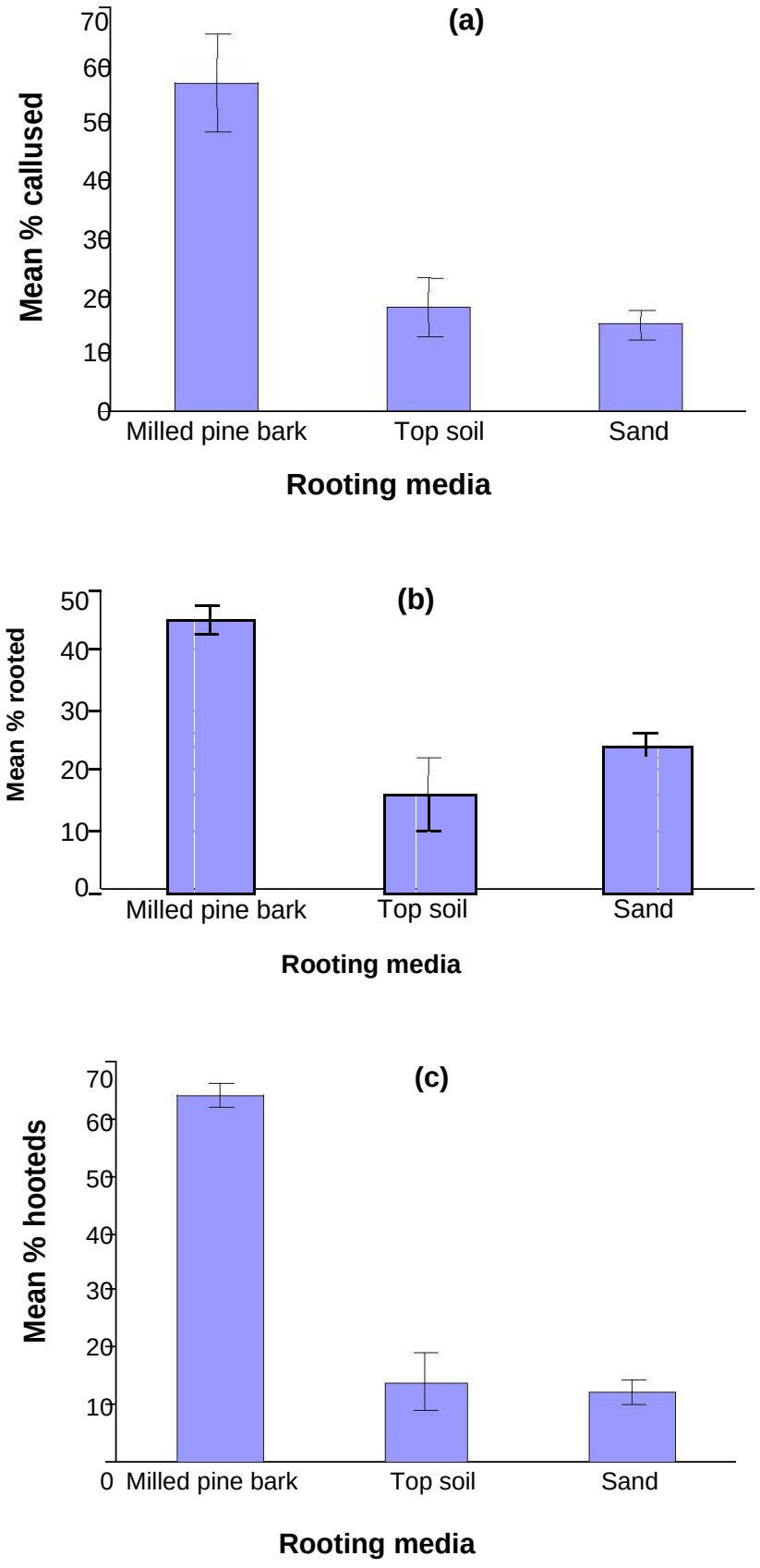

Figure 1. Mean percentage of (a) callusing, (b) rooting and (c) shooting of *W. ugandensis* stem cuttings in different rooting media.

