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Improving mass propagation of *Alstonia boonei* De Wild from stem cuttings: requirement for cutting dimension and growth promotants

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ABSTRACT

While Alstonia boonei De Wild is one of the West African tree species being extensively exploited for use in traditional medicine, a regeneration scheme to avert an imminent genetic erosion of the species is limited. Urgent efforts are therefore required to facilitate its large-scale propagation towards production in home-gardens, plantation establishment, and/ or re-introduction into the natural forests. We tested the effects of selected artificial hormones (Indole Butyric Acid, Naphtalene Acetic Acid) and natural hormone, a mix of Indole-3-acetic acid (IAA) and coconut water at different concentrations on sprouting and rooting of A. boonei stem-cuttings, collected from the Botanical garden, University of Ibadan, Nigeria. In total, 1,440 cuttings of 5, 10, 15 and 20 cm in length were treated with IBA and NAA at 0, 100, 200 and 300 mg L^{-1} concentrations and coconut water at 0, 50, 75 and 100% concentrations. We used both top soil and river sand as planting medium. Percentage sprouting, rooting and survival of cuttings were monitored every week for 60 days. Data were analyzed using both the Descriptive statistics and Analysis of Variance (ANOVA) with Duncan Multiple Range Test for separation of significant means.Hormone concentration and cutting length significantly affected leaf sprouting of A. boonei cuttings at $\alpha_{0.05}$. Highest leaf sprouting was observed in 20cm cuttings treated with 100% coconut water using topsoil as medium. No sprouting was observed in cutting lengths of 5 cm and 10 cm treated with rooting hormones, in either topsoil or river sand. Alstonia boonei sprouted using 100% coconut water at 20 cm cutting lengths. The results presented here may be complemented by further investigation, especially the survival and vigor analyses of the rooted propagules.

Keywords: Vegetative propagation, Organic hormones, Root induction, Plant multiplication

INTRODUCTION

Alstonia boonei De Wild is a tree species belonging to the family *Apocynaceae*, and one of the widely used medicinal plants of West African origin. It is popularly known as God's tree or 'Onyame dua' (Adotey *et al*. 2012). The common, English and trade names of this species are *Alstonia*, cheese wood, pattern wood and stool wood. This tree species naturally occurs in dry, peripheral semi evergreen forests and transitional rainforests of most West African countries, including Nigeria. Characteristic of the tree species is its high light demand, being a non-shade tolerant species that colonizes forest gaps. Meanwhile, there are indications that the species can tolerates shade, to some extent, at young developmental stage, and probably the reason for plenty of regeneration in young secondary forests, and medium sized to large gaps and abandoned plantations (*Orwa et al.*, 2009). Although, the conservation status/ extinction risk of *A. boonei* cannot be ascertained from the IUCN Red List (IUCN, 2015), evidence suffice that the species is especially in very high demand for use in African traditional medicine (Olajide *et al*., 2000; Betti, *et al.*, 2013; Betti and Ambara, 2014).

Many parts of *Alstonia boonei* have been traditionally used for its anti-malaria, aphrodisiac, anti-diabetic, antibiotic, anti-microbial and anti-pyretic activities, which have also been proved scientifically (Adotey *et al*. 2012; Betti and Ambara, 2014). According to Olajide *et al*. (2000) and Odeku *et al.* (2008), the bark of the stem of this species has been found to be effective in the treatment of several diseases such as fever, insomnia, chronic diarrhea and rheumatic pains. The tree is widely used in Nigeria for the treating of malaria and other ailments (Iyiola *et al*. 2011). It is therefore important to preserve the species in Nigeria and other West African countries in order to prevent genetic erosion, while ensuring availability of the species for the various cultural uses According to Orwa *et al.* (2009), there is sparse information on the flowering and fruiting of *A. boonei*, and the studies that exclusively address the natural regeneration of this species in its natural range are equally scarce.

Vegetative propagation, the reproduction from vegetative parts of the plant such as stem, leaves and roots, could provide a helpful basis for assisted regeneration of *A. boonei*. Plant organs could be excised and rooted in a suitable medium for root and shoot formation. According to Amri *et al*. (2010), this is possible because every cell of the plant contains the genetic information necessary to

regenerate the entire plant. Such vegetative propagation usually requires the presence of growth hormones which initiates root and/ or shoot induction. With the detection of available Cytokinins in some liquid plant extracts, like coconut water (Yong (2009); Wu and Hu (2009)), the curiosity to explore the potential of such naturally sourced hormones as growth promotants is clearly suggestive.

