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GERMINATION OF PROPAGULES IN CONDITION OF PRE-DISPERSION HERBIVORY OF *Laguncularia racemosa* (L.) C. F. GAERTN. (COMBRETACEAE) UNDER DIFFERENT SALINE CONCENTRATIONS

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Abstract: *Laguncularia racemosa* (L.) C. F. Gaertn. (Combretaceae) is a halophyte arboreal mangrove species, which grows under the influence of the floding by tides, on unstable, saline and anoxic soils characteristic of this ecosystem. The phases of germination and seedling establishment are critical stages, especially in the life cycle of the mangrove species, due to the intertidal environmental conditions, herbivory and high salinities that can become stress and imbalance factors mainly in degraded environments. Propagules in a situation of pre-dispersion herbivory of L. racemosa were evaluated regarding their ability to germinate in different salinity concentrations. The concentrations used were 0 (freshwater), 25, 40 (saline control), 50 and 75 ppt (%). Samples were collected in the mangroves of the municipality of Raposa, Maranhão. The assessed variables were: germinability, germination average time, germination sync calculation, germination average speed, germination speed index and germination average speed coefficient. The study showed that the propagules germinative capabilities were similar regardless of the condition in which they were; propagules that underwent lower salinities than those of the place in which they were collected of their matrices showed a high germinability and a high germination speed index. The successful germination of herbivorous propagules of *L. racemosa* suggests that they can have efficiency in degraded mangroves recovery used together to healthy propagules.
Keywords: Mangrove germination, seedling herbivory, salinity stress.

INTRODUCTION

Despite the ecological and socioeconomic importance of mangroves (Vannucci 2001), their conservation efforts have not been able to reduce the rate of destruction of this ecosystem (Duke et al. 2007; Polidoro et al. 2010; Richards & Friess 2016). Accelerated mangrove loss has been a matter of growing concern, with several authors encouraging stronger measures to contain it (Valiela et al. 2001; Duke et al. 2007; Giri et al. 2011). Ecological recovery is one of the most urgent measures for the socio-environmental sustainability of this ecosystem (Mochel 2016). A careful analysis of site characteristics and species ecology is required before attempting to restore degraded mangrove sites (Jayatissa et al. 2008). Therefore, the germination and seedling establishment phase is a critical stage for mangrove species due to the intertidal environmental conditions, as they must overcome unstable, anaerobic substrates, and high salinities (Mckee 1995). In this sense, in addition to salinity playing important roles in regulating the growth and distribution of mangrove plants (Wang et al. 2011), it can also become a strong stress and imbalance factor, especially in degraded environments.

In addition to salinity, herbivory in propagules is also a stressor, as it can influence patterns of recruitment dynamics and plant populations (Fenner 1992; Chambers & Macmahon 1994). Herbivory can negatively affect plant aptitude, the absorption and distribution of nutrients and energy in food chains and the diversity of organisms within communities (França et al., 2020). Herbivores limit recruitment by reducing the supply of propagules, often consuming the fruits on the parent plant or in the post-dispersal environment (Silva, 2014).

Post-dispersal herbivory of propagules is a significant structuring factor in mangrove communities around the world (Smith 1988; Smith et al. 1989). Pre-dispersal herbivory has received less attention in mangrove studies (Robertson et al. 1990; Rabinowitz 1977). According to Farnsworth & Ellison (1997), pre-dispersion herbivory is a ubiquitous feature in mangroves all over the world and the estimate of available propagules capable of natural regeneration or assisted recovery must take into account the loss due to these very common agents. Mangrove recovery studies such as the one by Menezes (1999) have been using the recommendations of Goforth & Thomas (1979) for the selection of healthy propagules. Therefore, those found in herbivores are not selected, being left out of the recovery work.

In order to contribute to research on the possibility of mangrove recovery using herbivorous propagules and in different salinity regimes, this work addresses the investigation of the germination capacity of mangrove propagules. *L. racemosa* in a pre-dispersion herbivory situation in different saline concentrations. Among the typical mangrove plant species, *Laguncularia racemosa* is a halophyte arboreal species, having epigeal germination that starts with elongation of the hypocotyl-radicle axis, as soon as the propagule detaches from the tree and is very representative in this ecosystem (Tomlinson 1986).

