

Growth and Survival of *Avicennia marina* (Forssk.) Vierh. and *Bruguiera cylindrica* (L.) Blume in Different Substrates

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ABSTRACT

Mangrove nursery protocols are essential for sustainable reforestation. Seedling generation of various mangrove species is required to re-establish the coastal and mangrove biological systems. This research assessed the growth and survival of *Avicennia marina* and *Bruguiera cylindrica* in a nursery using different substrates: sand (sand) as Treatment I, mangrove soil (clay) as Treatment II, and their mixtures (sandy-clay-loam) as Treatment III for 18 weeks. The experiment was arranged in a randomized factorial design, with two mangrove species, three substrates, and five replicates. Each replicate has 10 mangrove seedlings or propagules. Analysis of variance showed a significant difference in survival between sand ($82 \pm 20\%$) and sand-clay-loam ($48 \pm 08\%$) in *A. marina* ($p < .05$). Sand also yielded significantly higher survival ($93 \pm 6\%$) than mangrove soil ($67 \pm 23\%$). The total height, number of axils, and number and lengths of internodes in *A. marina* were not significantly affected by substrate types ($p > .05$). However, the number of leaves ($p < .05$) and internodes ($p < .01$) produced by *B. cylindrica* were higher in mangrove soil. Mangroves *A. marina* and *B. cylindrica* have been successfully grown in all substrates tested. More importantly, the two species' seedling performances differed among substrates. Therefore, it is recommended to use sand soils for the nursery of *A. marina* and *B. cylindrica* when conducting actual plantations because the survival rate was significantly higher in the sand than in the other substrates. In contrast, results suggest that mangrove soils might positively affect the growth of *B. cylindrica* in terms of leaf and internode production; hence, they could be used alternatively.

Keywords: Axil, internode, mangrove soil, mangroves, nursery, Philippines

Introduction

Through the years, Philippine mangroves have experienced significant decreases in total cover (Long et al., 2013; Primavera & Esteban, 2008). These were attributed to many factors, such as illegal logging, human settlements, and the conversion of mangrove areas for aquaculture (Melana et al., 2005; Primavera, 1993; Primavera, 2000; White & De Leon, 2004). The lack of awareness of the importance of mangroves and other coastal ecosystems has resulted in continued detrimental practices (Albarico et al., 2021; Duarte et al., 2008). The importance of mangroves as bio-shields were further realized during Super Typhoon Haiyan (Yolanda) when the Philippines experienced massive casualties, providing strong evidence of the importance of mangroves in wind and wave dissipation (Long et al., 2016; Primavera et al., 2016). Hence, the government and various sectors take moves for mangrove restoration, rehabilitation, and conservation. However, many have depended on rooted wild mangrove seedlings or propagules (also known as wildlings) to be transplanted in rehabilitated areas. However, using wild seedlings/propagules reduces the chances of natural regeneration. Since natural mangrove vegetation was found to be more resilient during calamities than mangrove plantations, it is essential to establish mangrove nurseries rather than using wildlings for rehabilitation (Primavera et al., 2016).

To sustain reforestation projects, a mangrove nursery is necessary to reduce dependence on wildlings. Compared to direct planting commonly practiced in *Rhizophora* spp., restorations using nursery-reared mangroves increase seedling survival (Kairo, 1995; Toledo et al., 2001). Studies conducted on the growth and survival of mangrove seedlings *Avicennia marina*, *A. germinans*, *Bruguiera gymnorhiza*, *Ceriops tagal*, *Languncularia racemosa*, *R. apiculata*, *R. mangle*, and *Sonneratia alba* (Costa et al., 2016; Kirui & Huxham, 2008; Patterson et al., 1993; Terrados et al., 1997; Toledo et al., 2001) in both nursery and mangrove forests show that nursery requires an appropriate substrate type to promote optimum seedling growth. There are limited studies dealing with the effects of substrates on the development and survival of mangroves such as in *R. mangle* (Castanheira &

