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Enhancing *Barringtonia racemosa* (L.) Streng. Stem Cutting Propagation for Restoration Efforts: Influence of Cutting Position and Substrate Type

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ABSTRACT

Barringtonia racemosa (L.) is a common native tropical tree species that grows in adjacent areas of tidal riverbanks, which are slightly beyond the influence of saline waters. This species is commonly associated with mangrove plants, which help prevent erosion, protect water quality, and nourish terrestrial and aquatic habitats. Nevertheless, the seed production of this species has become limited as a result of the severe degradation of riparian areas, thereby making seedling production *via* micropropagation costly. Thus, this study tested the ability of *B. racemosa* to undergo vegetative regeneration in a non-mist poly-propagator under 90% shading in the nursery for three months. The observation primarily focused on the growth performance of cuttings, specifically the shoot and root development, depending on the treatments used. Three different stem cutting positions were used: apical, median, and basal, as well as three types of substrates: sand, cocopeat, and a mixture of sand and cocopeat (1:1). All cuttings were treated with dissolved indole-3-butyric acid (IBA) hormone. The results showed cuttings at the median and basal positions with a mixture of sand and cocopeat sprouted the most with 97.22 ± 4.81% and 83.33 ± 8.36%, respectively. Cuttings at the basal position with the same mixture also rooted the most (97.22 ± 4.81%), followed by the median position with sand (89.00 ± 9.62%). Contradictorily, the apical cuttings in the mixture substrate had lower sprouting and rooting success (13.89 ± 4.82% and 13.98 ± 12.73%). This study concluded that *B. racemosa* can be efficiently propagated using basal and median stem cuttings with suitable substrate type, providing a practical and cost-effective approach for restoration efforts in degraded riparian areas.

Keywords: *Barringtonia racemosa*, non-mist poly-propagator, restoration, seedling production, stem cuttings

INTRODUCTION

Barringtonia racemosa (L.) is a mangrove-associated plant species that belongs to the Lecythideceae family and flourishes well under humid and moist conditions (Kabir *et al*., 2013). This species is distributed along tropical and sub-tropical coasts in various regions, from East and South Africa, Madagascar, the Seychelles Islands, India, Sri Lanka, Myanmar, Thailand, throughout Southeast Asia (including Malaysia), and the Pacific Islands to Northern Australia (Osman *et al*., 2015). It typically occurs near watery areas such as riverbanks and freshwater swamps, occasionally in less sane areas of mangrove swamps (Kabir *et al*., 2013). It has been revised that the *Barringtonia* genus has a total of 69 species, but only three of the species, *B. asiatica*, *B. acutangula*, and *B. racemosa*, are common and occur in lowlands near coastal or along streams and dispersed by water (Aluri *et al*., 2019; Prance, 2012). This tropical higher plant species is native to Malaysia (Hussin *et al*., 2009) and locally known as *Putat Ayer* or *Putat Sungai* in Borneo, Sabah (Soepadmo *et al*., 2002).

B. racemosa is a small to medium sized tree that can grow up to 20 meters tall. Its leaves are arranged in alternate and clustered at the ends of thick twigs. The leaves are characterized by their large size, with shape ranging from obovate-oblong to lanceolate. They typically measure approximately 8 to 35 cm in length and 4 to 13 cm in width. It has a pointed tip with slightly toothed edges and prominent veins. The flowers are attractive, have whitish-pink colours, and are attached to the staminal tube. It has four white petals surrounded by a mass of white filaments, which are arranged in long spikes emerging from the centre of leaf clusters. The fruit is approximately 9 cm long and has an egg-like shape. The bark is typically smooth and greyish (Chantaranothai, 1995; Soepadmo *et al*., 2002; Osman *et al*., 2015; Prance, 2012). In Malaysia, this species has been classified as an under-utilized crop due to a lack of effort in promoting its development and commercialization (Malaysian Agricultural Research and Development Institute (MARDI) and Ministry of Agriculture of Malaysia, 2007). *B. racemosa* is one of the species that have been extensively documented to function as food and medicine (Chan *et al*., 2017; Kong *et al*., 2020).

This species has a high tolerance for floods and vaguely saline environments, making it a viable option for mangrove wetlands restoration and an important windbreaker in the coastal zone. According to Liang *et al*. (2022), the riparian zone and the estuarine soil may contain high concentrations of cadmium and lead as a result of sediment contamination, affecting the plant community composition. Hence, it was found that *B. racemosa* has the potential for phytoremediation of cadmium and lead-contaminated soil. However, many riparian forest areas have been reported to be degraded over the last few decades, resulting in soil erosion, habitat loss, poor water quality, and disturbance of terrestrial and aquatic ecosystems (Kabir *et al*., 2013). These challenges, compounded by habitat destruction, have significantly reduced the seed production of *B. racemosa,* hindering large-scale planting stock production necessary for restoration efforts (Kulip *et al*., 2020; Valentine & Chan Kim Lian, 2021).

