

Full Length Research Paper

The effects of different concentrations of indole-3-butyric acid (IBA) on leafy stem cuttings of four tropical timber species

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The effects of different concentrations of indole-3-butyric acid (IBA) on the rooting of leafy stem cuttings of *Albizia zygia*, *Blighia welwitschii*, *Lophira alata* and *Pterocarpus soyauxii* were investigated using non-mist propagators. Four hormone concentrations were used; 16000, 8000, 4000 and 0 $\mu\text{g ml}^{-1}$ (control). Results indicated that *Albizia*, *Blighia* and *Lophira* cuttings rooted best when treated with 16000 $\mu\text{g ml}^{-1}$ IBA (80, 100 and 40% respectively) and their control cuttings did not root. *Pterocarpus* stem cuttings treated with 8000 and 4000 $\mu\text{g ml}^{-1}$ had 100% rooting while those treated with 16000 $\mu\text{g ml}^{-1}$ and control was 80%. The highest root number was noted in cuttings of the various species treated with 16000 $\mu\text{g ml}^{-1}$ IBA and there were significant differences only from treatments of *Blighia* and *Pterocarpus* species. Stem cuttings treated with IBA at 16000 $\mu\text{g ml}^{-1}$ also gave the fastest rooting time which ranged from 18 to 78 days. Stem cuttings of *Blighia*, *Albizia* and *Lophira* species treated with 16000 $\mu\text{g ml}^{-1}$ IBA also had the longest roots (2.48, 1.23 and 1.45 cm respectively). The longest root length of *Pterocarpus* was observed in cuttings treated with 8000 $\mu\text{g ml}^{-1}$ of IBA (3.72 cm). Vegetative production of seedlings of these species is essential for reforestation programmes.

Key words: Timber species, indole -3- butyric acid, leafy stem cuttings, rooting time, percentage rooting.

INTRODUCTION

Tropical forests contain much terrestrial biodiversity, provide food, shelter, health care, protect water and soil resources store carbon in biomass and maintain the delicate composition of the atmosphere (Neuwinger, 2000). One very important material provided by forests is wood which is used in construction, as fuel, in making furniture and other implements. Tropical timbers are preferred as a source of wood because of their natural durability and good working properties (Miller and Wiedenhoef, 2002). Despite these important uses, tropical forests are threatened by unsustainable land and resource use (De Capua, 2005). The greatest constraint to forest regeneration projects is the lack of good planting material (materials that are viable and free from pest and

diseases). For example, the viability of *Terminalia* spp. is about 0.1%, *Balakata baccata* 25%, *Michelia baillonii* 31% and *Erythrina subumbrans* 39% (Mostacedo and Fredericksen, 1999). In a study on seed viability, seed of some tree species were found to lose viability rapidly; *Swietenia macrophylla* seeds were viable for only 6 weeks. Viability is also affected by storage conditions, thus, seeds of *Araucaria hunsteinii* will die if allowed to dry out (Blakesley et al., 2002). Seed availability can be a problem in tropical trees. For example, *Triplochiton scleroxylon* trees only fruit once in 4 to 7 years (Howland and Bowen, 1977) and the seeds are only viable for 2 to 3 weeks while *Lophira alata* would fruit once every 5 or even more years (Bonner, 1992). Many other tree species have a long period of juvenility before they become sexually mature and capable of flowering and fruiting, and this can have a range of 5 to 50 years. The tree species *Baillonella toxisperma* would start flowering at the age of 50 to 70 years but regular fructification would

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start later when the bole is about 70 cm in diameter (Louppe, 2005). Furthermore, tree seeds typically suffer from pre-germination mortality caused by herbivores, diseases or other pathogens.