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Carrasco, 2004) and *R. mangle*, *A. geminans*, and *L. racemosa* (Costa et al., 2016). Mangrove nursery studies have also utilized alternative asexual techniques, but were unlike successful due to poor survival (Castillo Elías et al., 2021). Studies on *A. marina* and *B. cylindrica* in nursery conditions were relatively scarce, and there is a need to improve mangrove rehabilitation and reforestation strategies. This research assessed the growth and survival of *A. marina* and *B. cylindrica* in different substrates. It was also noted that *A. marina* is resilient against typhoons and strong waves making it a suitable species for mangrove rehabilitation along seafront areas (Primavera et al., 2016). On the other hand, studies on *B. cylindrica* in the Philippines are limited, especially regarding their growth and survival. *B. cylindrica* is considered rare (Tomlinson, 1986; cf. Vartak & Shindikar, 2008) due to its low abundance in many mangrove forests. However, it is distributed in the Old World in the eastern hemisphere.

It is known that survival rates at different substrates support the zonation of *A. marina* nearshore along sandy areas and *B. cylindrica* inward mangrove forests with muddy substrates (Primavera et al., 2004). Moreover, on-site observations of the study area provided evidence that *A. marina* assemblages were common nearshore along sandy areas while *B. cylindrica*, where propagules had been collected, were found only in the middle of the forest, along muddy substrates, without strong tidal influence. Thus, *A. marina* may grow best in the sand, while *B. cylindrica* in muddy substrates. Whether these species prefer specific substrates would help improve existing nursery protocols for better survival. Currently, mangroves in nurseries are planted in any

substrate available, regardless of the species. However, substrate types can significantly affect mangrove growth in nursery stages (Costa et al., 2016). Thus, this research used different substrates such as sand, mangrove soil (clay), and a mixture of two (sandy-clay-loam) as they are the general substrate types found in mangrove areas and used in mangrove nurseries.

Methods

Study Area

The study was undertaken at the Northern Negros State College of Science and Technology—Mangrove Civil Reservation Area (NONESCOST-MCRA) situated 10°94'80.91"N and 123°41'73.69"E, approximately 6 km away from the City of Sagay, Province of Negros Occidental. It is located within Sagay Marine Reserve, the largest reserve in the country (David-Lagutin et al., 2022; Silva et al., 2020). The nearest river system is the Himogaan River, approximately 1.5 km from the nursery (Figure 1). Tidal fluctuation is semi-diurnal. The nursery was situated in an open area beneath *Rhizophora* assemblages. The site has diverse mangrove assemblages with *A. marina*, *Rhizophora* spp., and *Sonneratia alba* as the dominant species. Other species observed were *Acanthus* spp., *Aegiceras floridum*, *A. officinalis*, *A. rumphiana*, *Brugiera cylindrica*, *Ceriops zepelliana*, *Excoecaria agallocha*, *Lumnitzera racemosa*, *Nypa fruticans*, *Pemphis acidula*, *Scyphiphora hydrophyllacea*, *Xylocarpus granatum*, and *X. moluccensis* (Albarico, 2020, 2022). It was also observed that only a few mature (less than 10) *B. cylindrica* with a height of more than two (2) meters were distributed throughout the study area.