Coconut water, for instance, contains a variety of nutrients including vitamins, amino acids, enzymes, and growth hormones that include IAA and cytokinins (Yong *et al*. 2009). Coconut water has been previously used in tissue culture as an additive to growth medium, and has been shown to significantly increase shoot proliferation and growth in tissue cultured species (Wu and Hu 2009; Soares *et al*. 2011), and enhance adventitious root formation in herbaceous ornamental tropical species (Agampodi and Jayawardena, 2009). Cytokinins are a group of hormones that regulate growth, development and ageing. They regulate cell division and influence the rate at which plant age. Wu and Hu (2009) obtained as much as 150nM cytokinin in young green fruits of coconut, and up to 0.78 µg mL^{-1} in mature green fruits (Yong, 2009). These available studies have fairly demonstrated the crucial need to evaluate the performance of this naturally sourced Cytokinins in coconut water against other synthetic counterparts in stimulating growth of the stem cuttings of *A. boonei*. This study, therefore, investigated the appropriate combination of cutting length, rooting hormone and hormone concentration best suitable for root and shoot induction of *A. boonei* using stem cuttings.

MATERIALS AND METHODS

Source of stem cuttings and experimental site

Stem cuttings for the macro-propagation of this species were collected from mother trees in University of Ibadan campus, Ibadan. The experiment was conducted at the Nursery of the Department of Forest Resources Management, University of Ibadan which 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. Climate is West Africa Monsoon with dry and wet seasons. The dry season is usually from November through March and is characterized by dry cold winds of harmattan (Akinyele, 2010). The wet season usually starts from April to October with occasional strong winds and thunderstorms.

Collection of stem cuttings

Stem cuttings were collected by carefully cutting randomly selected branchlets from plus mother trees with the use of a sharp cutlass. Stem cuttings were soaked in water to prevent dehydration during transportation to the nursery. Our experiments proceeded in two major parts as described under the section tagged experimental set-up.

Experimental set-up

Effect of hormone concentration and cutting length on sprouting of A. boonei stem cuttings

Collected branchlets of *A. boonei* were divided into multinodal cutting lengths of 5, 10, 15 and 20cm, respectively. A total of 480 cuttings were replicated 5 times for a total of 2,400 cuttings used in the experiment. IBA and NAA were applied at different concentrations: 0 mg L^{-1}, 100 mg L^{-1}, 200 mg L^{-1} and 300 mg L^{-1} and coconut water (Table 1) was applied at 0%, 50%, 75% and 100% concentrations, using the "quick dip" method (Oni, 1987; Akinyele, 2010) where the base of the stem cuttings were dipped into prepared medium for 30 seconds and transferred into germination trays filled with 2kg of topsoil as a rooting medium. The study was arranged in a 3 x 4 x 4 factorial experiment in completely randomized design (CRD) with cutting length at 4 levels, type of hormones at 3 levels and hormonal concentrations at 4 level. Cuttings were misted and irrigated twice daily using a knapsack sprayer, and were monitored daily for 60days. Leaf sprouting, rooting and survival of cuttings were recorded throughout the study period. One-way Analysis of Variance (ANOVA) and descriptive statistics were used to analyze data. A follow up test for significant means was carried out using Duncan Multiple Range Test (DMRT). The factors that were considered in this study are: cutting lengths, type of hormones and hormonal concentrations.

Effect of growth media, hormone concentration and cutting length on sprouting of A. boonei stem cuttings

Based on the findings in 'A' above, *A boonei* cuttings at 15 cm and 20 cm were introduced into two rooting media namely topsoil and sterilized river sand. Two hundred and forty (240) cuttings were introduced to each rooting media and replicated 3 times making a total of 1,400 cuttings that were used for the experiment. Cuttings were monitored daily for 5 weeks. The first day of sprouting was also noted. At the end of the experiment, ANOVA and Descriptive Statistics were used to analyze the data generated. A follow up test for ANOVA was carried out using DMRT. The experimental design was 3 x 4 x 2 x 2 factorial design in Completely Randomized Design where Factor A is hormone type at 3 levels, Factor B is hormone concentration at 4 levels and Factor C is cutting length at 4 levels and Factor D is growth media at 2 levels.