Another constraint to tree regeneration is pest attack. An example of this is the seedling growth of *Milicia excelsa* and *Milicia regia* that is seriously affected by gall-forming *Phytolyma* spp. (iroko gall flies) which kill the seedling apex (Ofori et al., 1996). Teak seeds are attacked by a number of fungi especially *Aspergillus* spp. and *Fusarium* spp. These fungi also caused both pre-emergence and post-emergence damping-off disease on seeds of *Albizia lebeck* and *Rhizoctonia solani* (Mostacedo and Fredericksen, 1999). Thus, there are many reasons why it is important to screen some of these important species for their amenability to vegetative regenerate using juveniles stem cuttings. Vegetative propagation provides a unique opportunity of avoiding the problem of poor seed viability and recalcitrant seeds which are predominant in tropical tree species. It is also an assembly of germplasm for base populations to maintain certain desirable characteristics (Amri, 2010; Leakey and Simons, 2000). The four species used for this study were: *Albizia zygia* (D. C.) Macbr (small leaf), *Blighia welwitschii* (Hiern) Radlk, *Lophira alata* Banks ex C. F. Gaertn (Azobe or iron wood) and *Pterocarpus soyauxii* Taub (African Padouk or cam wood). They were selected based on commercial, conservational and cultural importance. *A. zygia* produces a moderately durable timber, is a nitrogen fixer, thus important in agroforestry. Its wood is used for indoor construction, light flooring, furniture, canoes, implements, carving, veneer and plywood. It is commonly used as firewood and for charcoal production (Burkill, 1995). The wood pulp is suitable for paper production (Kpikpi, 1992). Its bark produces gum that is useful in the food, cosmetic and pharmaceutical industries (Odeku, 2005). In traditional medicine, bark sap is instilled in the eyes to treat ophthalmia (Neuwinger, 2000). *B. welwitschii* (Sapindaceae) produces class four timbers and can be planted as an ornamental; thus it is a goods candidate for the greening of towns. Its seeds are used as fish poison and are not toxic to other organisms. Its bark is used in the treatment of measles (Neuwinger, 2000).

L. alata (Ochnaceae) produces a very durable timber that is highly commercialized (Laird, 1999). It is popular for heavy construction work, harbour works and railway sleepers (UNEP-WCMC, 1999). The species is used locally in traditional medicine, for treating backache, toothache, respiratory and stomach problems and yellow fever (Neuwinger, 2000). The leaves are used in termite control, and oil from the seeds is used as food and to make ointments and soaps (Laird, 1999). Its timber, commonly called "Azobe" is rich in silica, resistant to marine borer, and it has been classified as vulnerable on the IUCN Red List (IUCN, 2009). *P. soyauxii* (Fabaceae)

produces a very durable timber and is classified as vulnerable on the IUCN Red List (IUCN, 2009). It is highly commercialized (Laird, 1999). The wood contains alkaloids and is also rich in tannins (Richter and Dallwitz, 2000). It is a preferred wood for furniture, carving, canoes, stools, musical instruments and agricultural implements (Laird, 1999). The bark has antifungal activity against some pathogenic fungi. The ground stem is of cultural and medicinal importance associated with childbirth and marriages (Laird et al., 1997). The low cost macropropagation is a solution to forestry and agroforestry projects and for the conservation of biodiversity especially threatened tree species.

MATERIALS AND METHODS

Study site

This study was carried out in the shade house (87.6% full sunlight, measured with digital Luxmetre (MS 6610 Mastech®) at the University of Buea, Cameroon. Buea is situated in the Southwest region of Cameroon between latitude 3° 57' and 4° 27' N and longitude 8° 58' and 9° 25' E (Tchouto et al., 1997). It has a humid tropical climate with a mean annual rainfall of about 2500 mm, most of which is between June and September (unpublished data). The mean annual temperature is 28°C, relative humidity average ranges from 80 to 86% and annual sunshine between 900 to 12000 h per annum (Ndam, 1998). The soil type is basically volcanic, rich in mineral content but due to continuous cropping, the soil fertility has reduced drastically.