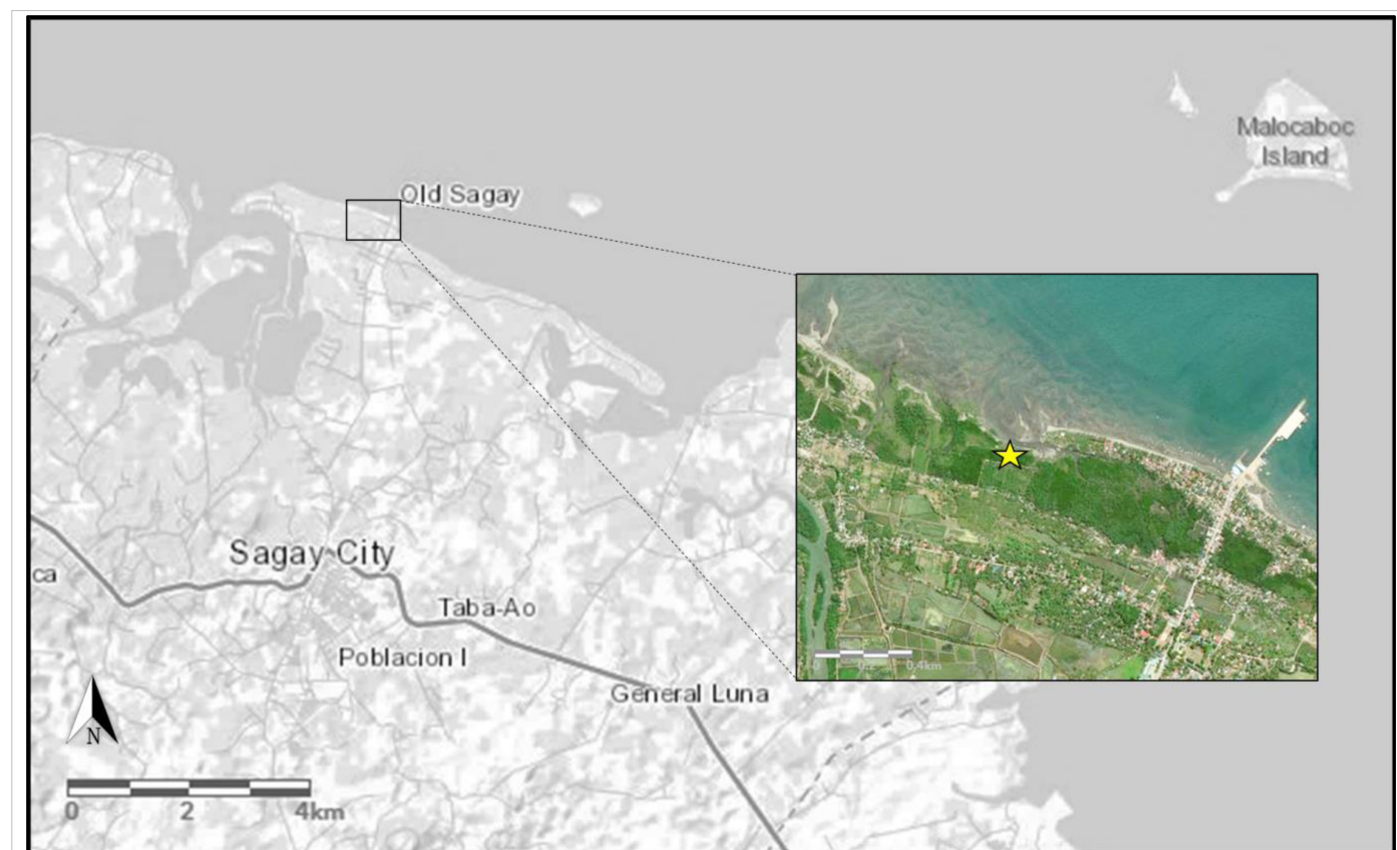


Figure 1.
Map of the Site in Old Sagay, Sagay City, Indicating the Location of the Nursery.

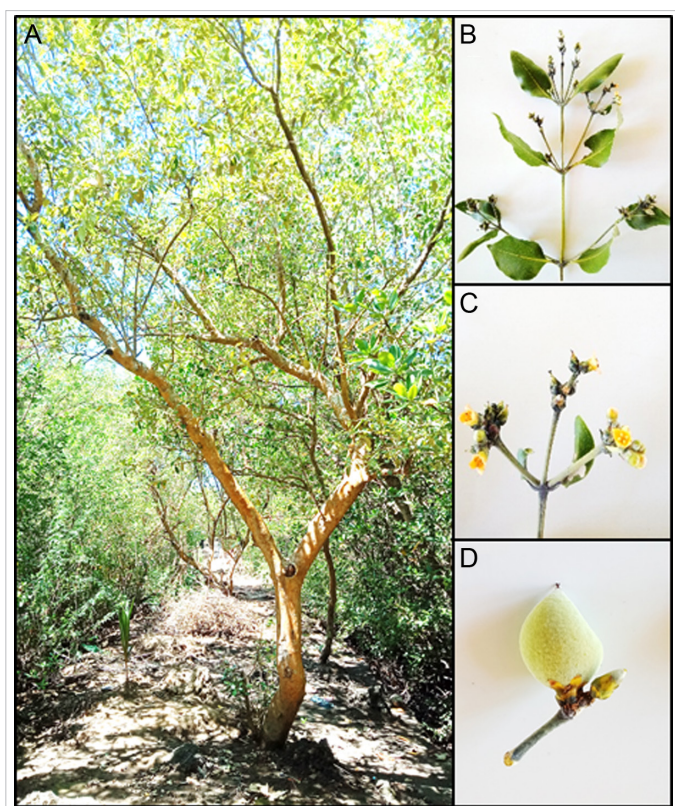


Figure 2.
 Photos of *A. marina* Tree (A), Leaves (B), Flower (C), and Maturing Seed (D).