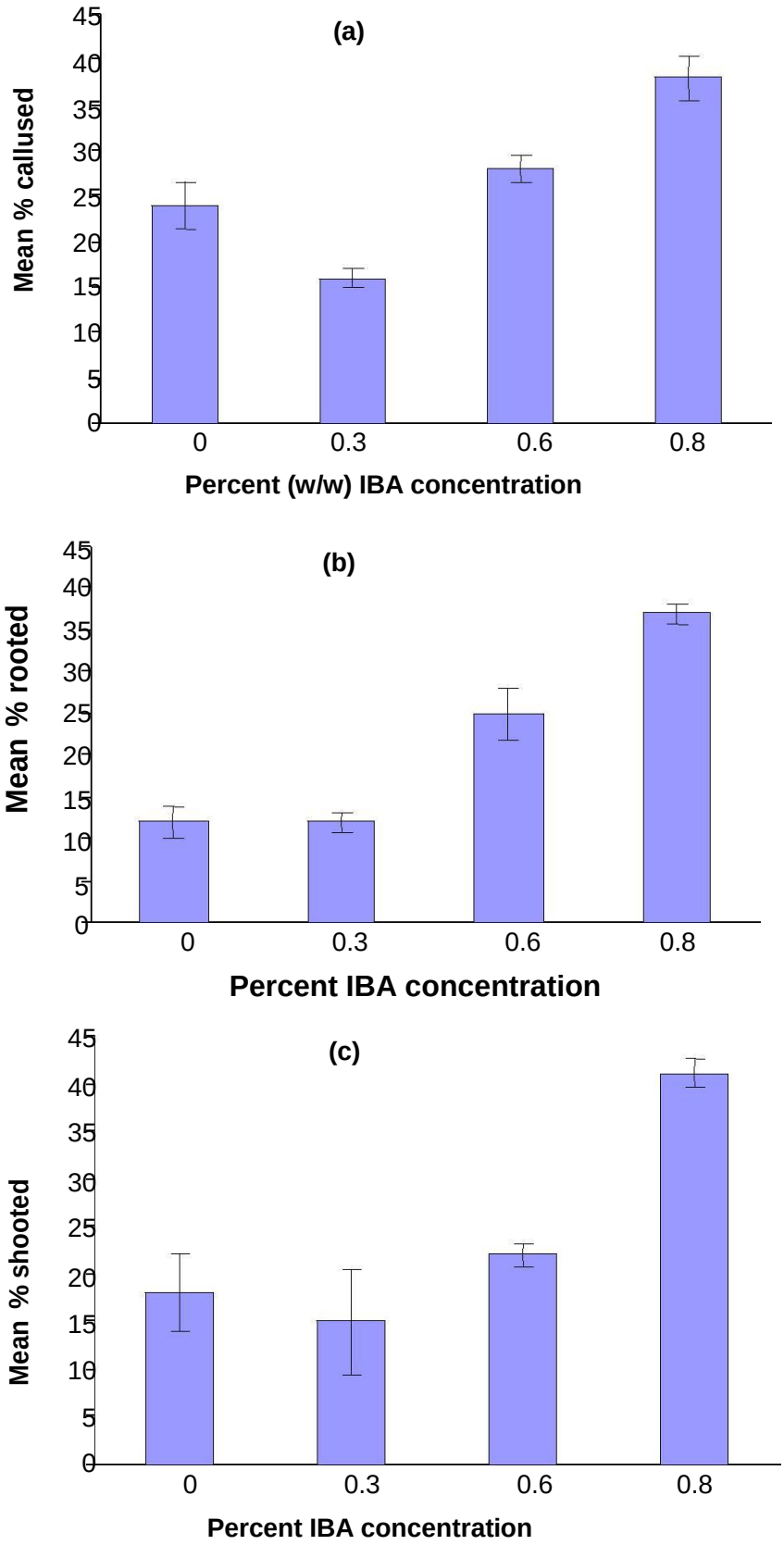

Figure 2. Mean percentage (a) callusing, (b) rooting and (c) shooting of *W. ugandensis* stem cuttings treated with the different concentrations of IBA rooting hormone.

Table 3. Performance of *W. ugandensis* stem cuttings under three different rooting medias and IBA concentrations.

Rooting media	IBA concentration	Mean number of roots	Mean root length (cm)	Mean number of shoots	Mean shoot length (cm)
Milled pine bark	0.0	0.10	0.420	0.30±0.12^{b}	0.55±1.20^{c}
	0.3	0.03	0.003	0.27±1.10^{b}	0.51±1.31^{c}
	0.6	0.57	0.110	0.80±0.31^{b}	1.10±0.24^{b}
	0.8	1.13	1.000	2.27±0.62^{a}	3.30±0.10^{a}
Top soil	0.0	0.10	0.210	0.17±0.25^{b}	0.14±0.33^{c}
	0.3	0.00	0.000	0.00±0.00^{c}	0.00±0.00^{c}
	0.6	0.00	0.000	0.00±0.00^{c}	0.00±0.00^{c}
	0.8	0.20	0.130	0.07±0.20^{c}	0.05±0.01^{c}
Sand	0.0	0.00	0.000	0.00±0.00^{c}	0.00±0.00^{c}
	0.3	0.00	0.000	0.00±0.00^{c}	0.00±0.00^{c}
	0.6	0.00	0.000	0.00±0.00^{c}	0.00±0.00^{c}
	0.8	0.23	0.063	0.17±0.24^{b}	0.22±0.12^{c}
LSD (P < 0.05)		ns	ns	0.64	0.49

Letters in superscript in the same column indicate no significant difference at p < 0.05 using Tukey's pairwise comparison (LSD). ns = non significant.

Table 4. Effect of rooting media on number and length of roots and shoots.