MATERIAL AND METHODS

STUDY AREA

The area where the propagules were collected is located in the mangroves of Praia do Mangue Seco, Municipality of Raposa northwest of the municipality of São Luís, Ilha de São Luís, Maranhão. It is inserted between the coordinates: 2º27'06.86" and 2º27'21.81" S and 44º09'20.33" and 44º09'45.76" W. (Figure 1)

According to the classification by Köppen (1900), the region between the municipalities of São José de Ribamar and Raposa has a climate of the AW Tropical Rainy type, with an average rainfall of 1954 mm³. The rainy season runs from January to June and the dry season starts in July and lasts until December. The area is occupied by sandbanks, dunes and mangroves concentrated at the mouths of rivers and streams. The Mangue Seco channel has high salinities, averaging 40‰ of salinity.

PROPAGULE COLLECTION

The mature propagules of *Laguncularia racemosa* were manually collected directly

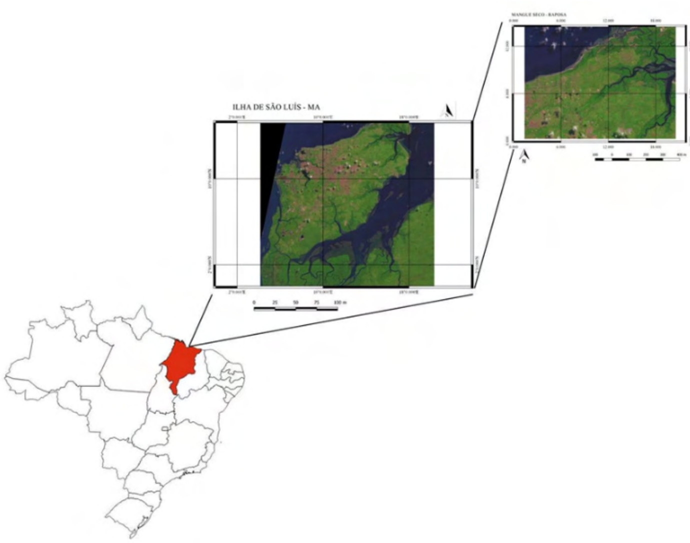

Figure 1. Detail of the area where the collection of propagules located between the municipalities of São José de Ribamar and Raposa, Ilha de São Luís, Maranhão. Landsat 8 OLI Sensor images. Source: USGS – Earth Explore.

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from the tree branch and transported to the Mangrove Recovery Center (CERMANGUE) of the Department of Oceanography of the Federal University of Maranhão. Healthy propagules from those in pre-dispersion herbivory were selected. Healthy propagules were selected as per Goforth & Thomas (1979). For herbivorous propagules, those that showed signs of grazing on their surface and/or those that contained perforations were selected (Figure 2). Those who were in an intense stage of herbivory, with more than 50% of their tissue consumed, were discarded.

SALT STRESS

To investigate the influence of salt stress, the propagules were placed to soak and germinate in sterile plastic vats, previously labeled. The seawater used in the experiment was collected in the same area as the propagules. The five salinity concentrations used were 0 (fresh water), 25, 40 (control salinity), 50 and 75 (%). Concentrations were measured with a refractometer model Q767-3 Quimis – Portable refractometer. The volume of solution used in the containers was 1,000 ml, monitored daily to correct the salinity concentration if necessary.

The experiment consisted of three replicates of 30 healthy propagules and 30 herbivorous propagules for each salinity treatment. Germination was evaluated daily at the same time, for 7 days, using the propagules that presented radicles larger than two millimeters, measured on the caliper, as a criterion for evaluation.

PHYSIOLOGICAL MEASUREMENTS OF GERMINATION

The variables evaluated were: germinability (G%), mean germination time (TMG) (t;days), germination synchrony calculation (CSG), mean germination speed (VMG) (v; days -1), index of germination speed (IVG) and mean germination speed coefficient (CVG; %).