Experimental Design

This study employed a randomized factorial design with two mangrove species, three substrates, and five replications. Seedling growth and survival of the two species were evaluated in three different substrates for 18 weeks (>3 months). These three treatments include sand as Treatment I (sand), mangrove soil (clay) as Treatment II, and a mixture of sand and mangrove soil (sandy-clay-loam) as Treatment III. Each treatment was replicated five times, with 10 plants per replicate having 50 plants per treatment.

Substrate Collection and Preparation

Collecting substrates, sand, and mangrove soil was done early in the morning. Sand came from area nearshore approximately 30 m from the nursery, while the mangrove soil was collected from the center of the mangrove area beside the nursery setup. The substrates were placed in separate containers and mixed thoroughly using a clean shovel. After 30 minutes of mixing, equal volumes of sand and mangrove soil were combined to create the third test substrate (referred to as a sandy-clay-loam substrate). Substrate composition was based on soil triangle substrate particle size comparison scales (Ditzler et al., 2017). The collected sand substrate was made up of 90% sand and 10% silt (referred to here as sand); the so-called mangrove soil substrate was made up of 60% clay, 30% silt, and 10% sand (referred to here as mangrove soil or clay); and the mixture of both substrates was made of 50% sand, 30% clay, and 20% silt (sandy-clay-loam).

A total of 300 7.5 × 7.5 × 15 cm polyethylene plastic pots, packed with different substrates, were prepared for *A. marina* and *B. cylindrica*. Each plastic pot contained approximately 675 cm³ of substrates. The substrates were compacted in each plastic pot, all about 12 cm in height.

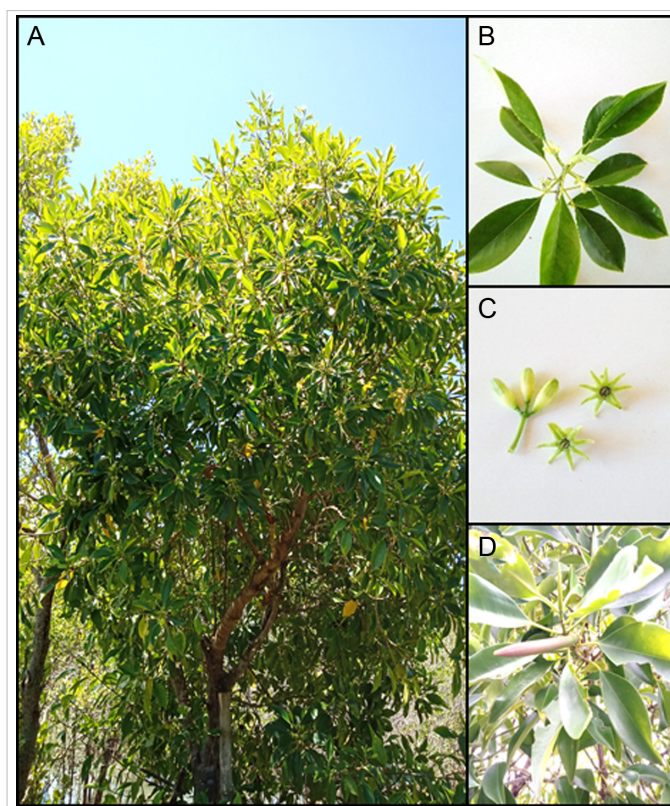


Figure 3.
 Photos of *B. cylindrica* Tree (A), Leaves (B), Flower (C), and Matured Seed (D).

The remaining 3 cm of plastic above the substrate was sliced vertically, allowing excess water to leach on the side and to avoid water logging.

