***Original Research Article***

# Effect of salinity and substrate on the emergence and growth of propagules of the mangrove species *Rhizophora racemosa* in the Sassandra-Dagbego Ramsar complex (Côte d'Ivoire)

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**ABSTRACT**

This study aimed to evaluate the behaviour of seeded propagules of *Rhirophora racemosa* on different substrates and under different salinity levels. Three substrates including sand, mud and a mixture of the two were tested together with tree salinity levels (low 5%, moderate 10% and high 25%). *R. racemosa* seedlings were more likely to emerge and grow under moderate and low salinity conditions. The propagules had significant early growth in mud compared to sand and mud-sand mixture. The combined effect of salt and substrate influenced significantly propagules performance in nursery (P<0.001). High propagules emergence and growth was observed in the combination mud and salt treatments as compared to sand and san-mudmixture substrates. These results provide valuable information for the management and restoration of mangroves, highlighting the optimal environmental conditions for the successful regeneration of this species.


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*Keywords:Climate resilience,Rhizophora racemosa, Mangrove restoration, Early growth, Côte d'Ivoire*


## 1. INTRODUCTION

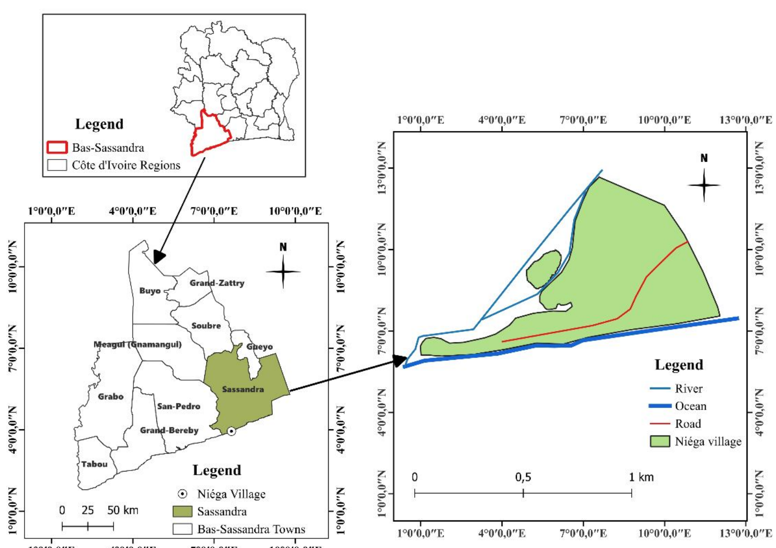
Mangroves are primarily found in tropical and subtropical coastal areas, usually in sheltered estuaries and bays where wind and wave energy are lower [1,2]. They provide various ecosystem functions and benefits, such as carbon sequestration, storm and wave buffering, and the preservation of marine and coastal biodiversity [3-5]. Despite the ecological importance of mangrove forests, their global coverage has been steadily declining. In Côte d'Ivoire, efforts have been made to restore mangroves, albeit minimally [6], by planting hundreds of hectares of degraded mangroves using direct seeding techniques and transplanting seedlings from nurseries [7]. As mangroves are easy to rehabilitate, restoration efforts often involve hasty or belated plantations of inappropriate species in unsuitable locations [8]. These activities are frequently coupled with poor post-planting monitoring [9] and lack of active involvement of local communities [7]. Consequently, these endeavors often result in failures characterized by high mortality rates and stunted plant growth [10], suggesting effective strategy be deployed. The design of effective conservation and management plans usually requires knowledge of the system's functioning [11]. Beyond the inefficacy of conservation measures, the failure of rehabilitation or restoration projects necessitates scientific investigations into the system's functioning and species' physiological responses to key stress factors, including salinity [9]. Although mangrove forests are typically located in sheltered coasts with tranquil environments, the establishment and early root development of seedlings are sensitive to the physical composition of sediments in mangroves [12]. Recent research suggests that environmental properties (soil and water) influence mangrove vegetation, specific composition, and structure [13]. According to Sinsin *et al.,* [14], variations in NaCl also affect mangrove seedling growth. Salinity is one of the most critical factors influencing mangrove health, distribution, growth, and productivity [15]. It often exhibits strong spatial and temporal fluctuations caused by freshwater and seawater inputs, flooding, groundwater infiltration, and evaporation [16-18]. Salinity was reported to determine the survival and growth of planted mangrove seedlings in restoration projects across various sites [19-22]. However, these factors are rarely considered in mangrove regeneration initiatives and their subsequent monitoring and evaluation operations. In Côte d'Ivoire, numerous studies have previously highlighted the species richness, anthropogenic pressures leading to mangrove degradation [23,7], mangrove rehabilitation, and the ecosystem services they provide to surrounding populations [7]. However, little is known about how substrate and salinity affect the emergence and growth of mangrove seedlings. Yet, this knowledge is crucial for a sound decision-making and a better monitoring of nurseries in mangrove restoration program. Therefore, the present study focusing on *R. racemosa* nursery production was initiated to assess the behavior of propagules sown on different substrates and subjected to varying salinity levels. More specifically, the study aimed to (i) identify the appropriate substrate type for the emergence and early growth of *R. racemosa* propagules and (ii) evaluate the effect of different salinity levels on the emergence and early growth of *R. racemosa* propagules and (iii) determine the combined effect of substrate type and salinity level on the emergence and early growth of *R. racemosa* propagules.

