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Vegetative propagation of *Afzelia africana* Sm. Ex Pers.: a multipurpose and threatened tree

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ABSTRACT

Afzelia africana is at the verge of extinction from continuous exploitation without replacement, thus propagation of this tree species is expedient. Vegetative propagation is a reliable means of propagation of tree species. This study therefore describes a protocol for the vegetative propagation of *Afzelia africana* by using stem cuttings from its mature tree (20 years old) and saplings (2 years old). Herbaceous (non - lignified portion of the stem) and semi – hard wood cuttings (lignified portion of the stem) (with and without leaves) were treated with high and low concentrations of Indole butyric acid (IBA) solution via the quick dip and the soaking method of application and planted on forest soil and river sand in a humidifier. Cuttings from mature tree planted on forest soil and river sand did not respond to any treatment. Also, no response was observed on cuttings collected from saplings and planted on forest soil; however, cuttings planted on river sand sprouted. The presence and absence of leaves and IBA treatment had an interaction effect on root number (0.02) and root length (0.0001). The response from herbaceous cuttings was significantly higher than from semi – hard wood cuttings with respect to root number (1.00) and root length (5.82 cm). Cutting type and IBA treatment also had an interaction effect on root number (0.05) and root length (0.0007). Herbaceous leafy stem cuttings of *A. africana* treated with 400 ppm IBA and planted on river sand is suitable for rooting stem cuttings of *A. africana* for raising seedlings for reforestation, afforestation and other conservation purposes.

Keywords: Rooting, cuttings, *Afzelia africana,* hormone, humidifier.

INTRODUCTION

Afzelia africana Sm. Ex Pers. known as African mahogany or African oak is a large deciduous tree. It is a leguminous tree which belongs to the family Leguminosae or Fabaceae, sub-family Caesalpiniaceae. It is widely distributed in many African countries including Benin, Burundi, Cameroon, Central African Republic, Cote d'Ivoire, Gambia, Ghana, Guinea-Bissau, Kenya, Liberia, Mali, Nigeria, Senegal, Sierra-Leone, Sudan, Tanzania and Uganda (Orwa et al., 2009). *Afzelia africana* is found in the humid and dry forests, especially in the forest-savanna borders or semi-deciduous forest. *Afzelia africana* prefers sandy soil in well-watered sites but it can tolerate seasonally hydromorphic and lateritic soils. In Nigeria, it is called *akpalata* in Igbo, *apa* in Yoruba, *yiase* in Tiv, *ukpo* in Idoma and *kawa* in Hausa.

Tender leaves and shoots of *Afzelia africana* are added in yam dishes and eaten as vegetables alone or mixed with ground cereals

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before cooking, (Igwenyi and Akubugwo, 2010). The nitrogen-rich leaves also serve as soil improver especially when mulching. A leaf decoction mixed with *Syzygium guineensis* leaves and *Xylopia* fruit forms a drink used to treat oedema (Orwa et al., 2009). The flowers are used as condiment in sauces. In traditional medicine, an infusion of the bark is used against paralysis; and as a decoction against constipation. The crushed bark mixed with honey is used in veterinary medicine while the ash can be prepared with shea butter as soap and used against lumbago. The root of the plant can be pulverized and employed in the treatment of various ailments and disease conditions such as hernias, gonorrhea, and stomach ache.

Afzelia africana is one of the non-conventional vegetables obtained from forests, and supplements the conventional ones obtained from farms and home gardens. *Afzelia africana* seeds contain extractable oil (Palgrave and Palgrave, 2002) and the physicochemical and fuel properties of the methyl esters shows its potentials in biodiesel production. The oil can release high amount of heat on combustion and can ignite easily in a combustion engine. The iodine and peroxide values shows increased stability of the oil during storage and transportation (Igwenyi et al., 2011). Despite the usefulness of this plant, it is on the verge of extinction, owing to population explosion, urbanization and the resultant degradation of natural forests; and the preference of farmers to produce the conventional vegetables (Umedum et al., 2014).