Preparation of leafy stem cuttings and planting medium

Seeds of *B. welwitschii*, *A. zygia* and *P. soyauxii* were sown in seed boxes filled with top soil. The germinated seeds were pre-transplanted into black polybags filled with top forest soil as the potting medium. The seedlings of these species were raised in the nursery and the main stems were used for stem cuttings when they were 7 months old with mean height range of 45.5 to 65.8 cm depending on the species. Wildlings of *L. alata* that were about 1 year old were collected from the Limbe Botanic Garden. The mean temperature in the shade was about 22°C and the seedlings were watered three times a week. Leafy stem cuttings were made from firm but young stems in slants using a secateur. 64 stem cuttings of about 6 cm were collected from each of the four species. The number of stem cuttings per shoot of the seedling ranged from 4 to 6 and these were one node leafy cuttings. The uppermost part of the stem was cut and discarded. A total of 256 one node leafy stem cuttings were used and there were four replicates per treatment. The leaves on the stems were reduced with a scissor to less than half their sizes with an area of about 25 cm². This was to reduce the transpiration rate of the stem cuttings. Coarse sand was boiled at 100°C for 8 h and allowed to cool for 24 h in the laboratory. It was then used to fill small perforated trays. The rooting hormone Indole-3-butyric acid (IBA) was used and different concentrations were prepared as described by Leakey et al. (1990). The IBA formulation was prepared using 95% ethanol diluted to 50% with distilled water. The various concentrations were: 16000, 8000, 4000 and 0 µg ml⁻¹ (control) 50% diluted alcohol only. The cut ends of one node leafy cuttings were dipped into the solutions according to the different concentrations and fan to evaporate the alcohol and thus increasing the concentration of IBA at the cutting base. A hole was made in

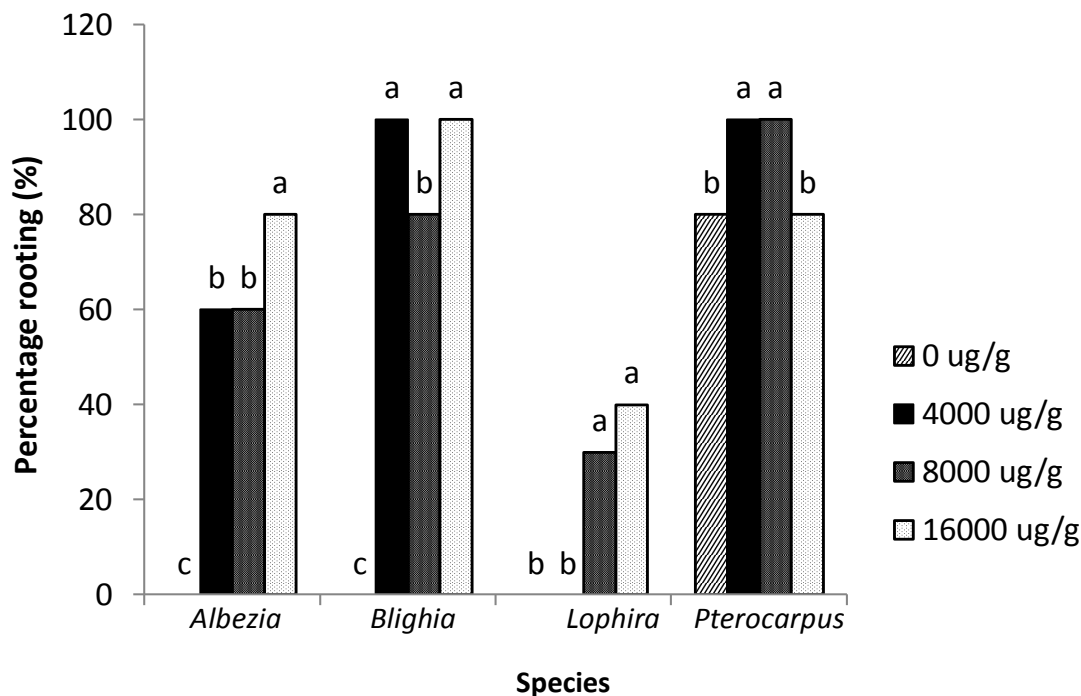


Figure 1. The effect of various hormonal treatments on rooting percentage of leafy stem cuttings of the various timber species. Values grouped by the same letter for each species are not significantly different at $P < 0.05$.

the rooting medium (sterilized sand) with a pencil and the leafy stem cutting inserted into it. Stem cuttings of each treatment were inserted in a separate chamber of the trays.