Table 1: Chemical Composition of Coconut Water used for the study

Ca (g/kg)	Mg (mg /l)	K (mg/1)	Na (mg/1)	Mn (mg/1)	Fe (mg/1)	Cu (mg/1)	Pb (mg/kg)	Cd (mg/kg	P (mg/kg)
62.70	106.00	881.00	690.00	2.34	34.6	0.36	1.466	12.25	25.61

Source: Department of Agronomy, University of Ibadan.

RESULTS

Effect of hormone concentration and cutting length on leaf sprouting of A. boonei stem cuttings

Leaf sprouting of *A. boonei* cuttings was observed at 14 days after planting. Leaf sprouting was observed in stem cuttings of 15 and 20 cm lengths. Highest percentage leaf sprouting (100%) was recorded in 20 cm cutting lengths treated with 100% coconut water (Figure 1). Table 2 indicated that there was significant difference ($p<0.05$) in the effect of cutting lengths (CL), hormone types (HO), hormone concentrations (HC) and the interaction between hormone types and hormone concentrations on leaf sprouting.

Stem cuttings of 20 cm length treated with coconut water at 100% concentration had the highest mean leaf sprouts, 3.8; 75% concentration had 3.4 leaf sprouts, 50% concentration had 2.6 leaf sprouts while control had 1.4 (Table 3). Stem cuttings of 20 cm length treated with IBA at 300mg L^{-1} had 1.8 leaf sprouts, 200mg L^{-1} and100mg//L had 1.6 leaf sprouts. The 20 cm long cuttings treated with NAA at 200mg L^{-1} had 1.8 leaf sprouts while 300mg L^{-1} and 100mg L^{-1} had 1.6 leaf sprouts.

Stem cuttings of 15 cm length using 100% coconut water as a rooting hormone the highest leaf sprouts (3.20). 75% concentration had mean value of 2.6 leaf sprouts and 50% concentration had 1.8 leaf sprouts. Cuttings treated with IBA at 300mg L^{-1} had mean value of 1.4 sprouts, while 200 and 100mg L^{-1} had 1.0 and 0.8 leaf sprouts respectively. The 15cm long cuttings treated with NAA of 300mg L^{-1} and 0mg L^{-1} concentrations both had mean value of 1.2 leaf sprouts each, while 200mg L^{-1} and 100mg L^{-1} concentration had mean values of 1.00 and 0.80 leaf sprouts respectively. However, no rooting was observed in all stem cuttings (Plate 1). No leaf sprout was observed on stem cuttings of 5 and 10 cm length treated with all hormones and concentrations Furthermore, all sprouted cuttings exhibited 'die back' after 6 weeks, due to lack of root initiation.

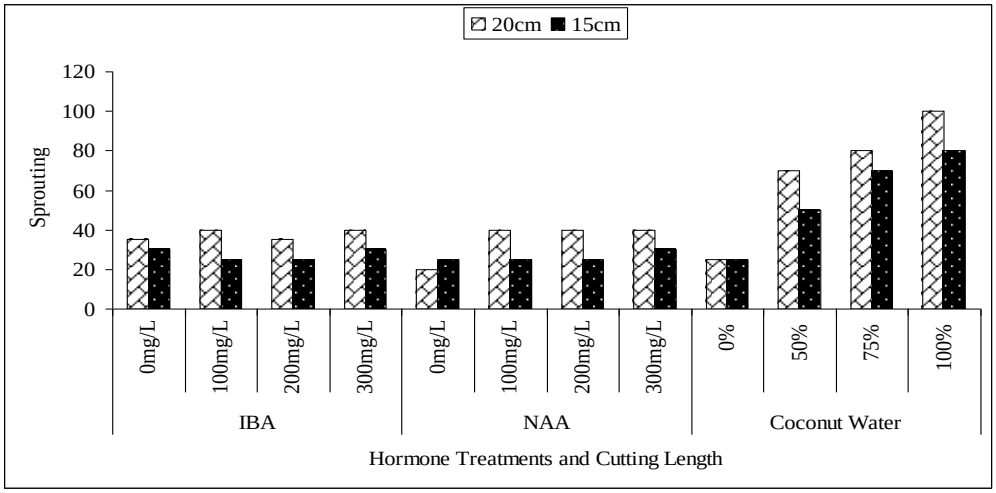

Fig. 1: Percentage Sprouting of *Alstonia boonei* of 15cm and 20cm cutting length