## 2. MATERIAL AND METHODS

### 2.1 Materials

#### 2.1.1 Study site

This study was carried out in Niéga, located in the town of Sassandra and part of the Sassandra-Dagbego Ramsar complex (Fig. 1). Niéga has sandy soil, although in mangrove stands it is mainly silty-clay soil. Salinity varies greatly in the river depending on the geographical location and the measurement season. The tropical climate with average monthly temperatures ranging from 23 to 28°C [24] and an average annual rainfall of about 1,600 mm [25] is characterized by four seasons: two rainy seasons and two dry seasons. The main rainy season is from April to July. The short rainy season is from October to November. The main dry season runs from December to March and the small from August to September. Two of the three mangrove species found in the country are present : *Rizophora racemosa* (red mangrove), *Avicennia germinans* (white mangrove).



## 2.2Methods

### 2.2.1 Seedling Production

The nursery was set up near the lagoon at low tide close to brackish water. The experiment was conducted in a semi-controlled environment for better management of predators and watering [26,14]. The fruiting peak of *R. racemosa* species in the region varies between November and March. Given this, propagules were collected for this study in February. All propagules were collected on the tree according to different criteria used by Sinsin *et al.,* (2021) [14]. The length ranged from (12 cm to 40 cm), circumference (3 cm), colour (brown or green) and the presence of a bud. Mature propagules that are still attached to the tree are identified because they drop off easily on contact. Three salinity levels were tested : high salinity (sea water), moderate (lagoon water) and low (mixture lagoon water - drilling water). The high level (H) corresponds to 25% salinity against 10% and 5% respectively for the moderate level (M) and low level (L). Propagules were planted in polyethylene pots filled with one of three types of substrates all taken from the vicinity of the lagoon: sand-mud mixture (Mi), sand (S), mud (Mu), according to the volumetric ratio of 1:1 (MI). One propagule was planted directly in the substrate of each pot.

### 2.2.2Experimental Design, Sampling, Measurement

The experiment was set in completely randomized and based on a 3 x 3 factors, three salinity levels and three substrate types with 3 repetitions. The nursery spanned 8 $m^2$, and we utilized 270 polyethylene pots (13 cm in diameter and 24 cm in height). We employed 90 pots for each repetition, totaling 30 pots per treatment. Spacing of 50 cm between repetitions and 43 cm between treatments were observed.

**Comment [I2]:** Please rephrase and clarify the sentence “Spacing of 50 cm between repetitions and 43 cm between treatments were observed”.

### 2.2.3Statistical Analysis

The collected data were analyzed using the RStudio software. All data were tested for normality. Descriptive statistics were performed and histograms were drawn to illustrate the evolution of emergences and growth parameters as a function of time, treatments and factors.The differences in emergence between the three salinity levels and between the three substrates were explored by a non-parametric alternative to the single-factor ANOVA test (the Kruskal-Wallis test). Dunn test was done to identify different groups. The leaf number, stem height variables were compared between the fixed salinity and substrate factors using a two-way ANOVA. The outputs are presented as means ± standard deviations, while differences between treatments were considered significant at the 5% ($p<0.05$) level.