A non-mist propagator (Leaky et al., 1990) of dimensions length $100 \times 60 \times 40$ cm was used for this experiment. The leafy stem cuttings were sprayed with fine drops of water after every 48 h using a 2 L capacity hand sprayer. There were 16 cuttings per treatment and a total of 64 per species. The cuttings were evaluated for rooting every 7 days for 90 days. The non-mist propagator is placed in a shade with about 60% of the full sun light and mean temperature of 20°C . The percentage of rooted leafy stem cutting for each treatment was calculated as a percentage of

RESULTS

The effects of hormonal concentrations on the percentage rooting of leafy stem cuttings

Rooting was affected by IBA concentration in all species (Figure 1). Leafy stem cuttings of *Albizia* treated with $16000 \mu\text{g ml}^{-1}$ of IBA rooted the most (80%) while control did not root and this significantly different at $P \leq 0.05$. *Blighia* leafy stem cuttings had the highest rooting percentages of 100% with IBA concentration of 16000 and $4000 \mu\text{g ml}^{-1}$ while the control had 0% and these were significantly different. *Lophira* leafy stem cuttings had the highest rooting percentage in cuttings treated with 8000 and $16000 \mu\text{g ml}^{-1}$ IBA (40%) while those

the total leafy stem cuttings used. The length of the longest root was measured with a ruler to the nearest 1.0 mm from the point of emergence to where it ends (when all treatments had either rooted or decayed). Diameter of roots was measured with an electronic digital caliper (Shenzhen[®], to the nearest 0.1 mm) and root number was counted. The means of these were all calculated. Rooting time is the number of days it took the leafy stem cuttings to root (presence of root primodium on the leafy stem cuttings) with root diameter and length of at least 0.2 and 5 mm respectively. Analysis of variance was carried out on the resulting data using the MINITAB Version 15 statistical package to assess the best hormonal concentration. All analyses were carried out at 95% interval. treated with 4000 and $0 \mu\text{g ml}^{-1}$ did not root. Leafy stem cuttings of *P. soyauxii* treated with 8000 and $4000 \mu\text{g ml}^{-1}$ IBA each gave the highest percentage rooting (100%) and the lowest was in cuttings treated with $16000 \mu\text{g ml}^{-1}$ of IBA and control (80%) and these were significantly different ($P \leq 0.01$) (Figure 1).

The effects of IBA concentrations on the number of roots on leafy stem cuttings

The mean number of roots of leafy stem cuttings per treatment was affected by the different concentrations of IBA (Figure 2). *Albizia* cuttings treated with $16000 \mu\text{g ml}^{-1}$ of IBA had the highest mean number of roots (5) compared to those treated with 8000 and $4000 \mu\text{g ml}^{-1}$ IBA which had the least number of roots (4 roots),

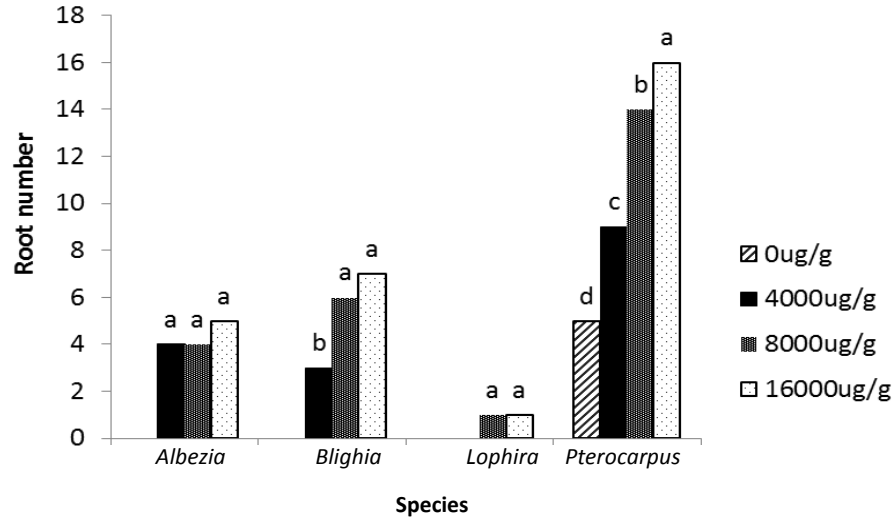


Figure 2. The effect of various hormonal treatments on root number of leafy stem cuttings of timber species. Values grouped by the same letter for each species are not significantly different at $P < 0.05$