Treatment	Mean number of roots	Mean root length (cm)	Mean number of shoots	Mean shoot length (cm)
Milled pine bark	2.87±0.26 (0.001)	2.93±0.1^{a}	3.85±0.13^{a}	2.45±0.23^{a}
Top soil	0.32±0.08 (^{b}	0.23±0.1^{b}	0.54±0.07^{b}	0.12±0.05^{b}
Sand	0.53±0.18^{b}	0.12±0.06^{b}	0.73±0.4^{b}	0.64±0.16^{b}
LSD (P<0.05)	1.6	0.34	1.48	1.28

Letters in superscript in the same column indicate no significant difference at p < 0.05 using Tukey's pairwise comparison (LSD). ns = non significant.

Table 5. Effect of hormone concentration application on number and length of stem cuttings.

IBA concentration	Mean number of roots	Mean length of roots (cm)	Mean number of shoots	Mean length of shoots
0.8	2.00±0.27^{a}	2.59±0.75^{a}	2.90±0.16^{a}	1.79±0.26^{a}
0.6	0.65±0.16^{b}	0.80±0.03^{b}	0.98±0.13^{b}	0.75±0.21^{b}
0.3	0.02±0.02^{c}	0.06±0.01^{c}	0.5±0.09^{c}	0.26±0.16^{c}
0.0	0.34±0.1^{b}	0.31±0.01^{b}	0.83±0.1^{b}	0.60±0.18^{b}
LSD (P<0.05)	0.61	1.15	1.2	0.5

Letters in superscript in the same column indicate no significant difference at p < 0.05 using Tukey's pairwise comparison (LSD). ns = non significant.

(1990) . Sand was also identified as the best rooting media for *Gongronema latifilia* stem cuttings by Agbo and Omaliko (2006). However, there is variation in response to different rooting media that has been reported for many tree species. For example, in Southwest Cameroon and Ghana, the highest rooting percentages of *Irvingia gabonensis* (Shiembo et al., 1996), *Milicia excelsa* (Ofori et al., 1996) and *V. paradoxa* (Yeboah and Amoah, 2009) have been reported for stem cuttings propagated in sawdust. The differences in rooting ability of various species propagated in different rooting media could be explained by their xeromorphic or hydromorphic status (Loach, 1992) and the effects of this status on the water relations of the cuttings (Mensen et al., 1997). While there is need for further investigation, the relatively low level of success for *W. ugandensis* stem cuttings propagated in sand may point to the existence of significant variation in the total physical characteristics of

the media (sand).

Soil recorded the least percentage, number and length of roots developed probably due to very low aeration and porosity (Amri et al., 2009). Soil resistance to root penetration that is highly dependent upon water content, bulk density, structure and strength of the soil could also have been responsible for the resultant low percentages, number and length of roots (Bradford, 1986). According to Amri et al. (2009), soil has not got enough required aeration porosity for sufficient gas exchange which can lead to rotting of the cuttings. In this study, the mortality of *W. ugandensis* stem cuttings was highest in soil compared to milled pine bark and sand. Apart from poor rooting of stem cuttings in soil as a rooting media (Amri et al., 2009), poor rooting of cuttings in soil also reflects anoxia associated with high water content (Hartmann et al., 2002).

The high rooting percentage of the *W. ugandensis* stem cuttings treated with 0.8% IBA concentration is concordant with the findings by Aminah et al. (2006) in which application of 0.8% IBA recorded the highest rooting percentage of leafy stem cuttings of *Shorea parvifolia* and *Shorea macroptera.* This could be due to the effect of auxins that have been reported to enhance rooting through the translocation of carbohydrates and other nutrients to the rooting zone (Milleton et al., 1980). According to Davis and Hassig (1990), the production of adventitious roots in plants through cell division, multiplication and specialization is also controlled by plant growth substances especially auxins. This implies that treating stem cuttings with auxins can increase the percentage of rooting, root initiation and number of roots. Even then, application of optimal hormone concentration is very important for successful rooting of cuttings (Leakey et al., 1982). In some instances, however, optimum application of hormone concentration has resulted into failures for stem cuttings to develop roots. An example is the propagation of *Ulmus parvifolia* stem cuttings treated with 0.8% IBA that failed to root (Griffin and Schroeder, 2004). This suggests that root formation in such species is highly sensitive to auxin formulation. The decline in rooting percentage with IBA concentrations greater than 0.2% is also a clear indication that some levels of hormone concentrations are inhibitory to root initiation, as has been recorded in a number of other tree species (Leakey et al., 1990). Thus, apart from ensuring optimal hormone concentration, the sensitivity of hormone to adventitious root formulation for a particular species has to be taken into account when rooting stem cuttings.

CONCLUSIONS AND RECOMMENDATIONS

Although there are various materials that can be used for rooting stem cuttings, the use of milled pine bark appears to be most appropriate for rooting *W. ugandensis* stem cuttings. Rooting of *W. ugandensis* softwood stem cuttings treated with 0.8% IBA concentration in milled pine bark should be promoted for mass propagation of this tree. In order to promote mass production of *W. ugandensis* , mixtures of other rooting materials may also be tried out in situations where milled pine bark is in short supply.

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