A study by Behbahani *et al*. (2007) successfully regenerated *B. racemosa* from leaf explants using *in vitro* propagation, with successful establishment achieved after transplantation into mixed soil under greenhouse conditions. While tissue culture has demonstrated potential as a preservation method for tropical tree species, its applications on a large-scale may constrained by high costs and technical demands. Alternatively, *B. racemosa* has been reported to propagate effectively through seeds or stem cuttings (National Parks Board (NParks), 2024). Research on a closely related species, *Barringtonia procera*, has further demonstrated that while seed propagation is common, propagation using leafy stem cuttings in a poly-propagator system is both feasible and efficient (Pauku, 2006). These findings highlight the practicality and cost-effectiveness of stem cutting propagation for *B. racemosa*, making it a compelling alternative to tissue culture for large-scale restoration efforts.

Therefore, this study aimed to establish standardized propagation techniques by examined the influence of stem cutting positions (apical, median, and basal) and substrate type (sand, cocopeat, and a mixture of sand and cocopeat). The goal was to assess the growth performance and root development of *B. racemosa* seedlings using a non-mist poly-propagator under shaded nursery area.

MATERIALS AND METHODS

Study Site

The experiment was conducted from August to November 2023 under 90% shaded nursery of the Faculty of Tropical Forestry, Universiti Malaysia Sabah (6°2'28.68" N, 116°7'46.73" E). Plant material was collected from natural vegetation near the Petagas riparian area in Putatan, about 20 km south-west of Universiti Malaysia Sabah. Both locations experience similar rainfall patterns, with mean annual rainfall ranging from 50 mm and 345 mm. Monthly temperatures typically ranged from 22.9°C to 32.2°C, while relative humidity averages between 78.5% to 83.0% (Malaysia Meteorological Department, 2023).

Plant Material

The plant material consisted of stem cuttings taken from seedlings measuring 20 to 45 cm in height, which had been acclimatized for six months in the nursery at the Faculty of Tropical Forestry, Universiti Malaysia Sabah. Prior to planting, the substrate was treated with Thiram to prevent fungal and parasitic infections. No fertilizer was applied, as the study aimed to develop cost-effective propagation method minimizes environmental disturbances to riparian and aquatic during seedling transplantation. The poly-propagator was secured to prevent pest intrusion, and pesticide was applied selectively as needed. The cuttings were monitored daily to ensure favourable conditions and promptly address any pest or disease issues.

Preparation of Cuttings

The seedlings were watered 24 hours prior to taking the cuttings. Stem were excised 2 cm above the collar region using sterilized secateurs. Each stem was divided into three parts: 1) apical,

2) median, and 3) basal (Figure 1). The average length of these cuttings ranged from 5 to 12 cm (Murugan, 2007), and the average diameter ranged from 4 to 12 mm. Each cutting had at least two axillary buds. For the apical part, two leaves were retained and trimmed to half their size, while the median and basal cuttings were left without leaves. The variation in cutting size can be attributed to the limited availability of suitable plant material due to the scarcity of wildings in its natural habitat and the challenges in sourcing seedlings from the nursery. Consequently, the available plant material was utilized to maximize the potential for successful propagation.

Figure 1. Different stem cuttings position of *B. racemosa* used for propagation; apical cuttings (a), median cuttings (b), and basal cuttings (c)

Experimental Design and Treatments

The experiment was designed as a 3x3 factorial arrangement using a randomized complete block design within a modified non-mist poly-propagator, which inspired by the designs of Leakey (2014), Kouakou *et al*. (2016), and Dao *et al*. (2020). Two factors were tested: 1) cutting position (apical, median, and basal) and 2) substrates (sand, cocopeat, and mixture of sand and cocopeat (1:1)) with nine treatments applied (3 cuttings position x 3 substrates). Each treatment included 36 cuttings arranged in three replicates, with 12 cuttings per replicate. As a treatment, the basal bottom end of each cutting was treated with indole-3-butyric acid (IBA) hormone, briefly dipped into 2.5 g of the IBA dissolved in 1 litre of distilled water for 5 seconds before being placed in the potting trays (Kouakou *et al*., 2016) measuring 54 x 28 x 4 cm (105 holes). While IBA has limited solubility in water, Kroin (1992) suggested that dissolving IBA in water is more effective for rooting compared using alcohol-based solutions, as supported by Kouakou *et al*. (2016).

Nine potting trays were placed inside the non-mist poly-propagator, each containing three types of substrates for each cutting position (Figure 3). The non-mist poly-propagator, measuring 100 x 350 x 80 cm, was positioned on a planting bed under 90% of black plastic netting in the nursery (Figure 2) to protect cuttings from excessive temperatures and direct sunlight (Aminah *et al*., 1997). The non-mist poly-propagator was built using PVC pipe and covered with a transparent polythene plastic sheet to maintain high humidity around the cuttings while allowing light to penetrate. The average temperature and relative humidity recorded inside the non-mist poly-propagator were 29 □ ± 2 □ and 93%, respectively. In order to retain moisture in the substrate, the cuttings were occasionally sprayed with a fine mist of water, 2-3 times per week.

Figure 2. Closed non-mist poly-propagator consisted of cuttings and placed under 90% shading at the nursery

The substrates−sand, cocopeat, and a mixture of sand and cocopeat−were treated with Thiram 80% w/w fungicide to prevent fungal and parasite growth in the substrates (Kodoh *et al*., 2018). Cuttings were planted vertically, with the apical end inserted approximately 3 cm into the substrates (Dao *et al*., 2020) and spaced 3 cm between cuttings. The distance between the trays was set at 9 cm (Figure 3). After 90 days of planting, the cuttings were evaluated for viability, including sprouting percentage, rooting percentage, and growth traits such as shoot length, root collar diameter (RCD), number of shoots, number of leaves, leaf area index (LAI), and root development metrics such as number of primary and secondary roots and primary root length.