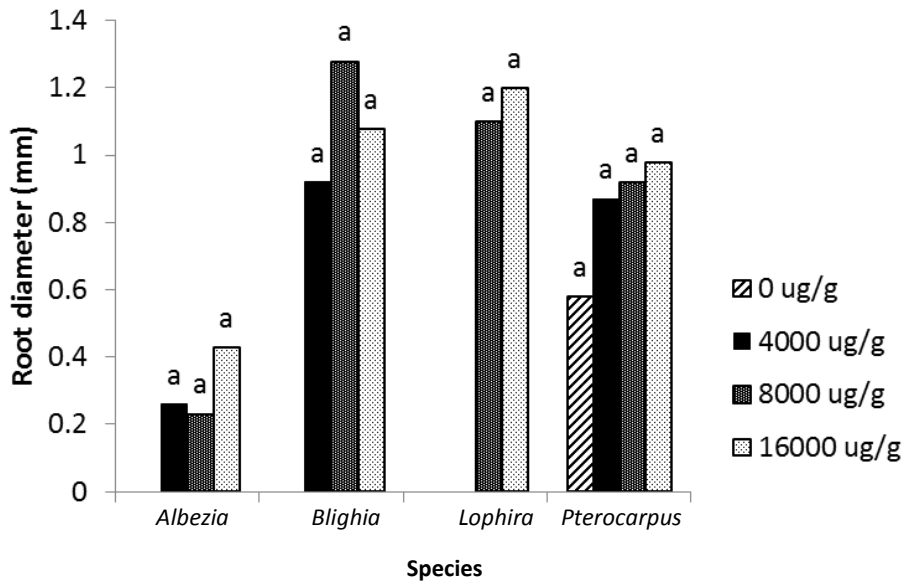


Figure 3. The effect of various hormonal treatments on root diameter of leafy stem cuttings. Values grouped by the same letter for each species are not significantly different at $P < 0.05$.

although the numbers did not vary significantly at $P \leq 0.05$. Leafy stem cuttings of *Blighia* and *Pterocarpus* treated with $16000 \mu\text{g ml}^{-1}$ of IBA had the highest mean root number of 7 and 16 respectively. Untreated cuttings of *Pterocarpus* had the least root number (5) and it was significantly different between the different treatments ($P \leq 0.05$) while *Blighia* cuttings treated with $4000 \mu\text{g ml}^{-1}$ had the least root number (Figure 2).

The effects of hormone concentrations on root diameter of rooted cuttings

Root diameter was unaffected by IBA treatments (Figure 3). The root diameter had a range of 0.23 to 1.28 mm and the maximum and the least diameter were observed in *Blighia* and *Albizia* leafy stem cuttings treated with $8000 \mu\text{g ml}^{-1}$ IBA respectively.