**Comment [I3]:** Please explain the statistical test used to compare means.
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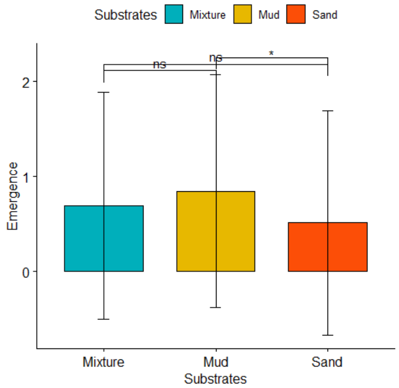
## 3. RESULTS AND DISCUSSION

## 3.1Results

### 3.1.1 Emergence of propagules following the substrate type

For the different substrate types, emergence rates were substantially equal, showing that the emergence of propagules was not significantly influenced by the substrate type. From the 30 days observation period, the rate of emergence of propagules was higher in mud, followed by mud-sand mixture and sand (Fig. 2). The emergence on sand-mud mixture and mud started simultaneously earlier (7th day) to reach respectively a higher rate of 69 and 86%, respectively at 28 and 25th days. The emergence of sand seeded propagules was low and reached its maximum (51%) just at 21th day (Fig. 3).



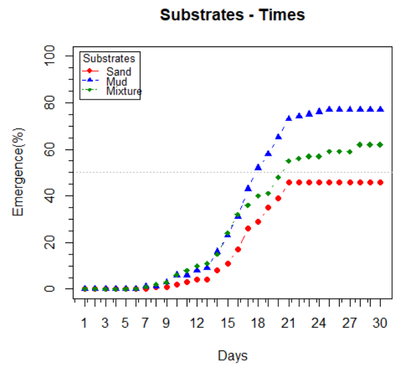
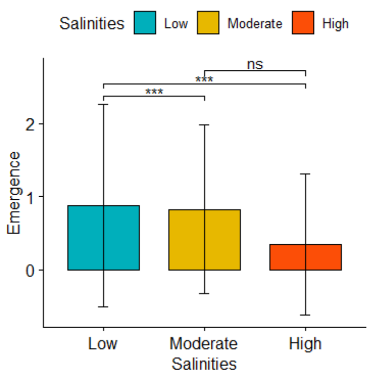




**Fig. 2. Emergence of the seedlings of R. racemosa with different substrate**

**Fig.3. Evolution of emergence rates of the seedlings of R. racemosa with different substrate**

## 3.1.2Emergence of propagules as a function of salinity

After 30 days of observation, the rate of emergence of propagules had varied with the treatments though no significant between treatments . The emergence of propagules was lower in high salinity treatment (Fig. 4). The emergence of high salinity propagules began late and a low rate (34%) was achieved. On the other hand, moderate and low salinity levels started a little earlier (6th and 9th days) to reach respectively a higher rate of 82 and 89%, respectively (Fig. 5).