Mangrove Seed Collection and Planting

To ensure that all seeds came from the same parent plant, above the dike, where only one tree grew, 1–2-month-old *A. marina* seeds were collected. Root lengths were measured, and the number of roots was counted. Seedlings having more or less six individual roots with an average length of 1.0 cm were used. The mean lengths of the hypocotyl of *A. marina* seedlings were 1.1 ± 0.15 cm. Matured propagules (dark brown) of *B. cylindrica* with an average length of 12.0 cm were collected from the same parent plant. Parts of *A. marina* and *B. cylindrica* are presented in Figures 2 and 3, respectively.

Mangroves were planted individually in plastic pots filled with different substrates. *A. marina* seeds were planted on the substrates with the roots and hypocotyl buried while cotyledons were exposed on the surface, pointing upward. *B. cylindrica*, on the other hand, was planted with one-third of its length buried on the substrates.

Mangrove Nursery

The nursery is situated at the center of the NONESCOST Mangrove Civil Reservation Area. It is inundated during high tide and is drained during low tide with minimal water current. The nursery was made of an elevated platform to standardize the elevation of plants from the ground. The platform was made of bamboo stakes with 5–8 cm spacing. Each replication was placed inside a black tray to stabilize the plants and prevent them from falling off the nursery. The tray was made-up of polyethylene plastic with equally spaced holes of 2.0 cm in diameter

Table 1.
Percent (%) Mean Survival for *A. marina* and *B. cylindrica* After 18 Weeks

Species	Treatment I (Sand)	Treatment II (Mangrove Soil)	Treatment III (Sandy-Clay-Loam)
<i>A. marina</i>	82 ± 20 ^a	66 ± 26 ^{ab}	48 ± 8 ^b
<i>B. cylindrica</i>	93 ± 6 ^c	67 ± 23 ^d	84 ± 14 ^{cd}

Note: a, b and c, d are significantly different ($p < .05$).

throughout the bottom and all sides, while the top is fully open. In this manner, all plants had no access to nutrients from the ground and only received nutrients equally during high tide. The plants were exposed to an average 8-hour photoperiod and received similar environmental parameters throughout the experiment. No watering was done throughout the study.

Measurements

Seedling survival was monitored every week except the second week for 18 weeks. The first record for *B. cylindrica* was obtained after apical bud elongation was observed (4 weeks after planting). *B. cylindrica* seedlings without growth were temporarily recorded as mortality, and when apical meristem (shoots) elongated, previous records were rectified and considered survival. Plants that did not show any shoot elongation (five in sand, one in mangrove soil, and seven in sandy-clay-loam substrate) were excluded from the survival analysis.

Growth was quantified for the surviving seedlings based on the total height, internode lengths, the number of leaves, and the number of axils at the 18th week. In addition, the growth was also calculated for each survived seedling by measuring the height from the base of the

substrate to the tip of the apical meristem for *A. marina* (Kirui & Huxham, 2008) and the base of the hypocotyl to the tip of the apical bud for *B. cylindrica*. Growth rate per week was determined using the formula: growth rate/week (in cm) = height (this week) – height (previous week). A small bamboo stake was inserted to mark the substrate and avoid overestimation of heights due to eroded soils.

Statistical Tools and Analysis

The analysis focused on intraspecific variations as both species have different growth patterns. Similarly, the seedlings and propagules used were not of the same stages. A homogeneity test was done before statistical analysis. Growth parameters and survival of each mangrove species in three different substrates were analyzed using Analysis of Variance followed by Tukey's post hoc test. Statistical analyses were computed using Microsoft Excel 2016 Analysis Toolpack and confirmed using Statistical Package for Social Sciences 2.0 (SPSS Inc.; Chicago, IL, USA).