Afzelia africana is propagated by seeds and vegetative technique through budding. Natural regeneration is poor because seed predation by animals is usually high (Amusa, 2011). *Afzelia africana* seeds are dormant and they become recalcitrant on storage (Orwa et al., 2009). The rate of seed germination in the wild is low and its seedlings rarely develop into saplings. The stock of *A. africana* species has been badly depleted. In addition, the species is not fire resistant (Amusa, 2010). Exploitation for timber is locally severe, for example in Ghana, and conservation measures have been recommended. *Afzelia africana* has been categorized as a threatened species by the International Union of Conservation of Nature (IUCN, 2012).

Cloning forestry (establishing forests through vegetative propagation) was proposed as an alternative propagation method comparable to conventional seedling - based forestry. This technique offers a unique opportunity of avoiding the problem of recalcitrant seeds predominant in tropical tree species. Also, it circumvents long juvenile periods.

The multiple use of *A. africana* in West Africa, particularly the use of the seeds has led to a permanent pressure on natural populations. However, propagation by stem cuttings offers a means of rapid regeneration and conservation of its germplasm, hence this study. Therefore, the objective of the study was to develop a protocol for vegetative propagation of *A. africana* through investigating the effect of different IBA treatments on leafy and non-leafy herbaceous and semi – hard wood stem cuttings of saplings and mature tree. Achieving this is inevitable in reversing the status of this tree species from threatened to widely cultivate.

MATERIALS AND METHODS

The experiment was conducted in the Department of Botany, Obafemi Awolowo University, Ile – Ife, Nigeria (Lat 7 ^{0} 32 N Long 4 ^{0} 31 E), between March 2017 and August 2017. It was set up in a humidifier placed under partial shade. The rooting medium was forest top soil and river sand.

Preparation of cutting materials

Herbaceous and semi – hard wood stem cuttings were obtained from mature tree and 1 – 2 years old saplings of *A. africana* in the Obafemi Awolowo University estate. Stem cuttings of 8 – 10 cm length were used with a set partially defoliated (with leaves) and another set completely defoliated (without leaves).

Application of growth hormone

Herbaceous (non - lignified portion obtained from the terminal end of the branch) and the semi – hard wood cutting (lignified portion obtained towards the base of the branch) were collected and treated with (0, 100, 200 and 400) ppm liquid formulation of Indole Butyric Acid. These cuttings were soaked for eighteen hours in the hormone solution. Another set of cuttings were treated with (1000, 2000, 3000, 4000 and 5000) ppm IBA via the quick dip method. The cuttings were rinsed and air dried after the hormone treatment, and then planted and watered every two days for 8 weeks, except during the rainy season when they were watered twice a week.

Experimental design and statistical analysis

The experiment was set up in a completely randomized design with two replicates of four (for low concentration of IBA) and five (for high concentration of IBA) treatments with each consisting of five cuttings. Four categories of stem cuttings (from both mature tree and sapling) were used in the study and they are - leafy herbaceous, non - leafy herbaceous, leafy semi - hard wood and non - leafy semi – hard wood. Growth parameters such as sprouting percentage, rooting percentage, number of roots, length of roots, number of shoots and length of shoots were measured after 8 weeks. The data was subjected to square root transformation, after which a one and two-way analysis of variances were carried out and the means separated with least significant difference (LSD) at $P < 0.05$.

RESULTS

Effect of IBA treatment, presence and absence of leaves and type of medium

Stem cuttings collected from mature tree; herbaceous and semi – hard wood cuttings with and without leaves, treated with low and high concentrations of IBA and planted on forest soil and river sand did not respond to any treatment. However, herbaceous and semi – hard wood cuttings collected from saplings and treated by soaking for 18 hours in low concentration of IBA (0, 100, 200 and 400) ppm, responded within 8 weeks on river sand (Table 1). No response was observed when these cuttings were subjected to the same treatment and planted on forest soil.