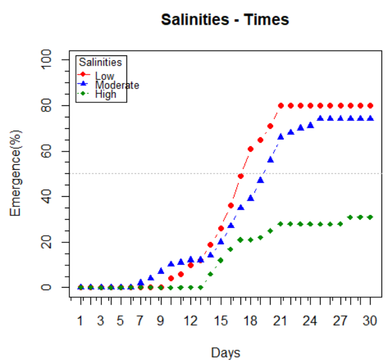




**Fig. 4. Emergence of the seedlings of R. racemosa with different salinities**

**Fig. 5. Evolution of emergence rates of the seedlings of R. racemosa with different salinities**

## 3.1.3Combined effect of salinity and substrate on propagule emergence

The emergence of propagules in the sand substrate reached its maximum, 73 and 77% for low salinity (5%) and moderate salinity (10%), respectively. High salinity gave a very low emergence rate (3%). The emergence of the propagules began on the 7th and 10th day for moderate and low salinity levels, respectively. On the other hand, it started only after 14th day for the high salinity level (Fig. 6a). Similarly, for the mud-sand mixture, the lowest emergence rate was recorded for high salinity (25%). The emergence rates varied and reached its maximum between 80-100% for the moderate and low salinity levels. The emergence was earlier and began on the 6th day for the moderate salinity and 9th day for low salinity. The emergence rate did not change from the 21st day for the low salinity level and from 24th day for the moderate salinity level (Fig. 6b). Moreover, the mud substrate presented remarkable rates of emergence irrespective of the levels of salinity. The emergence of propagules from low and moderate salinity levels began earlier (6th day). They reached 90% compared to 73% for the high salinity level that began 7 days later (Fig. 6c). Therefore, sand and mixture sand - mud, are not suitable for the emergence of propagules with high salinity. These substrates are suitable only for moderate and low salinity levels. Conversely, the mud was suitable for all three salinity levels.

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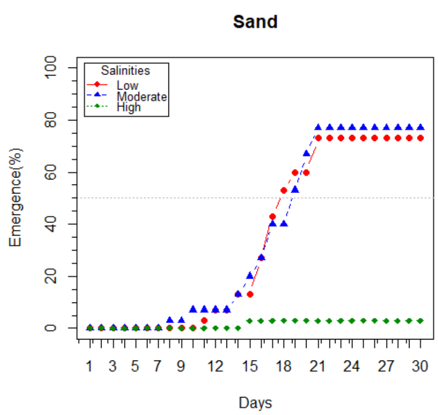


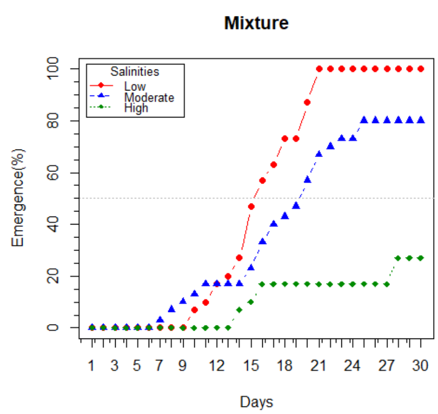


**Fig. 6a. Evolution of the emergence of R. racemosa propagules in sand substrates following different salinity levels for 30 days**

**Fig. 6b. Evolution of the emergence of R. racemosa propagules in mixture substrates following different salinity levels for 30 days**

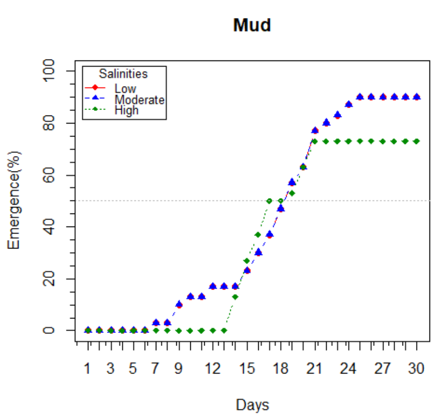

**Fig. 6c. Evolution of the emergence of R. racemosa propagules in mud substrates following different salinity levels for 30 days**

### 3.1.4Effect of treatments on propagule height growth

Significant variations were observed for *R. racemosa*seedlings height regardless of the type of treatments (Table 1). The smallest increase in height were recorded for treatments (HS, HMi, HM) between 0 and 1.80 cm at 5 weeks. The highest increase in height were observed for treatments (LMu and MS) with 9 and 8 cm achieved at 5 weeks (Fig. 7). Thus, a treatment consisting of low salinity with mud as substrate is more suitable for *R. racemosa* seedling growth at an early stage of its life cycle.

**Comment [I6]:** seedlings'

**Comment [I7]:** treatment

**Comment [I8]:** Please rephrase and clarify the sentence “The highest increase in height were observed for treatments”.