Results, Discussion, and Conclusions

Survival Patterns in *A. marina* and *B. cylindrica* Seedlings in Three Substrates

A higher survival rate of *A. marina* was observed in the sand (Table 1; Figure 4). Analysis of variance showed a significant difference in survival between sand (82 ± 20%) and sandy-clay-loam (48 ± 08%) in *A. marina* ($p < .05$), while no significant difference was observed between clay (66 ± 26%) and other substrates. Survival was significantly higher in the sand (93 ± 06%) than in clay (67 ± 23%) for *B. cylindrica* at $p < .05$. Survival patterns in both species were found best in the sand. Survival of *A. marina* in sand slowly dropped throughout the study. However, survival in mangrove soil was slightly lower, dropping to the 10th week

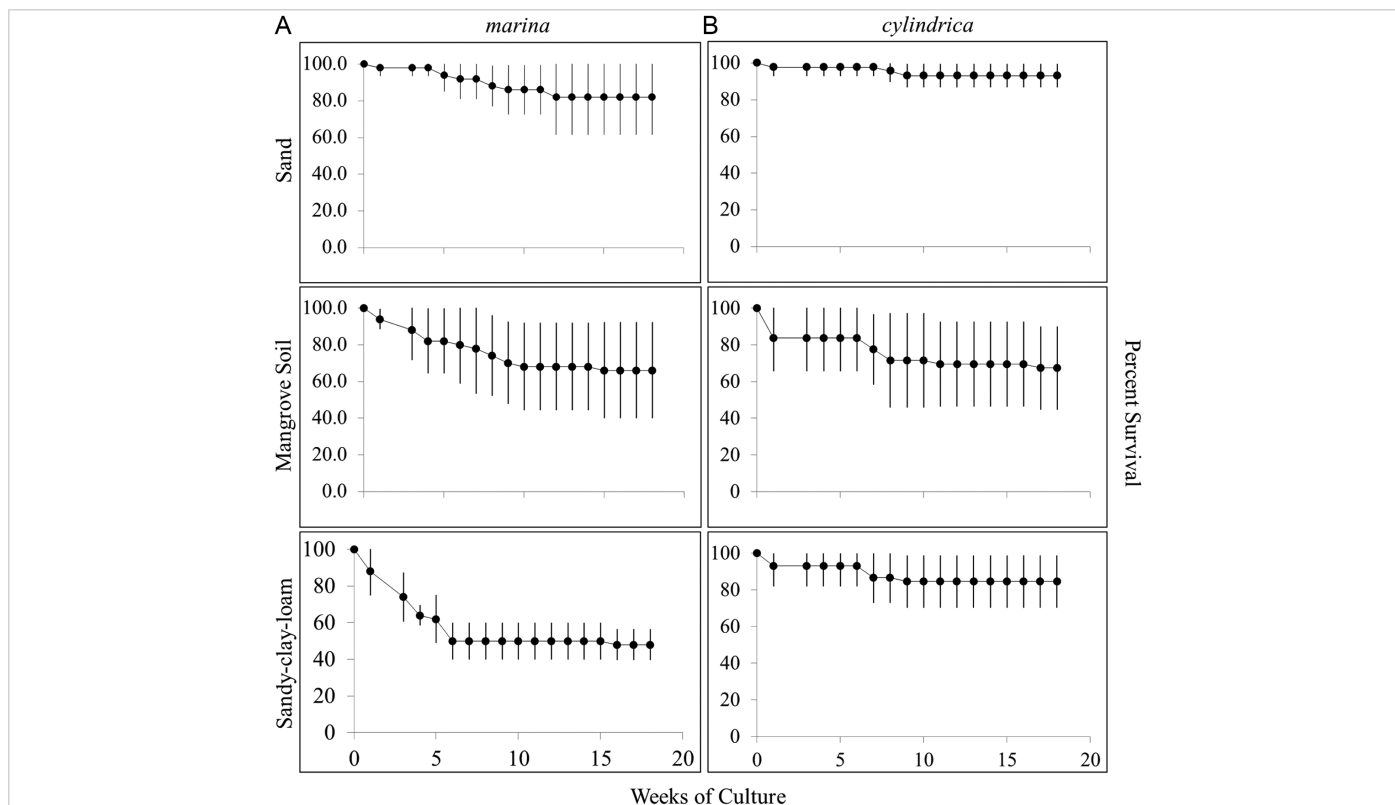


Figure 4.
Survival Patterns of *A. marina* and *B. cylindrica* in Different Substrates. Vertical Lines Show the Standard Deviation.