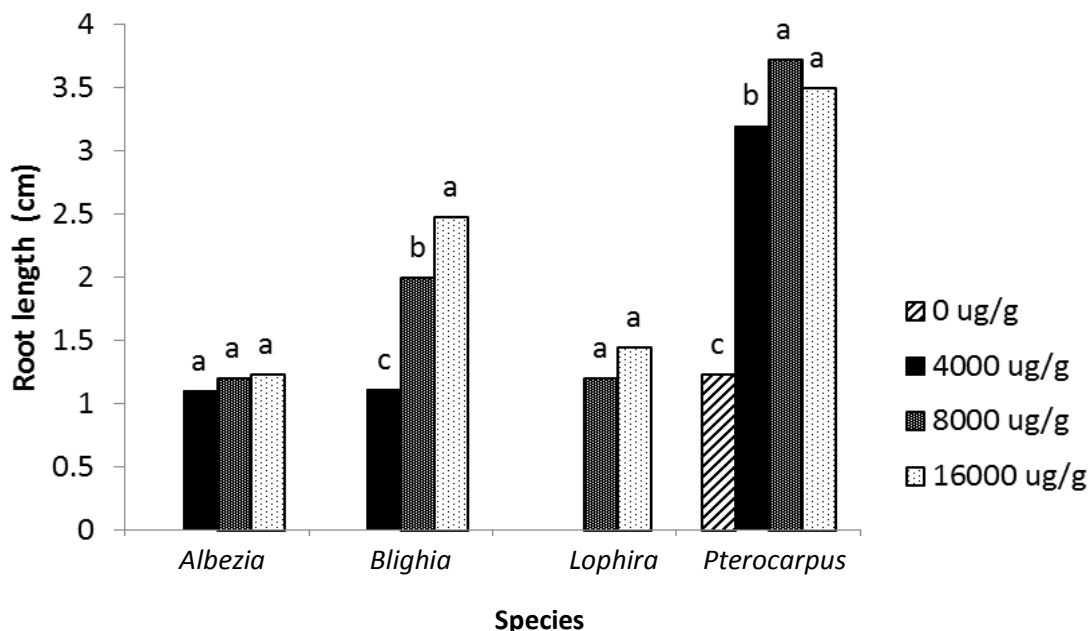


Figure 4. The effect of various hormonal treatments on root length of leafy stem cuttings of timber species. Values grouped by the same letter for each species are not significantly different at $P < 0.05$.

The effects of hormone concentrations on root length of rooted cuttings

Root length was affected by the different concentrations of IBA (Figure 4). Leafy stem cuttings of *Albizia*, *Blighia* and *Lophira* treated with $16000 \mu\text{g ml}^{-1}$ IBA had the longest mean roots length (1.23, 2.48 and 1.45 cm respectively) at the end of 90 days. The least root lengths for *Albizia* and *Blighia* species was recorded in cuttings treated with 4000 of IBA with values of 1.10 and 1.11 cm respectively while the shortest root length for *Lophira* species was noted in cuttings treated with $8000 \mu\text{g ml}^{-1}$ IBA. These lengths did vary significantly between treatments at $P \leq 0.05$ only for *Blighia* species. The mean longest roots on *Pterocarpus* leafy stem cuttings were those treated with $8000 \mu\text{g ml}^{-1}$ IBA (3.72 cm) and the shortest were noted in the control (1.23 cm) and this was significantly different between the treatments at $P \leq 0.05$.

The effect of hormone concentrations on the rooting time

The speed of rooting varied between species and of leafy stem cuttings of the different species. Leafy stem cuttings of *Albizia*, *Blighia*, *Lophira* and *Pterocarpus* treated with $16000 \mu\text{g ml}^{-1}$ IBA had the least rooting time (25, 38, 72 and 18 days respectively) and there was significant difference between the treatments at $P \leq 0.05$ except for *Lophira* that was not significantly different for the IBA concentrations (8000 and $1600 \mu\text{g ml}^{-1}$ IBA). The highest

rooting time for *Lophira* cuttings was 78 days while that of *Pterocarpus* was recorded in control (38 days) (Figure 5).