**Table 1. Results of the ANOVA for effect of duration and treatments on seedling height**

| Source of variation | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| ***Weeks*** | **1** | **103.23** | **103,23** | **982,46** | **< 2e-16 ***** |
| ***Treatments*** | **8** | **218.66** | **27.33** | **260.12** | **< 2e-16 ***** |
| ***Weeks : Treatments*** | **8** | **41.83** | **5.23** | **49.77** | **3.55e-14 ***** |
| ***Residuals*** | **27** | **2.84** | **0.11** | | |
| **---** | | | | | |
| ***Signif. codes*** | **0 ‘***’** | **0.001 ‘**’** | **0.01 ‘*’** | **0.05 ‘.’** | **0.1 ‘ ’ 1** |

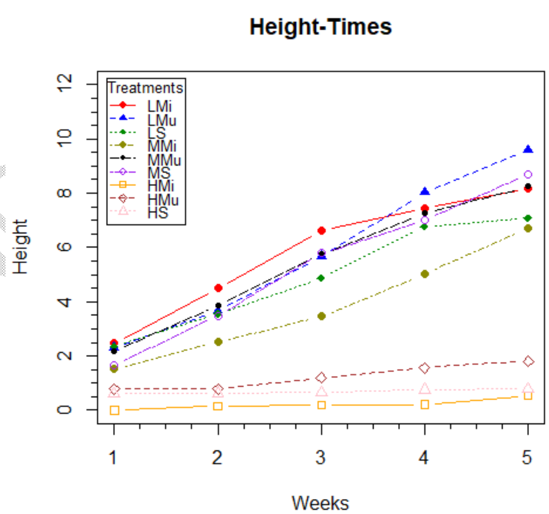


**Fig. 7. Height growth of R. racemosa for 5 weeks after propagule emergence**

### 3.1.5Effect of treatments on propagule height growth

There were significant variations for seedling height among the different treatments (Table 2) High numbers of leaves (Table 2) were observed for treatments LS, LMi, LMu, MS, MMi, MMu (up to 4 leaves). Although having given less leaves, the appearance of the leaves under the treatment HMu, HS happened at the same time as the others. Only HMi treatment showed late onset of leaves (Fig. 8)

**Table 2. Results of the ANOVA for effect of duration and treatments on seedling height**

| Source of variation | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|---|---|---|---|---|---|
| ***Weeks*** | 1 | 19.6 | 19.600 | 63.00 | 1.57e-08 *** |
| ***Treatments*** | 8 | 30.4 | 3.800 | 12.21 | 3.36e-07 *** |
| ***Weeks : Treatments*** | 8 | 5.6 | 0.700 | 2.25 | 0.0551 |
| ***Residuals*** | 27 | 8.4 | 0.311 | | |
| ***---*** | | | | | |
| ***Signif. codes*** | 0 '***' | 0.001 '**' | 0.01 '*' | 0.05 '.' | 0.1 ' ' 1 |

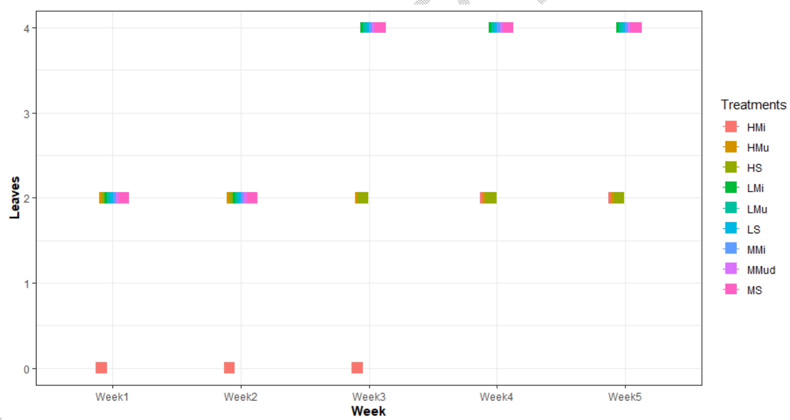


**Fig. 8. Number of leaves of R. racemosa for 5 weeks after propagule emergence**

### 3.1.5Effect of treatments on propagule height growth

Emergence of propagules of *R. racemosa* reduced at high salinities of 25%. Growth (first 30 days after emergence) is possible only for low to moderate (5–10%) salinities. Seawater salinities of 25% restrict the emergence and growth of seedlings of *R. racemosa*. However, a salinity of 5% is adequate for nursing because it enhances both emergence and growth. Mud is the best substrate whatever the salinity. He gives best emergence and growth. The best treatment for seedling is the combination of low salinity and mud. If substrate salinity is low or high where the seedlings will be transplanted later, water of low or moderate salinity would be suitable in nurseries to avoid the risk of dieback from osmotic shock after transplantation. The results may be useful to non-governmental organizations (NGOs), governments and institutions engaged in mangrove restoration through plantations.