Leaf area index (LAI) was measured using Easy Leaf Area software, an automated digital image analysis tool. This method was selected to minimise disturbance to actively growing leaves, ensuring accurate and consistent measurements throughout the study period. Conventional methods for LAI measurement often require destructive sampling, which was deemed unsuitable for this research as it aimed to monitor ongoing leaf development. This software has been demonstrated to provide an accurate and efficient alternative for estimating leaf area from digital images (Easlon & Bloom, 2014), making it a reliable and practical choice for this study. Cuttings were considered to have sprouted or rooted when they had developed at least one bud. To assess the rooting, the rooted cuttings were carefully removed from the substrate (Dao *et al*., 2020). The sprouting was monitored weekly, while the rooting was evaluated at monthly intervals. The final mean data and percentages were calculated at the end of the experiment.

Data Analysis

Means and standard deviations were calculated for each of the parameters based on cutting position and substrates. The effect of the different factors was assessed using one way analysis of variance and their interaction effects between factors were analysed using two way analysis of variance (ANOVA). All statistical analyses were conducted using R software version 12.1 (2023). Tukey's Honestly Significant Different (HSD) test was applied to identify differences between means. Correlation coefficients were analysed using "metan" package in R. One way analysis of variance (ANOVA) was performed to compare the different levels of coppicing nodes.

Figure 3. Sprouted cuttings of *B. racemosa* planted in non-mist poly-propagator; apical cuttings (a), median cuttings (b), basal cuttings (c) with different substrate arranged randomly in replicate block

RESULTS

Effect of Stem Position on Sprouting and Rooting Growth of *B. racemosa*

The result (Table 1) shows that the stem cutting positions significantly influenced the shoot and root development of *B. racemosa* ($p<0.05$) across all parameters. Stem cuttings taken from basal positions presented the highest percentage of sprouting (87.00 ± 14.50%) and rooting (88.90 ± 12.50%). Basal cuttings also presented the greatest number of shoots (0.89 ± 0.17), longest shoot length (4.36 ± 1.70 cm), widest collar diameter (1.59 ± 0.57 mm), largest number of leaves (1.30 ± 0.33), and highest leaf area index (1.24 ± 0.62 cm^2) compared to apical and median position. The formation of roots also presented the best growth when using basal cuttings with highest number of primary roots (3.80 ± 1.58) and secondary roots (6.61 ± 3.44) and longest primary root length (2.96 ± 0.87 cm).

Effect of Substrate on Sprouting and Rooting Growth of *B. racemosa*

Table 2 presents the influence of substrate types on sprouting and rooting growth of *B. racemosa* stem cuttings. The result shows there were no significant differences among the substrate types on the shoot and root growth parameters ($p>0.05$). Despite that, the highest mean sprouting (75.00 ± 17.20%) and rooting (79.60 ± 18.70%) percentages were observed in sand. The number of shoot (0.75 ± 0.17) and number of leaves (1.26 ± 0.43) mean were also presented the highest in sand substrate. In contrast, the shoot length (2.39 ± 2.22 cm), collar diameter (0.87 ± 0.84 mm), and leaf area index (0.87 ± 0.75 cm^2) presented the best in mixture of sand and cocopeat. As for the root formation, the primary (2.85 ± 2.02) and secondary (4.46 ± 4.10) root were observed the best in cocopeat substrate, while primary root length (2.38 ± 1.44 cm) were obtained the better result in a mixture of sand and cocopeat.

Interaction Effect of Cutting Position and Substrate on Sprouting and Rooting Percentage of *B. racemosa*

The interaction effect between cutting position and substrate factors were statistically significant on sprouting ($F = 4.555$, $p = 0.0102$) and rooting ($F = 27.308$, $p = 0.001$) percentage, indicating that the cutting position and substrate on sprouting and rooting percentage were independent. However, Figure 4 shows that there were no significant different among substrates for basal cuttings. Despite that, basal cuttings planted in mixture of sand and cocopeat had the highest sprouting and rooting percentage (97.22 ± 4.81%, 97.22 ± 4.81%, respectively). Conversely, the lowest sprouting and rooting percentage were observed at the apical cuttings with mixture of sand and cocopeat (13.89 ± 4.82 % and 13.98 ± 12.73 %).

Interaction Effect of Cutting Position and Substrate on Growth Traits of Stem Cuttings

Based on Table 4, stem position and substrate significantly affected the number of shoots and shoot length in *B. racemosa* stem cuttings ($p<0.05$). However, no significant effects were observed on collar diameter, number of leaves, and leaf area index ($p>0.05$). The result revealed that the highest mean values for shoot number (1.03 ± 0.09) and shoot length (4.97 ± 1.22 cm) occurred in basal cuttings grown in a sand and cocopeat mixture. Although other growth parameters did not exhibit significant interactions between stem position and substrate, the overall highest mean values for collar diameter (1.86 ± 0.38 mm) and leaf area index (1.55 ± 0.58 cm^2) were recorded in basal cuttings with a sand and cocopeat mixture. Additionally, the number of leaves had the highest mean (1.47 ± 0.48) in basal cuttings grown in sand substrate. Conversely, cuttings at apical part with mixture of sand and cocopeat show the lowest mean value among the parameters observed, whereas substrate with sand alone generally yielded better mean values for the measured parameters.