The concentration of IBA treatment and the presence or absence of leaves on stem cuttings had no significant effect at $P < 0.05$ on the mean shoot number. Herbaceous cuttings with leaves treated with 200 ppm IBA had the highest mean shoot height (3.16^{a}) with 60% of the cuttings sprouting and 20% rooting. Herbaceous cuttings with leaves treated with 400 ppm IBA produced the highest mean number of root (1.00^{a}) and the longest roots (5.82^{a}) (Figure 1) with 80% of the cuttings sprouting and rooting.

The general trend observed was that for herbaceous cuttings without leaves, the mean shoot number decreased as the concentration of IBA treatment increased up to 200 ppm, whereas for herbaceous cuttings with leaves, mean shoot number increased as IBA treatment increased (Table 1). A similar trend was observed with respect to mean shoot height, mean root number and mean root length in herbaceous cuttings without leaves, a decrease was observed as the concentration of IBA increased. In the case of cuttings treated with 0 ppm IBA, there was a significant decrease between this treatment and other IBA treatments with respect to mean root length. In herbaceous cuttings with leaves, the mean number of roots increased when the IBA treatments increased with the highest number of roots formed in cuttings treated with 400 ppm IBA (1.00^{a}), while in herbaceous cuttings without leaves, more roots were formed at lower concentrations of 0 ppm (0.80^{ab}) and 100 ppm (0.40^{ab}) with no rooting at higher IBA concentrations of 200 and 400 ppm. Also with respect to mean root length, herbaceous cuttings without leaves treated with 0 ppm IBA produced the longest roots; (3.54^{b}), after which a significant decrease at $P < 0.05$ occurred as the concentration of IBA treatment increased, while in herbaceous cuttings with leaves, a significant increase occurred as concentration of IBA increased, the longest roots (5.82^{a}) formed when cuttings were treated with 400 ppm IBA.

In order to consider if an interaction effect exists between the presence and absence of leaves on cuttings and IBA treatment on the growth parameters of stem cuttings, a factorial analysis of variance was carried out and is presented in Tables 2 and 3. The presence or absence of leaves had no significant effect on all the parameters observed at $P < 0.05$ (Table 2). However, the presence of leaves gave a better response with respect to mean root number and mean root length and the absence of leaves resulted to a better response with respect to mean shoot number and mean shoot length. IBA treatment had significant effect on only the root length with cuttings treated with 400 ppm responding better (1.56^{a}) (Table 3). Howbeit, an interaction effect (Leafiness - IBA treatment) was observed on the root number (0.02) and the root length (0.0001) at $P < 0.05$ (Table 3).

Effect of IBA treatment and cutting type

A comparative study was carried out between leafy herbaceous cuttings (herbaceous cuttings with leaves) and leafy semi hard wood cuttings (semi hard wood cuttings with leaves) to test the effect of IBA treatment and cutting type on rooting of cuttings (Table 4). In all the IBA treatments tested, there was no significant difference at $P < 0.05$ in the mean shoot number and mean shoot height of both cutting types (herbaceous and semi hard - wood). Semi – hard wood cuttings treated with 0 ppm IBA had the highest mean shoot height, (5.08^{a}) (Figure 2). The mean root number (1.00^{a}) and the mean root length (5.82^{a}) were observed to be highest in herbaceous cuttings with leaves treated with 400 ppm IBA. A significant difference was observed between this treatment and all others with respect to mean root length, and in mean root number with the exception of herbaceous cuttings with leaves treated with 200 ppm IBA.

To further elucidate the response of cuttings to the effect of IBA treatments and cutting type, a factorial analysis of variance was carried out to test if an interaction effect exists between the cutting type (leafy herbaceous and leafy semi – hard wood) and IBA treatment (Table 5). Cutting type had no significant effect at $P < 0.05$ on mean shoot number, mean shoot height and root number, although herbaceous leafy cuttings gave the better response for mean root number (0.85^{a}), while cutting type had significant effect at $P < 0.05$ on root length with leafy herbaceous cuttings responding better (1.16^{a}). IBA treatment had significant effect on mean root length with cuttings treated with 400 ppm producing the best response (1.69^{a}) (Table 6). Cutting type and IBA treatment considered singly had significant effect on only mean root length, and the interaction of both (cutting type - IBA treatment) also had significant effect at $P < 0.05$ on only mean root length.