Table 2.
Mean Growth Parameters of *A. marina* and *B. cylindrica* After 18 Weeks

Treatment	Total Height (cm)	Growth Rate (cm/ Week)	Number of Leaves	Number of Axils	Number of Internodes	Internode Lengths (cm)			
						First	Second	Third	Fourth
<i>A. marina</i>									
I	9.6 ± 0.9 ^a	0.28 ± 0.05 ^a	4.0 ± 0.7 ^b	9.0 ± 3.0 ^a	2.4 ± 0.1 ^a	4.2 ± 0.2 ^a	2.6 ± 0.3 ^a	1.6 ± 0.7 ^a	–
II	10.1 ± 1.5 ^a	0.27 ± 0.10 ^a	5.4 ± 1.1 ^a	15.4 ± 10.0 ^a	2.6 ± 0.4 ^a	4.1 ± 0.8 ^a	2.8 ± 0.7 ^a	1.9 ± 0.7 ^a	–
III	9.8 ± 0.9 ^a	0.30 ± 0.08 ^a	5.4 ± 1.7 ^{ab}	11.0 ± 3.0 ^a	2.7 ± 0.6 ^a	4.2 ± 0.5 ^a	2.5 ± 0.3 ^a	1.4 ± 0.9 ^a	–
<i>B. cylindrica</i>									
I	7.2 ± 1.1 ^c	0.40 ± 0.07 ^c	5.8 ± 1.1 ^d	6.8 ± 1.9 ^c	2.8 ± 0.4 ^f	0.7 ± 0.3 ^c	1.5 ± 0.7 ^c	1.8 ± 0.3 ^c	0.6 ± 0.4 ^c
II	8.4 ± 0.7 ^c	0.44 ± 0.05 ^c	7.4 ± 1.1 ^c	5.6 ± 4.3 ^c	4.0 ± 0.1 ^e	0.8 ± 0.5 ^c	1.7 ± 0.5 ^c	1.9 ± 0.4 ^c	1.2 ± 0.3 ^c
III	7.5 ± 2.2 ^c	0.34 ± 0.16 ^c	5.4 ± 0.5 ^d	7.6 ± 3.5 ^c	3.2 ± 0.2 ^{ef}	0.7 ± 0.4 ^c	1.7 ± 0.7 ^c	1.9 ± 0.6 ^c	1.0 ± 0.8 ^c
Note: a, b and c, d are significantly different at $p < .05$; e, f at $p < .01$.									

Note: a, b and c, d are significantly different at $p < .05$; e, f at $p < .01$.

and becoming more stable. Higher mortality rates were observed in sandy-clay-loam, where survival rates sharply declined to up 50% in the sixth week, which became stable. Similar to *A. marina*, survival patterns of *B. cylindrica* were best in the sand, with higher survival rates throughout the study than other substrates. Survival patterns were similar for sandy-clay-loam and mangrove soils but with higher survival rates on the former. These results agree with a recent publication on the better survival of *A. marina* in sandy substrate (Budiadi et al., 2022).

Higher survival rates in sandy substrates have been previously observed in other *Avicennia* species. Costa et al. (2016) reported higher survival of *A. germinans* seedlings in sand compared to *Rhizophora mangle* and *Languncularia racemosa*. The survival rates follow the pattern of their natural distribution as *A. marina* are mainly distributed nearshore (sandy substrate) and *B. cylindrica* (sandy to muddy substrate) inward mangrove forests (Primavera et al., 2004). The establishment of mangroves in a particular substrate plays a critical role in survival. Judd et al. (2017) compared the establishment of different developmental stages and observed that head-started seedlings like what was used in this study (*A. marina*) attained the highest survival. The authors also observed that plants without well-developed root systems had low survivorship. In this study, mortality increased after a few more weeks due to insect predation, damaged stems due to debris, and drying up of the meristems. However, the exact reason for mangrove deaths in this study concerning substrates cannot be ascertained since all treatments have been exposed to the abovementioned factors; extrinsic factors being the main reason.