Table 1. Effect of stem position on the mean sprouting and rooting growth of stem cuttings from *B. racemose*

Stem position	Apical	Median	Basal	Statistical
Sprouting (%)	35.20 ± 22.40^{b}	78.70 ± 15.70^{a}	87.00 ± 14.50^{a}	p<0.05
Rooting (%)	32.40 ± 25.80^{b}	75.00 ± 21.70^{a}	88.90 ± 12.50^{a}	p<0.05
Shoot number	0.35 ± 0.22^{b}	0.80 ± 0.15^{a}	0.89 ± 0.17^{a}	p<0.05
Shoot length (cm)	0.24 ± 0.16^{c}	1.81 ± 0.62^{b}	4.36 ± 1.70^{a}	p<0.05
Collar diameter (mm)	0.00 ± 0.00^{c}	0.65 ± 0.34^{b}	1.59 ± 0.57^{a}	p<0.05
Leaves number	0.49 ± 0.40^{b}	1.14 ± 0.45^{a}	1.30 ± 0.33^{a}	p<0.05
Leaf area index (cm^2)	0.28 ± 0.19^{b}	0.80 ± 0.41^{a}	1.24 ± 0.62^{a}	p<0.05
Number primary root	1.44 ± 1.21^{b}	2.50 ± 0.58^{ab}	3.80 ± 1.58^{a}	p<0.05
Number secondary root	1.30 ± 1.47^{b}	2.54 ± 2.37^{b}	6.61 ± 3.44^{a}	p<0.05
Primary root length (cm)	1.16 ± 0.81^{b}	2.30 ± 0.70^{a}	2.96 ± 0.87^{a}	p<0.05

*Values (means ± SD) within a column followed by different superscript letters are significant different at p<0.05 (Tukey's HSD test).

Table 2. Effect of substrate on the mean sprouting and rooting growth of stem cuttings from *B. racemose*

Substrate	Sand	Cocopeat	Sand + Cocopeat (1:1)	Statistical
Sprouting (%)	75.00 ± 17.20^{ns}	61.10 ± 27.60^{ns}	64.80 ± 39.00^{ns}	p>0.05
Rooting (%)	79.60 ± 18.70^{ns}	58.30 ± 29.80^{ns}	58.30 ± 40.80^{ns}	p>0.05
Shoot number	0.75 ± 0.17^{ns}	0.62 ± 0.28^{ns}	0.67 ± 0.41^{ns}	p>0.05
Shoot length (cm)	2.09 ± 1.91^{ns}	1.92 ± 2.08^{ns}	2.39 ± 2.22^{ns}	p>0.05
Collar diameter (mm)	0.68 ± 0.77^{ns}	0.69 ± 0.75^{ns}	0.87 ± 0.84^{ns}	p>0.05
Leaves number	1.26 ± 0.43^{ns}	0.74 ± 0.42^{ns}	0.94 ± 0.61^{ns}	p>0.05
Leaf area index (cm^2)	0.78 ± 0.57^{ns}	0.64 ± 0.44^{ns}	0.87 ± 0.75^{ns}	p>0.05
Number primary root	2.65 ± 1.02^{ns}	2.85 ± 2.02^{ns}	2.24 ± 1.45^{ns}	p>0.05
Number secondary root	2.06 ± 1.86^{ns}	4.46 ± 4.10^{ns}	3.93 ± 3.62^{ns}	p>0.05
Primary root length (cm)	2.07 ± 0.66^{ns}	1.97 ± 1.08^{ns}	2.38 ± 1.44^{ns}	p>0.05

*Values (means ± SD) within a column are not significant (ns) different between substrate at p>0.05 using Tukey's HSD test.

Table 3. Interaction effect of stem position and substrate on the growth of stem cuttings from *B. racemose*

Stem position	Substrate	Mean shoot number	Mean shoot length (cm)	Mean root collar diameter (mm)	Mean leaf number	Mean leaf area index (cm^2)
Apical	Sand	0.61 ± 0.13^{bc}	0.34 ± 0.14^{ab}	0.00 ± 0.00^{ns}	0.95 ± 0.29^{ns}	0.47 ± 0.07^{ns}
	Cocopeat	0.31 ± 0.10^{cd}	0.30 ± 0.16^{bc}	0.00 ± 0.00^{ns}	0.36 ± 0.21^{ns}	0.24 ± 0.16^{ns}
	Sand + Cocopeat	0.14 ± 0.05^{d}	0.07 ± 0.04^{c}	0.00 ± 0.00^{ns}	0.17 ± 0.09^{ns}	0.09 ± 0.05^{ns}
Median	Sand	0.83 ± 0.17^{ab}	1.81 ± 0.74^{a}	0.60 ± 0.46^{ns}	1.36 ± 0.46^{ns}	0.76 ± 0.42^{ns}
	Cocopeat	0.72 ± 0.21^{ab}	1.49 ± 0.75^{ab}	0.60 ± 0.41^{ns}	0.67 ± 0.30^{ns}	0.65 ± 0.33^{ns}
	Sand + Cocopeat	0.83 ± 0.09^{ab}	2.15 ± 0.27^{a}	0.76 ± 0.26^{ns}	1.39 ± 0.05^{ns}	0.97 ± 0.55^{ns}
Basal	Sand	0.81 ± 0.17^{ab}	4.13 ± 1.75^{a}	1.45 ± 0.26^{ns}	1.47 ± 0.48^{ns}	1.11 ± 0.90^{ns}
	Cocopeat	0.83 ± 0.17^{ab}	3.98 ± 2.48^{a}	1.47 ± 0.67^{ns}	1.19 ± 0.20^{ns}	1.05 ± 0.38^{ns}
	Sand + Cocopeat	1.03 ± 0.09^{a}	4.97 ± 1.22^{a}	1.86 ± 0.38^{ns}	1.25 ± 0.33^{ns}	1.55 ± 0.58^{ns}
	F	4.867	4.555	0.230	2.468	0.704
	p	0.008	0.010	0.918	0.082	0.5997