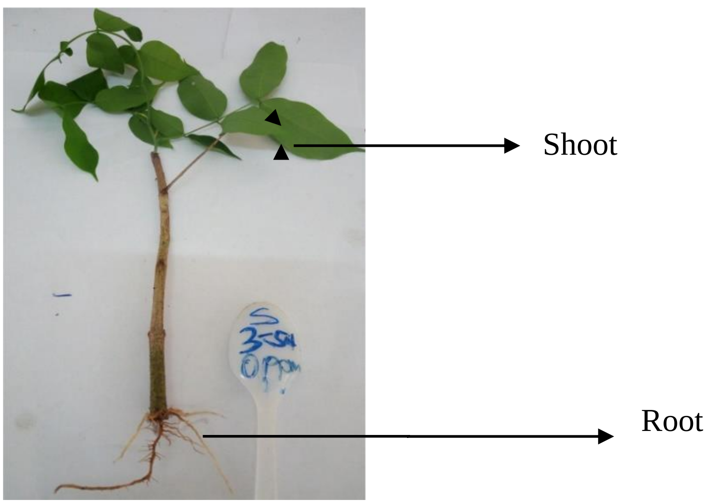

Figure 1: Sprouting of a leafy herbaceous cutting of *A. africana* Pre - treated with 400 ppm IBA after 8 weeks.

Table 1: Effect of different concentrations of IBA treatments on root and shoot induction on leafy and leafless herbaceous stem cuttings of *A. africana* saplings planted on river sand.

TREATMENT	MSN	MSH	SP (%)	MRN	MRL	RP (%)
0 ppm	0.8^{a}	3.14^{a}	80	0.80^{ab}	3.54^{b}	20
0 ppm L	0.2^{a}	0.48^{ab}	20	0.00^{b}	0.00^{c}	0
100 ppm	0.6^{a}	1.62^{ab}	60	0.40^{ab}	1.12^{c}	40
100 ppm L	0.2^{a}	0.26^{b}	20	0.00^{b}	0.00^{c}	0
200 ppm	0.2^{a}	0.56^{ab}	20	0.00^{b}	0.00^{c}	0
200 ppm L	0.4^{a}	3.16^{a}	60	0.20^{ab}	0.24^{c}	20
400 ppm	0.4^{a}	1.64^{ab}	40	0.00^{b}	0.00^{c}	0
400 ppm L	0.8^{a}	2.00^{ab}	80	1.00^{a}	5.82^{a}	80

*Values with the same superscript in the columns are not significantly different from each other at $P < 0.05$.
L - Presence of leaves, ppm – parts per million, MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number, MRL – Mean Root Length, SP – Shooting Percentage, RP – Rooting Percentage.

Table 2: Effect of presence or absence of leaves on root and shoot induction on herbaceous stem cuttings of *A. africana* saplings planted on river sand.

TREATMENT	MSN	MSH	MRN	MRL
Leafy	0.91^{a}	1.20^{a}	0.86^{a}	1.16^{a}
Non - Leafy	0.97^{a}	1.34^{a}	0.83^{a}	1.12^{a}

*Values with the same superscript in the columns are not significantly different from each other at $P < 0.05$.
MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number.

Table 3: Effect of different concentrations of IBA treatments on root and shoot induction on leafy and leafless herbaceous cuttings of *A. africana* seedlings planted on river sand.

TREATMENT	MSN	MSH	MRN	MRL
0 ppm	0.97^{a}	1.36^{a}	0.85^{a}	1.30^{ab}
100 ppm	0.91^{a}	1.09^{a}	0.81^{a}	0.93^{b}
200 ppm	0.83^{a}	1.26^{a}	0.76^{a}	0.77^{b}
400 ppm	1.02^{a}	1.38^{a}	0.95^{a}	1.56^{a}
Leafiness - IBA Pr >F	0.09	0.07	0.02	0.0001

*Values with the same superscript in the columns are not significantly different from each other at $P < 0.05$.
MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number.