Germination or germination percentage (G%):

$$G = (N/A).\ 100$$

where: G = germination percentage; N = number of germinated seeds; A = total number of seeds placed to germinate.

Average germination time:

$$TMG = (\Sigma niti) / \Sigma ni$$

where: TMG = average germination time; ni = number of germinated seeds per day; ti = time (days).

Germination synchrony calculation:

$$CSG = \Sigma Cn1,2/N \asymp Cn1,2 = ni(ni - 1)/2\ ;\ N = \Sigma ni(\Sigma ni - 1)/2$$

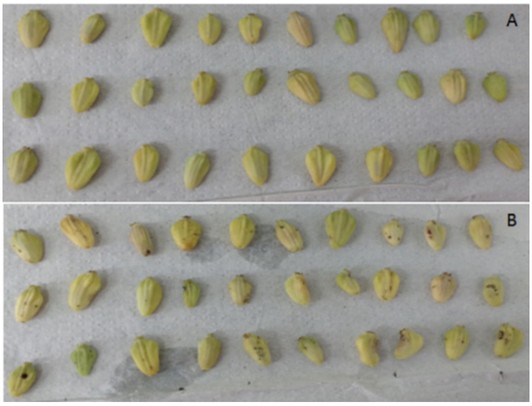

Figure 2. (A) Detail of selected healthy propagules, (B) Detail of propagules showing signs of herbivory.

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where: CSG = Germination synchrony calculation; $Cn1,2$: combination of seeds germinated in the *i*: umpteenth time; *ni*: number of seeds germinated over time *i*.

Average germination speed:

VMG = 1/tmg

where: VMG = average germination speed; t = mean germination time

Germination speed index:

$$IVG = \frac{G1}{T1} + \frac{G2}{T2} + \dots \frac{Gi}{Ti}$$

where: IVG = germination speed index; G1 to Gi = number of germinated seed per day; T1 to Ti = Time (days).

Germination Speed Coefficient:

$$CVG = \frac{G1 + G2 + \dots Gi}{G1T1 + G2T2 + \cdots GiTi} x\ 100$$

where: G1 a Gi = number of germinated seeds per day; T1 to Ti = time (days).

The variables germination, mean germination time, mean germination speed and germination synchrony calculation were evaluated by the GerminaQuant software - Version: 1.0. (Marques et al. 2015).

STATISTICAL ANALYSIS

To analyze the comparison of germination means between healthy and herbivorous propagules, the Kolmogorov-Smirnov statistical assumption of normality was used, followed by the Student's T test. In the analysis between salinity concentrations for healthy propagules and between salinity concentrations for herbivorous propagules, Levene's homogeneity assumption was used and they were evaluated by analysis of variance ANOVA, followed by Tukey's test to find where the difference was significant. To analyze the similarity between the physiological measures of germination evaluated and the similarity between the salinity concentrations, a multivariate analysis generating a cluster and a multidimensional scaling model (MDS) analysis using Student's t test were performed, respectively. To verify whether physiological measures of germination are associated as a function of salinity, principal component analysis (PCA) was applied. Statistically significant values were those with $p<0.05$. All analyzes were evaluated using the Past 3.0 program (Hammer et al. 2001).

RESULTS

GERMINATION CAPACITY BETWEEN HEALTHY AND HERBIVOROUS PROPAGULES

The statistical analysis showed that there were no significant differences in germination capacity regarding the condition of the propagules, that is, the propagules showed similar germinations regardless of the condition – healthy or herbivorous – in which they were found.

SALT STRESS

The germination rates were significantly different in relation to the salinity level to which the propagules were exposed. All the propagules, both healthy and herbivorous, which were submitted to the treatment with a salinity of 75% (ppt) did not germinate. This was an important behavior observed in the three repetitions of the experiment, which may suggest that this salinity is lethal for the germination of this species' propagules.

For healthy propagules, the analysis of variance showed that there was a significant difference (ANOVA: $P<0.000$) between the lowest and highest salinities, with the lowest concentrations where the most germinated propagules. The Tukey's test showed that the difference in germination was between the freshwater salinities (0) and 25 % in relation to the concentration of 50 %. For herbivorous propagules, the analysis of variance also showed that there was a significant difference

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(ANOVA: P<0.02) between the lowest and highest salinities. In this case, Tukey's test identified that the existing difference involved only the freshwater treatment (0) in relation to the 50 % treatment.