DISCUSSION

Auxins are known to promote adventitious root development of stem cuttings, through their ability to promote the initiation of lateral root primordia and enhance transport of carbohydrates to cuttings' bases (Hartmann et al., 1990). IBA induced rooting in all the species. This is in conformity with Abdullah et al. (2005) who observed between 65% (in 0.4% IBA) and 15% (in the control) rooting in (*Baccaurea sapida*) cuttings treated with different IBA concentrations. Cuttings of *Pterocarpus* not treated with IBA rooted successfully. This suggests that *Pterocarpus* leafy stem cutting had high levels endogenous auxins. A similar observation was reported by Magingo and Dick (2001) who recorded rooting percentages of 86 and 59% in cuttings of *Pterocarpus angolensis* and *Brachystegia spiciformis* respectively, without auxins. Successful rooting without hormones has also been reported in *Shorea macrophylla* (Lo, 1985) and *Nauclea diderrichii* (Leakey, 1990). Leafy stem cuttings of *B. welwitschii*, *A. zygia* and *L. alata* did not root in the absence of IBA. Probably, the amount of endogenous auxins within these species is not enough to initiate cell division. This is in conformity with reports of Mesén et al. (1997) who reported that the cuttings of *Cordia alliodora* failed to root in the absence of auxin treatment. The root number, root diameter and root length for the different

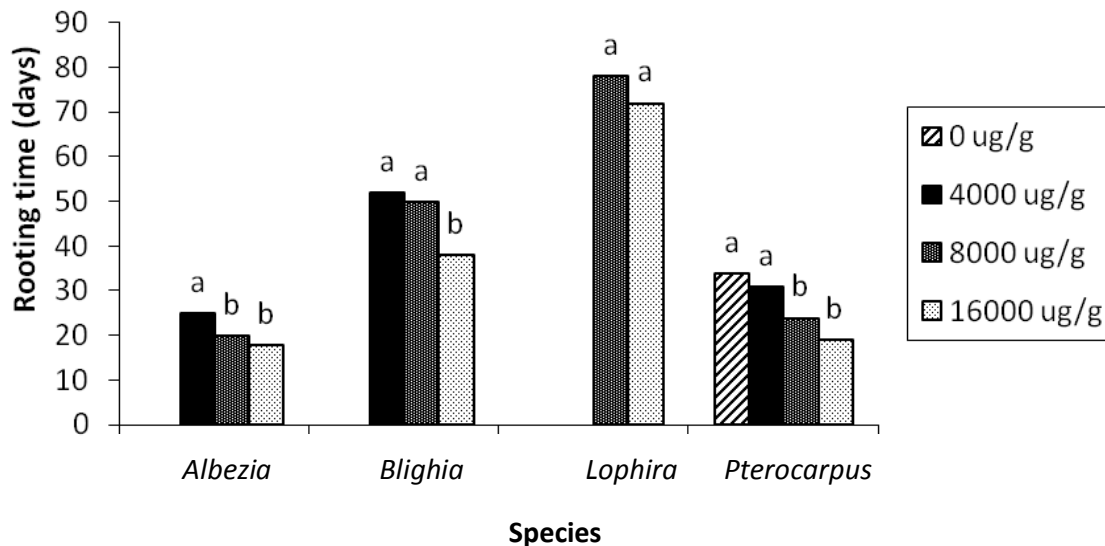


Figure 5. The effect of various hormonal treatments on rooting speed leafy stem cuttings of timber species. Values grouped by the same letter for each species are not significantly different at $P < 0.05$.

treatments did not vary significantly for the hormone treated stem cuttings of *B. welwitschii* and *A. Zygia*. The various IBA treatments did not affect the rate of rooting of *B. welwitschii*. These observations are in contrast to the findings of Abdullah et al. (2005) who registered significant differences in the number of root developed and root length with different hormone concentrations.

However, results obtained with seedlings of *P. soyauxii* show that the various IBA concentrations had significant differences on root length, root number and rate of rooting and it is in conformity with the findings of Abdullah et al. (2005). This was probably due to difference in the amount of polysaccharides hydrolyzed.

Conclusion

The hormone indole-3-butyric acid induced rooting in the different species studied. *Pterocarpus* cuttings can root successfully without the use of hormones. The IBA concentrations of 16000 and 8000 $\mu\text{g ml}^{-1}$ were good for the rooting of *A. zygia*, *B. welwitschii* while 16000 $\mu\text{g ml}^{-1}$ IBA was best for the rooting of *L. alata*. The production of vigorous and healthy seedlings by vegetative means would enhance forest management through enrichment planting especially for species that are threatened or those that reproduces once in 5 years or more and those that have poor recruitment rates from seeds.

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