*Values of means ± SD within a row followed by different superscript letters are significant different at p<0.05 (Tukey's HSD test) and are not significant (ns) different at p>0.005.

Table 4. Interaction effect of stem position and substrate on the root growth of stem cuttings from *B. racemosa* in a non-mist poly propagator

Stem position	Substrate	Mean primary root number	Mean secondary root number	Mean primary root length (cm)
Apical	Sand	2.50 ± 1.46^{ns}	2.28 ± 2.11^{bc}	2.02 ± 0.65^{abc}
	Cocopeat	1.11 ± 0.85^{ns}	1.28 ± 1.14^{c}	0.64 ± 0.27^{c}
	Sand + Cocopeat	0.72 ± 0.63^{ns}	0.33 ± 0.29^{c}	0.82 ± 0.71^{bc}
Median	Sand	2.50 ± 0.50^{ns}	0.95 ± 0.63^{c}	1.99 ± 0.79^{abc}
	Cocopeat	2.78 ± 0.54^{ns}	2.44 ± 1.64^{bc}	2.36 ± 0.47^{abc}
	Sand + Cocopeat	2.22 ± 0.75^{ns}	4.22 ± 3.35^{abc}	2.55 ± 0.91^{ab}
Basal	Sand	2.94 ± 1.27^{ns}	2.95 ± 2.43^{bc}	2.21 ± 0.80^{abc}
	Cocopeat	4.67 ± 2.40^{ns}	9.67 ± 1.17^{a}	2.91 ± 0.44^{a}
	Sand + Cocopeat	3.78 ± 0.63^{ns}	7.22 ± 2.33^{ab}	3.77 ± 0.59^{a}
	F	1.559	4.28	3.888
	p	0.228	0.013	0.019

*Values of means ± SD within a row followed by different superscript letters are significant different at p<0.05 (Tukey's HSD test) and not significant (ns) different at p>0.005.

Table 5. Effect of node position on the mean coppicing growth of stem cuttings from *Barringtonia racemose*

Node position	1	2	3	p
Mean shoot number	1.05 ± 0.05^{ns}	0.86 ± 0.24^{ns}	0.89 ± 0.19^{ns}	0.136
Mean shoot length (cm)	17.81 ± 3.55^{ns}	13.82 ± 7.93^{ns}	12.99 ± 6.69^{ns}	0.352
Mean root collar diameter (mm)	3.71 ± 0.66^{ns}	2.96 ± 1.47^{ns}	3.32 ± 1.33^{ns}	0.693
Mean leaf number	5.11 ± 0.90^{ns}	4.14 ± 2.49^{ns}	4.75 ± 1.95^{ns}	0.814
Mean leaf area index (cm^2)	3.94 ± 4.24^{ns}	2.45 ± 4.25^{ns}	4.74 ± 4.15^{ns}	0.814

*Values (means ± SD) within a column are not significant (ns) different at p>0.05 using Tukey's HSD test.

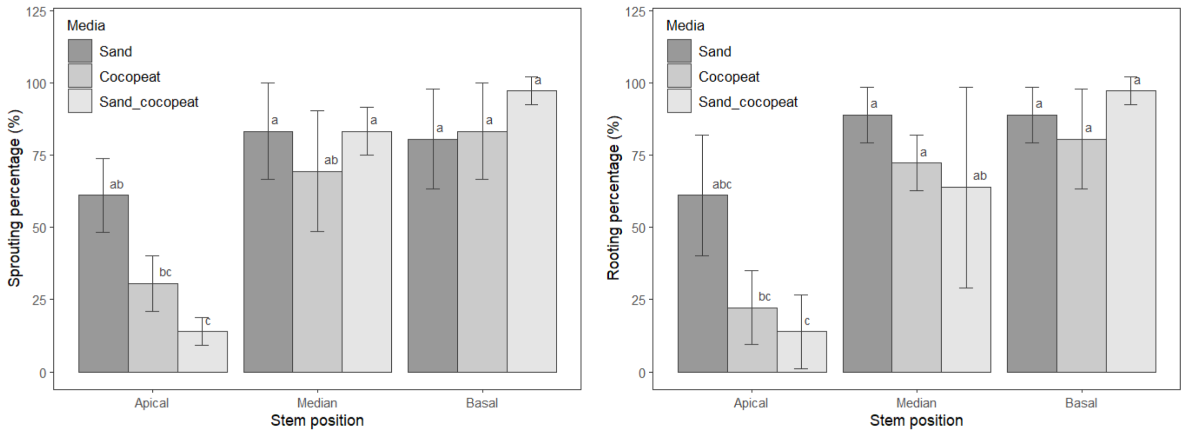

Figure 4. Effect of stem position and substrates on mean sprouting and rooting percentages of *B. racemosa* stem cuttings. The sprouting ($F = 4.555$, p = 0.0102) and rooting ($F = 27.308$, p = 0.001) percentage with different superscript letter were significant differences at p<0.005