According to these results, we can observe that germination in lower salinities had a greater success in relation to high salinities. Due to these results, we can also suggest that herbivorous propagules were more resistant to salinity than healthy ones.

PHYSIOLOGICAL MEASUREMENTS OF GERMINATION

The germination tests showed high germinability (G) at the lowest salinity concentrations. There was a significant reduction in germinability from 50 % salinity and lethality at 75 % salinity. Regarding the average germination speed, the results behaved homogeneously. (Table 1).

The mean germination time (TMG) did not show relevant differences between healthy propagules in different types of salinity. The same happened to the herbivorous propagules, however they germinated in a shorter time. The germination velocity coefficient (CVG) values did not show substantial differences for both healthy and herbivorous propagules, however, the latter showed higher values. (Table 1)

Healthy propagules at salinities 0 and 25 % presented a germination velocity index (IVG) with significant difference when compared to higher salinities. For herbivorous propagules, there was a significant difference only when compared to the concentrations of 0 and 50 %.

NON-PARAMETRIC ANALYSIS

The analysis of the multidimensional scaling model (MDS) grouped the salinities that showed the greatest similarity, taking into account the values of the physiological germination measures analyzed. Thus, it was noted that the values of the variables showed a tendency to differ depending on the concentrations. The lowest salinities – freshwater and 25 % – formed a distinct group from the higher salinities 40 % and 50 %. (Figure 3)

Through *cluster* analysis it was possible to obtain two large different groups based on the values of physiological measures of germination. The first group formed by germinability and the second by the other physiological measures of germination. It is important to note that there was no separation of groups between the variables in terms of the healthy condition – herbivore. (Figure 4)

Principal component analysis (PCA) was used to obtain the correlation between the germination variables and salinity. The first and second axis of the PCA accounted for 85.5% of the data variance. The contributions of the variables to the axes of this analysis show that most of the analyzed variables such as germinability, germination speed index, average germination speed and germination synchrony calculation, both for healthy and herbivorous propagules, are more associated with fresh water (0) and salinity 25 %. (Figure 5).

DISCUSSION

SALINITY

Salinity has been recognized as an important factor limiting mangrove growth and productivity (Clough & Sim 1989; Lin & Sternberg 1992; Ball 2002) yet this ecosystem is still highly adapted to soil salt concentrations exceeding those tolerated by most other plant species (Ball 1988).

Experiments show that the optimal salinity level for mangrove species is much lower than that of seawater, which is generally 35ppt (Clough 1993). In addition to this optimum, increasing levels of salinity can reach a tolerance limit, in which the mechanisms

	Salinity concentration %	Variable elements				
		G(%)[1]	VMG (days-^{1})	TMG (days)	CVG (%)	IVG[1]
Healthy	0	87,3 ^{a}	0,211	4,78	21,16	6,2456 ^{ab}
	25	74 ^{a}	0,195	5,15	19,5	4,844 ^{a}
	40	42 ^{b}	0,205	4,9	20,43	2,7193 ^{bc}
	50	16,53 ^{b}	0,219	4,98	21,96	1,2113 ^{c}
	75	-	-	-	-	-
Herbivores	0	86,33 ^{a}	0,2666	3,81	26,46	8,448 ^{a}
	25	69,66 ^{ab}	0,2233	4,55	22,33	5,4736 ^{ab}
	40	55,33 ^{ab}	0,23633	4,25	23,56	4,3486 ^{ab}
	50	31 ^{b}	0,21	4,84	21,03	2,1736 ^{b}
	75	-	-	-	-	-

1- Mean values followed by equal letters in the column do not differ by Tukey's test at 5%

Table 1. Results of the influence of salinity on the germination of healthy and herbivorous propagules of Laguncularia racemosa through the variables used.