Growth Patterns of *A. marina* and *B. cylindrica* Seedlings in Three Substrates

***A. marina* growth.** The mean growth parameters of the two mangrove species are found in Table 2. Different substrates showed no significant effect on the growth of *A. marina* in terms of total height, the number of axils, and growth rates between treatments. However, the number of leaves of *A. marina* in mangrove soil was significantly higher ($p > .05$) than in sand, in which the survival rate was highest. Sandy substrate has a lower water-holding capacity (Basso et al., 2013), which may have reduced leaf production (Clough & Mithorpe, 1975). The experimental setup may have affected the lower number of leaves produced in this study since it was elevated from the ground to standardize water and nutrient access in all experimental plants. Water was available only during major high tides of the spring tides, thus, limiting the water supply. Also, coarse sand has lower nutrient levels than mud, composed mainly of silt and clay (Costa et al., 2016), a critical nutrient source for

mangroves (Duarte et al., 1998). Similarly, substrates also showed no significant effects on *A. marina* growth in controlled conditions (Budiadi et al., 2022). Rather, the authors noted that salinity is a limiting factor having 5–15 ppt as the optimum condition for *A. marina* seedlings.

Internode development is a conservative trait for *Avicennia*, where the production of nodes is constant regardless of substrates and nutrient supply (; Duarte et al., 1998; Duke & Pinzon, 1992; Terrados et al., 1997). Based on the analysis of variance, no significant difference was observed in the number of nodes produced after 18 weeks (Table 2). No significant difference was also observed in the internodal heights of *A. marina* concerning the first, second, and third internodes (Table 2), conforming to earlier studies of Duarte et al. (1998), Duke and Pinzon (1992), and Terrados et al. (1997). These results indicate that internode development is conservative even during the early stages of *A. marina*, regardless of the substrates they have grown on. These were further supported by the results of Duarte et al. (1998, 1999) that even in substrates with less nutrients, *Avicennia* spp. continually produce internodes as they grow older, making it a good basis for determining age and development.

***B. cylindrica* growth.** The different substrates did not affect the height and number of axils produced by *B. cylindrica* (Table 2). Analysis of variance showed no significant differences in the total height, number of axils, and growth rates in all treatments. Lengths of the first, second, third, and fourth internodes were not significantly different in all treatments at $p > .05$. However, there were significantly different results in the number of leaves and internodes produced. Both the number of leaves ($p < .05$) and the number of internodes ($p < .01$) significantly increased in clay compared to other substrates (Table 2). Mangrove soil (clay) as the substrate is theoretically nutrient-rich compared to other substrates tested, consistently observed by Costa et al. (2016), who also used mangrove soil. The nutrient present in mangrove soil may have affected the ability of *B. cylindrica* to produce more leaves and internodes.

In other mangrove species such as *R. mangle* (Duke & Pinzon, 1992), *R. apiculata* (Duarte et al., 1998), *A. Alba*, and *Sonneratia caseolaris* (Duarte et al., 1999), internodal counts are used in estimating age and growth due to its conservative traits. However, their results were non-congruent in this study concerning the early development of *B. cylindrica* in the different substrates tested. The number of internodes produced was significantly higher ($p < .01$) for the seedlings in mangrove soil (4.0 ± 0.1 cm) than those in the sand (2.8 ± 0.4 cm). These findings

suggest that *B. cylindrica* has a distinct internodal development influenced by the substrate type despite belonging to the same Rhizophora family. This implies that the internode can be used as a basis for growth but not directly as an age determinant. Thus, an in-depth developmental study on a long-term basis should be conducted to verify this unique characteristic of *B. cylindrica*, different from previous mangrove species studied as well as with *A. marina*, which showed no significant difference in the number of internodes produced.

Mangroves *A. marina* and *B. cylindrica* have been successfully grown in sand, mangrove soil (clay), and mixtures of the two substrates (sandy-clay-loam). More importantly, the two species' seedling performances differed among substrates. Therefore, it is recommended to use sand soils for the nursery of *A. marina* and *B. cylindrica* when conducting actual plantations because the survival rate was significantly higher than in the other substrates. In contrast, the results of this study suggest that mangrove soils might have positive effects on the growth of *B. cylindrica* in terms of leaf and internode production, hence, could be used for *B. cylindrica*. Meanwhile, this study demonstrated that the internode development pattern of *B. cylindrica* changed depending on the substrates, implying that age estimation by counting the number of internodes needs to be paid more attention for *B. cylindrica*. This research also validates the conservative trait of internode development in *A. marina*, even in their early life stages. Since this study has been conducted in a water-limited environment, further research in different conditions is needed.

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