Table 2: ANOVA Table for the effect of cutting lengths, hormone types, and hormone concentration on the sprouting of *A. boonei*

Source of variation	df	Sum of Squares	Mean Square	F	Sig.
Cutting lengths (CL)	1	9.08	9.08	17.29	0.00*
Hormone types (HO)	2	34.52	17.26	32.87	0.00*
Hormone Conc. (HC)	3	11.03	3.68	7.00	0.00*
CL * HO	2	0.15	0.08	0.14	0.87ns
CL * HC	3	1.69	0.56	1.07	0.36ns
HO * HC	6	17.95	2.99	5.70	0.00*
CL * HO * HC	6	0.18	0.03	0.06	1.00ns
Error	96	50.40	0.53		
Total	119	124.99			

*- significant ($p<0.05$), ns- not significant ($p>0.05$)

Table 3: Mean for the sprouted *A. boonei* cuttings of lengths 20cm and 15cm

Cutting length (cm)	Levels		Hormones		
			IBA	NAA	Coconut water
20	0		1.40a	1.60a	1.40a
	100	50	1.60b	1.60a	2.60b
	200	75	1.60b	1.80b	3.40c
	300	100	1.80c	1.60a	3.80d
15	0		1.40a	1.20c	1.20e
	100	50	1.00d	0.80d	1.80f
	200	75	0.80e	1.00e	2.60b
	300	100	1.40a	1.20c	3.20g

Means with the same alphabet in the same column are not significantly different from each other. $p<0.05$

Plate 1: 20 cm stem cuttings of *Alstonia boonei* treated with 100% coconut water grown on river sand (A) and top soil (B)

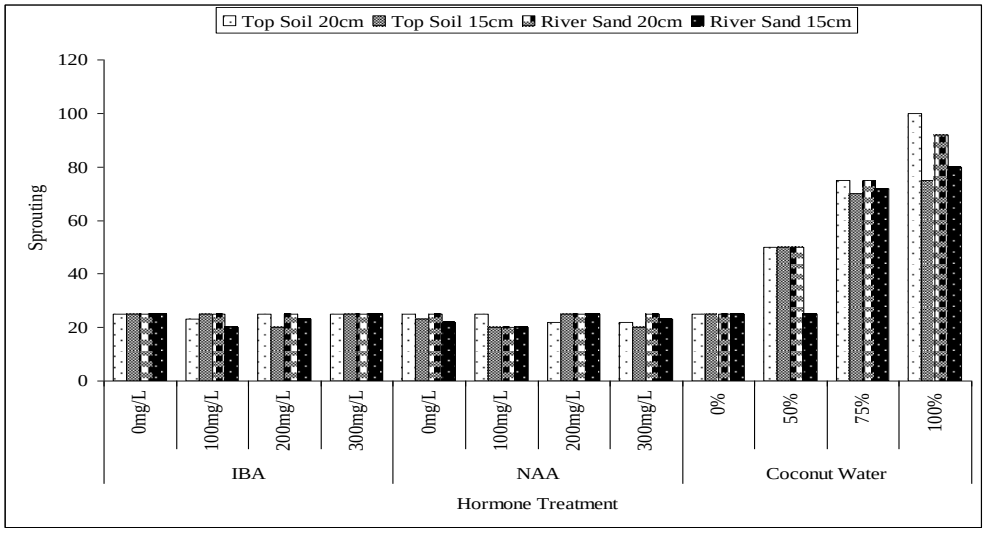

Figure 2: Percentage sprouting of 15cm and 20cm cutting length of *A. boonei* using top soil and river sand media treated with different hormones concentration

Effect of hormone type, hormone concentration, rooting media and cutting length on *Alstonia boonei* cuttings

There was significant difference in the effect of cuttings lengths, Hormone types, hormone concentrations and the interaction between Hormone types and Hormone concentration on leaf sprouts (Table 4). However, there was no significant difference ($p>0.05$) in the effect of rooting media (top soil and river sand) on leaf sprouts. Highest leaf sprouts (4.0) were recorded in

cutting length of 20cm treated with 100% coconut water (Figure 2) planted in topsoil. 75% concentration had 3.4 leaf sprouts, 50% concentration had 1.8 leaf sprouts, while 0% concentration had 1.0 (Table 5). Cuttings in topsoil treated with IBA at 200mg L^{-1} and 300mg L^{-1} concentrations had 1.0 leaf sprout each, while 100mg L^{-1} had mean value of 0.8 leaf sprout. Cuttings planted in topsoil treated with NAA at 200mg L^{-1} and 300mg L^{-1} concentrations had 1.0 leaf sprout each, while 100mg L^{-1} concentration had 0.8 leaf sprouts.