Interaction Effect of Cutting Position and Substrate on Root Dynamics of Stem Cuttings

A significant interaction was found between the stem cutting position and substrate on the root development for *B. racemosa* (p<0.05) (Table 5), except for number of primary roots (p>0.05). Despite the lack of statistical significance, basal cuttings with a cocopeat substrate exhibited the highest mean number of primary roots (4.67 ± 2.40). Similarly, the mean number of secondary roots were highest in basal cuttings with cocopeat (9.67 ± 1.17). In contrast, apical cuttings grown in a mixture of sand and cocopeat recorded the lowest mean values for both number of primary (0.72 ± 0.63) and secondary roots (0.33 ± 0.29). Regarding root length, the longest primary roots length (3.77 ± 0.59 cm) was observed in basal cuttings with a sand and cocopeat mixture, whereas the shortest primary roots length (0.64 ± 0.27 cm) were found in apical cuttings with cocopeat.

Correlation Relationship Between Growth and Rooting Traits of *B. racemosa*

The use of different significant levels ($p<0.05$, $p<0.01$, and $p<0.001$) highlights the varying degrees of statistical confidence in the observed relationships as shown in Figure 5. Lower values, such as $p<0.001$, indicate stronger evidence of a meaningful correlation, providing greater assurance of the strength and reliability of these associations. Significant ($p<0.01$) and highly significant ($p<0.001$) positive correlations, ranging from moderately high to very strong (r^2 = 0.49-1.00), were observed among eight (8) shooting and rooting traits based on Figure 5. The strongest positive correlation was between the percentage of sprouting and the number of shoots (r^2 = 1.00; $p<0.001$), followed by shoot length and root collar diameter (r^2 = 0.98; $p<0.001$). Meanwhile, the weakest, yet still positive and significant, was between the number of leaves and number of secondary roots (r^2 = 0.49; $p<0.01$).

Effect of Different Node Position on Coppicing Growth of *B. racemosa*

The formation of coppice shoots was not significantly ($p > 0.05$) influenced by the level of node position. However, the shoot produced by seedlings cut above node 1 was the longest (17.81 ± 3.55) and had the greatest mean number of leaves (5.11 ± 0.90) (Table 6). In addition, seedlings cut above node 1 exhibited the greatest root collar diameter (3.71 ± 0.66). Contradictorily, the mean of leaf area index (4.74 ± 4.15) was recorded the greatest at seedlings cut above node 3.

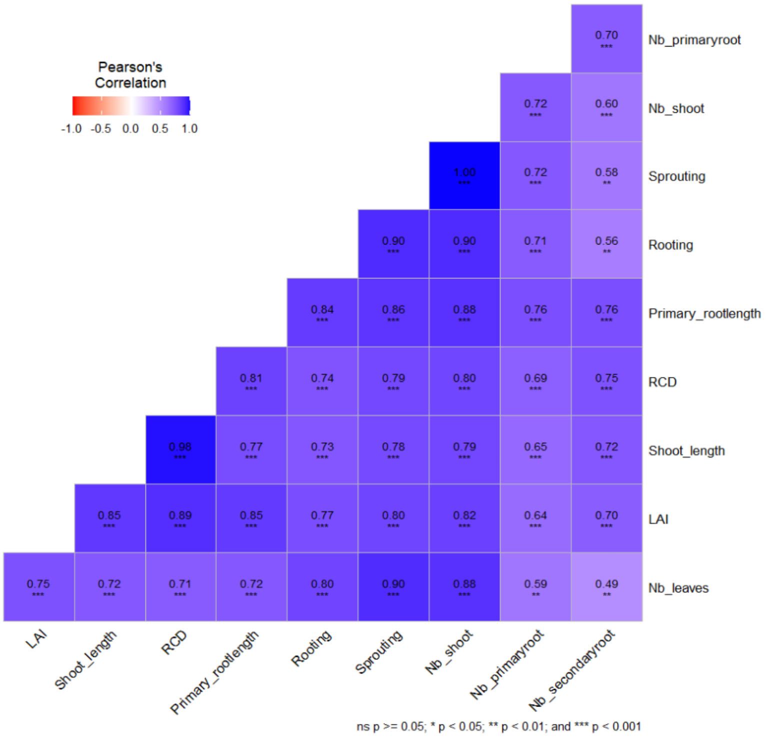

Figure 5. Correlation plot between shoot and root parameters of stem cuttings of *B. racemosa*