Table 4: Effect of different concentrations of IBA treatments on root and shoot induction on leafy herbaceous and leafy semi - hard wood stem cuttings of *A. africana* saplings planted on river sand.

TREATMENT	MSN	SP	MSH	MRN	RP	MRL
SHW 0 ppm L	0.8^{a}	80	5.08^{a}	0.00^{b}	0	0.00^{b}
SHW 100 ppm L	0.4^{a}	40	2.70^{ab}	0.00^{b}	0	0.00^{b}
SHW 200 ppm L	0.6^{a}	60	3.26^{ab}	0.20^{b}	20	0.86^{b}
SHW 400 ppm L	0.4^{a}	60	3.12^{ab}	0.20^{b}	20	0.76^{b}
HERB 0 ppm L	0.2^{a}	20	0.48^{b}	0.00^{b}	0	0.00^{b}
HERB 100 ppm L	0.2^{a}	20	0.26^{b}	0.00^{b}	0	0.00^{b}
HERB 200 ppm L	0.4^{a}	60	3.16^{ab}	0.20^{ab}	20	0.24^{b}
HERB 400 ppm L	0.8^{a}	80	2.00^{ab}	1.00^{a}	80	5.82^{a}

*Values with the same superscript in the columns are not significantly different from each other at $P < 0.05$.
L - Presence of leaves, SHW – semi hard wood, HERB – herbaceous, PPM – Parts per million, MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number, SP – Shooting Percentage, RP – Rooting Percentage.

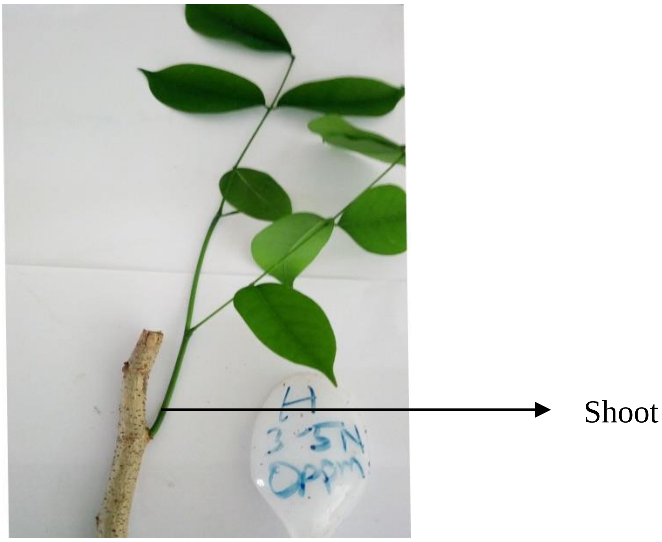

Figure 2: Sprouting of a leafy semi - hard wood cutting of *A. africana* Pre - treated with 0 ppm IBA after 8 weeks.

Table 5: Effect of cutting type on root and shoot induction on cuttings of *A. africana* saplings planted on river sand.

TREATMENT	MSN	MSH	MRN	MRL
Leafy herbaceous	0.91[a]	1.20[a]	0.85[a]	1.16[a]
Leafy semi- hard wood	0.99[a]	1.73[a]	0.76[a]	0.85[b]

*Values with the same superscript in the columns are not significantly different from each other at P < 0.05.
MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number.

Table 6: Effect of different concentrations of IBA treatments on root and shoot induction on leafy herbaceous and leafy semi – hard wood stem cuttings of *A. africana* seedlings planted on river sand.

TREATMENT	MSN	MSH	MRN	MRL
0PPM	0.97[a]	1.54[a]	0.71[b]	0.71[b]
100PPM	0.86[a]	1.16[a]	0.71[b]	0.71[b]
200PPM	0.83[a]	1.63[a]	0.81[ab]	0.92[b]
400PPM	1.02[a]	1.53[a]	1.00[a]	1.69[a]
Cutting type *IBA Pr >F	0.19	0.45	0.05	0.0007

*Values with the same Superscript in the Columns are not significantly different from each other at P < 0.05.
MSN – Mean Shoot Number, MSH – Mean Shoot Height, MRN – Mean Root Number.