Stem cuttings of 15 cm length in topsoil and treated with 100% coconut water the highest leaf sprouts (3.0), 75% concentration had mean value of 2.4 leaf sprouts, while 50% had 1.8 leaf sprouts. Cuttings treated with IBA at 200mg L^{-1} had mean value of 1.0 leaf sprout, while 100mg L^{-1} and 300mg L^{-1} concentrations had mean values of 0.8 leaf sprout each. Those treated with NAA at 0mg L^{-1}, 200mg L^{-1} and 300mg L^{-1} concentrations had mean value of 0.8 each, while 100mg L^{-1} concentration had mean value of 0.6.

Cutting lengths 20cm placed in river sand rooting medium and treated with 100% coconut water had the highest mean value leaf sprouts (3.8). 75% concentration had 3.0 leaf sprouts and 50% concentration had 1.8 leaf sprouts. Cuttings treated with IBA at 100mg L^{-1}, 200mg L^{-1} and 300mg L^{-1} concentrations had mean values of 0.8 leaf sprout each. Cuttings treated with NAA at 100mg L^{-1} and 300mg L^{-1} concentrations had mean value of 1.0 each, while 200mg L^{-1} concentrations had 0.8 each leaf sprout. Cutting lengths 15cm placed in river sand and treated with 100% coconut water had the highest mean leaf sprouts (3.2). 75% concentration had 2.8 leaf sprouts, 50% concentration had 1.0 while 0% had 0.6 leaf sprout. Cuttings treated with IBA at 300mg L^{-1} concentrations had mean value of 0.8, while 200mg L^{-1} and 100mg L^{-1} concentrations had mean value of 0.6 each. Cutting treated with NAA at 200mg L^{-1} and 300mg L^{-1} concentrations had mean value of 0.8 leaf sprout, while 100mg L^{-1} concentrations had mean value of 0.6 leaf sprout.

DISCUSSION

This study has demonstrated the potential of organic hormones, like coconut water as well as appropriate cutting dimension in promoting root and shoot induction in *Alstonia boonei*. The results from the experiment proved that coconut water could initiate leaf sprouts in the species. The approach considered in the study was better than other known methods of using inorganic hormones like IBA and NAA in this kind of experiment. However, the limitation in this approach is the inability of both the organic and inorganic hormones for root initiation.

Table 4: ANOVA table for the effect of cutting lengths, hormone types and hormone concentration on *Alstonia boonei* cuttings planted in topsoil and river sand

Source of variation	df	Sum of Squares	Mean Square	F	Sig.
Sowing media (SM)	1	0.34	0.34	0.62	0.43^{ns}
Cutting lengths (CL)	1	5.10	5.10	9.42	0.00^{*}
Hormones types (HO)	2	99.03	49.52	91.42	0.00^{*}
Hormones conc. (HC)	3	34.58	11.53	21.28	0.00^{ns}
SM * C L	1	0.00	0.00	0.01	0.93^{ns}
SM * HO	2	0.10	0.05	0.09	0.91^{ns}
SM * HC	3	0.21	0.07	0.13	0.94^{ns}
C L * HO	2	2.43	1.22	2.25	0.11^{ns}
C L * HC	3	0.11	0.04	0.07	0.98^{ns}
HO * HC	6	58.83	9.81	18.10	0.00*
SM * C L * HO	2	0.13	0.07	0.12	0.88^{ns}
SM * C L * HC	3	1.01	0.34	0.62	0.60^{ns}
SM * HO * HC	6	0.90	0.15	0.28	0.95^{ns}
C L * HO * HC	6	0.50	0.08	0.15	0.99^{ns}
SM * C L * HO * HC	6	1.00	0.17	0.31	0.93^{ns}
Error	192	104.00	0.54		
Total	**239**	**308.30**			

*- significant ($p<0.05$), ns- not significant ($p>0.05$)

Table 5: Mean for sprouted *Alstonia boonei* cuttings sown in topsoil and river sand.