DISCUSSION

The different cutting positions of *B. racemosa* seedlings had a significant effect on propagation growth in terms of sprouting and rooting ability. All shoot and root formation parameters increased gradually from the apical to basal cuttings, with the basal cuttings exhibiting the best shoot and root growth. However, this result contradicts the well-recognized convention that cuttings taken from the upper parts of the stem generally have a superior ability to produce new shoots and roots compared to those taken from the lower parts (Leakey, 2014). This phenomenon is probably associated with the interaction between different species and diameter of stem, which may have different effects on shoot and root development (Leakey, 1983; Dick *et al*., 2004; Kouakou *et al*., 2016). The result showed basal cuttings had the highest shoot and leaf growth as shown in Table 1 compared to apical cuttings, which had the least. The observation aligned with other findings, indicating root-shoot growth is better in the basal cuttings than in the apical cuttings (Zalesny *et al*., 2003; Husen & Pal, 2007b, Herastuti & EK, 2020). Saifuddin *et al*. (2013) also documented that the basal cuttings of *Peltophorum pterocarpum* had the highest survival rate, shoot number, leaf area index (LAI), and root initiation compared to apical cuttings which similar to the study results.

In this study, root formation varied depending on the cutting position. Cuttings taken from the basal position had a greater number of primary (3.80 ± 1.58) and secondary roots (6.61 ± 3.44), as well as longer root length (2.96 ± 0.87 cm), than those from the apical position (Table 1). Dao *et al*. (2020) suggested that basal cuttings of *Garcinia kola* superior growth are due to their higher carbohydrate content or food reserve and increased organogenic activity at different stem positions. Ezekiel (2010) similarly demonstrated that the basal cuttings contained more sugars, which were essential for supplying nutrients needed to initiate new root-shoot tips. Other previous studies indicated that storage capacity is affected by the thickness of stem, or cutting diameter (Leakey & Mohammed, 1985; Rianawati & Siswadi, 2020). Basal cuttings typically have a larger diameter than the apical cuttings, which may lead to better growth. Similar results were found by Rana and Sood (2012), where a larger diameter of *Ficus roxburghii* wall resulted in significantly better growth in the number of primary roots, number of shoots, shoot length, and number of leaves. These authors essentially demonstrated that thicker stems produce more roots number that help boost the water and nutrient absorption, which is essential for plant development.

Piñon *et al*. (2023) found that age-related auxin sensitivity, which is influenced by maturation or ontogenectic aging, has a significant impact. As a result, young and mature tissue respond differently to auxin application, even within the same plant (Pijut *et al*., 2011). The finding showed that basal cuttings have better rooting and sprouting abilities, which is similar to Maanik and Sharma's (2022) results. These abilities may also be attributed to the highest endogenous IBA levels and carbohydrate reserve typically found in the basal region, which play a crucial role in promoting root adventitious development and enhancing starch hydrolysis (Olaniyi *et al*., 2021). It helps facilitate the transport and absorption of carbohydrates and nutrients to the base of the stem. Hardwood cuttings treated with auxin perform better in producing root and shoot due to the auxin application, which enhanced the callus, tissue formation, and cell differentiation (Wendling *et al*., 2014). This variation may be partly explained by the differences in endogenous IBA levels across cutting positions.

Furthermore, the interaction between auxin and carbohydrates has been recognized as vital for root formation (Sorin *et al*., 2005; George *et al*., 2008; Herastuti & EK, 2020). IBA promotes root formation by hydrolyzing polysaccharides, which increases the metabolic activities necessary for the formation and elongation of root meristematic tissues and development of new shoot (Husen & Pal, 2007a; George *et al*., 2008; Kouakou *et al*., 2016). Therefore, the superior growth observed in mature and basal cuttings can be attributed to their higher carbohydrate reserves and the enhanced metabolic activities stimulated by IBA, both of which promote effective root and shoot development. Despite these findings, the reasons behind the decline or loss of rooting competence in physiologically mature cuttings compared to juvenile cuttings remain unclear, necessitating further investigation, potentially through molecular analysis (Piñon *et al*., 2023).

According to the findings of this study, varying maturity levels in different parts of the stem may influence the outcomes. Specifically, the basal part of the stem matures earlier than the apical part. This observation is supported by Corpuz (2013) research on *Hevea brasiliensis* propagation, which demonstrated that brown or older stem cuttings exhibited superior shoot and root formation than green stem cuttings. Rana and Sood (2012) also found that larger diameters of stems treated with IBA showed more successful propagation than smaller diameters of stems treated with IBA hormone. Since all the cuttings in this study were treated with IBA hormone, the effect of hormone might vary depending on the stem's maturity. These differences become more pronounced with increasing age disparity, highlighting significant variations in the physiological characteristics of cuttings at different developmental stages (Owusu *et al*., 2014).

According to Leakey (2014), the environment and traits of a parent plant are vital in determining the development of roots and shoots in cuttings. The high success rate of sprouting and rooting in the non-mist propagator is due to the constant high humidity and uniform temperature, regardless of stem position or substrate type (Singh *et al*., 2015; Kouakou *et al.*, 2016). The propagator is particularly provided with a favourable environment for better root growth (Selvarajan & Rao, 1982; Singh, 2014).