DISCUSSION

The differential response observed in the rooting ability of stem cuttings collected from mature tree with respect to cuttings collected from saplings is due to the age difference in the donor plants. Sitinjak (2017) reported a similar response in *Citrus amblycarpa* where the type of cuttings affected the growth of stem cuttings. Also, the ease of adventitious root formation declines with the age of donor plants due to changes such as decreased sensitivity of aging tissues to root promoters (hormones), accumulation of inhibitory substances which inhibit rooting (Hartmann et al., 2002, Husen and Pal, 2007) and the effect of decreased content of endogenous auxins (Leakay, 2004; Husen and PAL, 2007). The effect of age of donor on rooting ability has been reported in many trees (Bhardwaj and Mishra, 2005; Husen and Pal, 2007). The condition of parent plants from which cuttings are taken is also important, since only healthy plants that are disease free will produce high quality planting stocks (Wage, 2001).

The rooting media also plays a significant role in rooting ability of stem cuttings. The better response observed with the river sand in comparison with the forest soil in this study is due to the porosity of the former. The porosity of the river sand will allow proper aeration and supply of adequate oxygen for the developing rooting system (Baiyeri, 2005). Sand was the best rooting media for stem cuttings of *Gongronema latifolia* (Agbo and Omaliko, 2006) and *Delbergia melanoxylon* (Amri and Friedricks, 2006; Amri et al., 2009).

At lower IBA concentrations, the presence of leaves had an adverse effect on rooting while at higher IBA concentration rooting was promoted. The presence of leaves on stem cuttings pre - treated with 400 ppm IBA had a significant effect on the mean root length. The leafy cuttings treated with this concentration had significantly higher mean root length than the leafless cuttings. Pellicer et al. (2000) reported the positive effect of the presence of leaves in the growth of stem cuttings since they serve as carbohydrate source

An increase in the mean root number of semi - hard wood cutting occurred as the concentration of IBA increased. No roots formed when cuttings were pre - treated with 0 ppm and 100 ppm IBA, meanwhile at higher concentrations of 200 ppm and 400 ppm, roots formed. This could be as a result of low levels of endogenous hormones (auxin) in the cutting. Endogenous hormones are usually at sub – optimal levels in plant tissues (Pessarakli, 2002). Only higher concentrations of IBA treatment were capable of producing roots from semi - hard wood cuttings. Also, the more lignified the donor material is, the higher the hormone doses that should be applied (Hartmann et al., 2002).

Considering the effect of cutting type on rooting of stem cuttings, herbaceous cuttings with leaves treated with 400 ppm IBA had the highest mean number of roots and mean length of roots. The rootability of stem cuttings is dependent on its growth stage. In this study, the less mature growth stage cuttings enhanced rooting of stem cuttings with its establishment. This report contrasts that of Khan et al. (2006), who reported that the less mature the growth phase of a donor plant, the less easily it is able to root because it will lose water readily, dry off and consequently die. This could however be species dependent because there are reports of other species that root better with less mature cutting.

Conclusion

This study has provided a means of propagation of *Afzelia africana* species. Only cuttings from saplings planted on river sand responded to hormone application. Herbaceous cuttings responded better than semi-hard wood cuttings, and herbaceous cuttings with leaves responded better than herbaceous cuttings without leaves. Leafy herbaceous cuttings from saplings of *Afzelia africana* treated with 400 ppm IBA and planted on river sand is best for rooting stem cuttings of *Afzelia africana* because this resulted into the highest number of roots with the longest roots. However, for future research, natural products such as honey, coconut water and *Moringa oleifera* leaf extract (Sakpere et al. (2018) confirmed the extract to be effective for stimulating plant growth), which are readily available and more cost effective, should be used as an alternative to Indole butyric acid.

COMPETING INTERESTS

The authors declare that there is no competing interest.

AUTHORS' CONTRIBUTIONS

The conception, design, data collection, data analysis and the writing of the paper was done by ERO and AMS participated in the design of the study and supervised it. MOA took part in data collection and analysis.

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