Sowing media	Cutting lengths (cm)	Levels		Hormones		
				IBA	NAA	Coconut water
Top soil	20	0	0	1.00a	0.80 a	1.00a
		100	50	0.80b	0.80a	1.80b
		200	75	1.00a	1.00b	3.40c
		300	100	1.00a	1.00b	4.00d
	15	0	0	0.80b	0.80a	0.40e
		100	50	0.80b	0.60c	1.80b
		200	75	1.00a	0.80a	2.40f
		300	100	0.80b	0.80a	3.00g
River sand	20	0	0	1.00a	0.80a	1.00a
		100	50	0.80b	1.00b	1.80b
		200	75	0.80b	0.80a	3.00g
		300	100	0.80b	1.00b	3.80h
	15	0	0	0.80b	0.60c	0.60i
		100	50	0.60c	0.60c	1.00a
		200	75	0.60c	0.80a	2.80j
		300	100	0.80b	0.80a	3.20k

Mean value with the same alphabets in the column are significantly different from each other

Many authors (Gbadamosi and Oni, 2005; Tiwari and Das, 2010; Akinyele and Maradesa, 2013; Abdullahi and Akinyele, 2013; Onefeli and Akinyele, 2014) have worked on the use of hormones in vegetative propagation of tropical tree species. According to Awosan *et al.* (2014), hormone type did not have a significant effect on adventitious root length of cuttings of *Griffonia simplicifolia* (DC.) Baill. However, Akinyele (2010) observed that cuttings of *Buchholzia coriacea* Engl. rooted without any hormone. Results presented here indicated that use of 100% coconut water resulted in optimal sprouting of 15 and 20 cm long stem cuttings of *Alstonia boonei.* Result from this study is in agreement with the report by Jimoh *et al*. (2009) stating that various attempts to raise *Alstonia boonei* through macropropagation have not yielded good results. Many tropical trees and shrubs are difficult to propagate by stem cuttings. Onefeli and Akinyele (2013) did not succeed in rooting stem cuttings of *Pycnantus angolensis* (Welw.) and *Zanthoxylum xanthoxyloides* (Lam.) Waterman, although the cuttings showed signs of callus formation. Poor rooting ability among plant species has been attributed to the presence of growth inhibitors and lack of optimal hormone concentrations in tissues or rooting cofactors (Raviv *et al*, 1986). The presence of physical barriers (Edwards and Thomas, 1980; Ahmad and Hamzah, 1993) may also be responsible for poor rooting ability in many plant species. Ahmad and Hamzah (1993) also reported that several anatomical studies have suggested a correlation between difficulty in rooting and the presence of a pericylic sclerenchyma layer. A continuous sclerenchyma layer may act as a physiological barrier to adventitious root initiation or as a mechanical barrier to root emergence.

According to Davies and Hartmann (1988), woody cuttings' failure to develop root system may be due to physiological reasons arising from the failure of parenchymatous cells to differentiate into cells capable of forming root primordial, while Amissah, *et al*. (2008) and Lovell and White (1986) believed that lack of adventitious root development may be due to the failure of some necessary anatomical events to occur despite the fact that some root initiation sites may exist. Furthermore, they said that some anatomical changes may result only in vascular development and not in the development of root primordia. However, Davies *et al.* (1982) have a contrary view to all these because they were not able to find a relationship between the presence of a sclerenchyma sheath and root formation in mature and juvenile leaf bud cuttings of *Ficus pumila* L. Their conclusion therefore was that the differences in rooting capabilities were not as a result of restrictions by the sclerenchyma tissue but more closely related to the ease of root formation.

CONCLUSIONS

The study tested the use of organic and inorganic growth hormone in the sprouting of *Alstonia boonei* and discovered that coconut water at 100% concentration is the optimum concentration at which stem cuttings of *Alstonia boonei* can be best sprouted through macropropagation. A succint explanation on the root and shoot induction of coconut water mixed with Indole -3-acetic acid (IAA) in moderate or high concentrations facilitated rapid sprouting and root extension. Optimal cutting length is 20cm as observed from the study. Further investigation, especially on the survival and vigor analyses of the rooted propagules is suggested to complement this study.

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