The results of cuttings using three different substrates revealed that there was no significant effect on the shoot and root growth. However, the interaction of stem cutting position and substrates had a significant effect on the shoot and root parameters. All substrates produced nearly identical results for the shoot and root parameters. Nevertheless, the findings revealed that the substrate using a mixture of sand and cocopeat consistently produced the best results across all parameters (Table 2). Similar results were obtained by Netam *et al*. (2020). This mixture performed best in shoot formation, particularly at the basal stem position (Table 3), where it produced the most shoots (1.03 ± 0.09), shoot length (4.97 ± 1.22 cm), collar diameter (1.86 ± 0.38 mm), and leaf area index (1.55 ± 0.58 cm^2). These results were supported by Shrestha *et al*. (2023). Additionally, the mixture improves root development such as the number of primary and secondary roots and primary root length, particularly at the basal position (Kumar & Malik, 2019).

Overall, the combination of cocopeat and sand proved to be the most optimal substrate, offering a balanced environment that promotes robust root and shoot growth, as evidenced by the present study. This finding aligns with the requirements of an ideal rooting medium that provides sufficient porosity to ensure proper aeration, possesses high water retention capacity while maintaining adequate drainage, and remains free from any fungi and bacteria (Kontoh, 2016). Kontoh (2016) highlighted that mixing coarse river sand and composed oil palm fiber as a rooting medium in equal proportions effectively retained moisture and remained well-aerated. Conversely, a mixture of cocopeat and sand in equal proportions have proven effective for rooting, as demonstrated in the present studies. Similar results were found by Owusu *et al*. (2014) and Cahyo *et al*. (2019), who reported that a mixture of sand and cocopeat promotes the most sprouting and rooting development when compared to other substrate types. This combination may have provided favourable physical conditions and sufficient nutrients to the cuttings, which were necessary for activating enzymatic and metabolic activities (Wazir *et al*., 2003; Shah *et al*., 2021). As other studies have shown, ornamental plants or trees are likely suitable for propagation using a mixture of sand and cocopeat (Shokri *et al.*, 2014; Sedaghathoor *et al.*, 2016; Kumar & Malik, 2019; Shrestha *et al*., 2023).

Cocopeat alone demonstrated moderate to strong root development capabilities, although it was slightly less effective in promoting shoot formation than the mixture, yet root formation showed cocopeat had the highest number of primary and secondary root as shown in Table 2 and Table 4. Aparna *et al*. (2021) reported that cocopeat substrate has high water-holding capacity that helps more water and nutrient absorption from the substrate, which subsequently increases the number of primary and secondary roots. Furthermore, the substrate may protect endogenous IBA from degradation, thereby facilitating the differentiation of meristematic cells into root primordia and increases the number of roots (Rubasinghe *et al*., 2009). In contrast, sand exhibited moderate growth across all parameters, making it less effective than the other two substrates. Sand was reported to have high porosity, low absorptive capacity, and water retentive capacity (Boateng, 2014). Roots that grow in sand tend to develop as thick, brittle structures with minimal lateral branching, making them susceptible to damage during transplantation (Fatemeh & Zaynab, 2015). This could explain why sand substrate has moderate to low growth rates for both shoot and root growth, regardless of the position of the stem cuttings being used.

In terms of coppicing intensity, the results indicate that node position had no significant effect on coppice shoot formation (Table 5). However, other growth parameters varied notably depending on the node position. Seedlings cut above node 1 produced greater growth in shoot length (17.81 ± 3.55 cm) and number of leaves (5.11 ± 0.90), indicating optimal resource allocation for vigorous shoot growth and leaf development. This finding is consistent with Luostarinen and Kauppi (2005), who reported that less competition for resources leads to stronger and faster shoot development. Additionally, seedlings cut above node 1 had a larger collar diameter (3.71 ± 0.66 mm), indicating a stronger root system and increased nutrient uptake. Furthermore, seedlings cut above node 3 had the highest mean leaf area index (4.74 ± 4.15 cm^2), indicating that while these shoots may not be the longest or have the most leaves, they may have a larger overall leaf surface area, enhancing the plant's photosynthesis capacity (Tschaplinski & Blake, 1995; Kouakou *et al.*, 2016). These findings highlight the different effects of node position on various growth parameters, providing valuable insights for optimizing copping practices and seedling management.

CONCLUSION

This research indicates that *B. racemosa* is highly adaptable to vegetative propagation *via* stem cuttings, providing a viable method for large-scale seedling production aimed at reforestation. The result showed that substrate treatment alone had no significant effect on shoot and root formation. However, some of the shoot parameters and root development had significant interaction between the stem cutting positions and substrates. Basal cuttings with a sand and cocopeat mixture grew significantly faster than median and apical cuttings and adding the IBA application in an aqueous solution boosted growth even more, particularly in basal cuttings. This suggests that using basal in conjunction with IBA treatment is best for the vegetative propagation of *B. racemosa*. Seedling stock plants cut above nodes 1, 2, and 3 have no significant influence on the coppicing growth. However, seedlings cut above node 1 exhibit better shoot growth. Future research should focus on improving environmental and hormonal conditions to further enhance propagation efficiency. While tissue culture methods such as micropropagation have potential for large-scale restoration projects, they may not be feasible for small-scale applications due to resource and technical constraints. This study lays the groundwork for developing effective *B. racemosa* propagation protocols, which will help support long-term reforestation initiatives.

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