### 3.1Discussion

Plant seedling growth can be influenced by the environment conditions. In this study, we observed that highest emergence rate was achieved on the mud and mud-sand mixture as compared to sand substrate. These results are consistent with Clough (2011) [27], who obtained higher survival rates in the mangrove soil for *Avicenia germinans* and *Rhizophora mangle* as compared to sand substrate. Similarly, Abrahão (1998) [28] found higher emergence rate (up to 82%) of seedlings of *A. schauerian* grown in nursery using a mixture of sand and compost fertilizer (N, P, K). This suggests that mixture of composite soil may be more suitable for emergence of mangrove plant species seedling in nursery than sandy soil. Furthermore, our results indicated that with the mud substrate, the emergence rate was very high even under high level of salinity. This can be explained by the ability of the mud to retain excess salt in irrigation water thus facilitate the emergence and good growth of seedlings. Similar to the Sinsin *et al.,* (2021) [14], the best emergence rates were obtained with low and moderate salinity levels, confirming that the application of salt nutrient within this range can speed up propagules emergence in *R. racemosa* seedling production. Salinity influences seedling emergence through its effect on water and osmotic potential [29-31]. Previous studies have demonstrated that high salt concentration decreased emergence and growth rates in *Aegiceras corniculatum* (L.) Blanco, *Allophylus cobbe* (L.) Reausch, *Pemphis acidula* J. R. Forst. & G.Forst, and *Sonneratia caseolaris* (L.) Engl. Rhizophora species are considered to be less tolerant to salt than Avicennia [27,30]. Previous studies on Rhizophora showed that propagule emergence is also inversely correlated with salinity [32,20,33]. Our results showed that emergence is very low and almost inhibited under high salinity level (25%). This could be explained by the very low osmotic potential of propagules compared to that of water [27,34]. The optimal salinity range observed (5-10%) is not identical to that reported for other Rhizophora species. Biber (2006) [32], found that *R. apiculata*, *R. stylosa* and *R. mangle* all had optimal growth at 15% and Khan and Aziz (2001) [18] at 17%. Osmoregulation is generally considered the most important adaptation against higher salinity of media [34]. Salt tolerance mechanisms may vary among haplotypes and provenances. This suggests further studies to establish the interaction effect of provenances and salinity levels on the emergence of *R. racemosa* propagule. Differences were also noted between the height and the number of leaves following the water salinity levels. Reduced growth occurred at higher salinities with significantly lower plant height and leaf production, which can be interpreted as the result of an investment in tolerance mechanisms [16,35]. Thus, adequate salinity for the early stages of the seedling establishment in nursery life cycle remains uncertain. For many mangrove species, regardless of salinity tolerance, low salinity is recommended for the first five months. Kodikara *et al.,* (2018) [36] suggested that moderate salinity could improve mangrove plantations after the first 4-5 months of low salinity. Lugo et *al.,* (2007) [37] reported in Puerto Rico, in hypersaline lagoons, that tree height was proportional to salinity, resulting in stunted *R. mangle* trees.

**Comment [I9]:** environmental

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## 4. CONCLUSION

As part of the restoration of mangrove ecosystems, this study investigated the effect of salinity and substrate on the emergence and growth of propagules of the mangrove species *R. racemosa*. The results showed that these environmental factors play an important role in the development of seedlings. In terms of salinity, we found that *R. racemosa* propagules are more likely to emerge under low to moderate salinity (5-10%) conditions. The propagules of *R. racemosa* had a significant early growth in mud compared to sand and mud-sand mixture. The combination of the two factors gave a complex interaction between salinity and substrate. High salinity levels, associated with different substrates, were found to result in low but remarkable emergence and growth rates for mud.

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