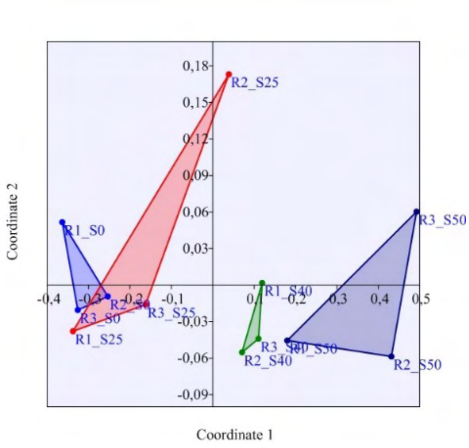

Figure 3. MSD analysis grouping more similar salinities according to physiological measures of germination.

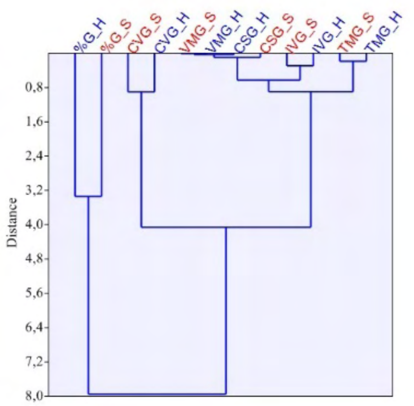

Figure 4. *Cluster* analysis showing the formation of two distinct groups based on the values of physiological measures of germination.

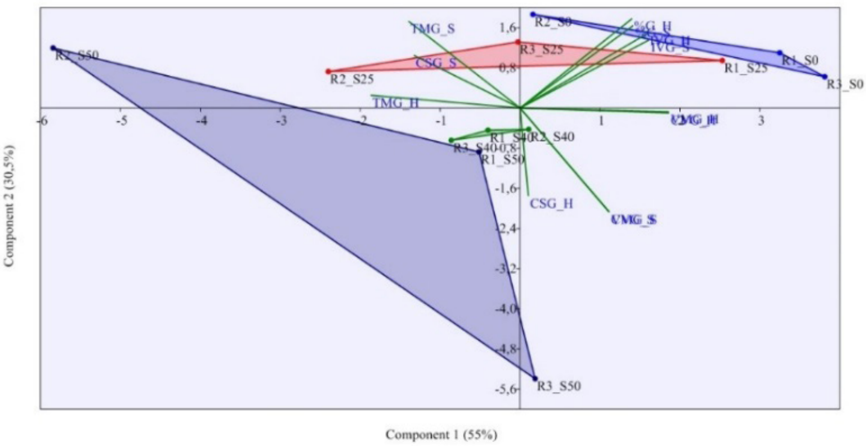

Figure 5. PCA analysis showing the correlation of physiological measures of germination as a function of salinity.

of acclimatization and adaptation to stress are overcome and can lead to plant death. (Larcher 2000).

According to the stress concept proposed by Lichtenthaler (1996), mangrove salinity can be considered a factor of eustress (stimulant stress) at low concentrations and distress (negative stress) even beyond the tolerance limit of the species. Our work showed that the propagules submitted to lower salinities than where they were collected from their matrices presented high germinability and a high rate of germination speed, different from propagules that were submitted to higher salinities such as 50 % which had a decrease in its germination values, as well as 75 % where no propagule could germinate.

The tolerance of halophyte seeds to salt stress is generally characterized by (1) germination in relatively high salinities, (2) dormancy induced or forced by saline conditions, and (3) ability to remain viable during soaking in saline conditions, germinating when the stress is relieved (Ungar 1995). Our study did not assess the recovery of non-germinated propagules at the end of the stress experiment, so we cannot answer whether propagules that did not germinate under high salinity conditions could germinate with stress relieved. Therefore, in future studies, it is recommended to evaluate the recovery of non-germinated propagules at the end of a stress experiment, which is an important factor to clarify doubts about the tolerance of these halophyte seeds.

It is noteworthy that the difficulty of isolating the effect of a single stress factor in ecological studies, due to the interaction with other factors, allows only relative comparisons (Larcher 2000).

HERBIVORY

According to the data obtained in the present work, the pre-dispersion herbivory condition did not affect the germinative capacity of the propagules of *L. racemosa*, since the values of the variables analyzed for both healthy and herbivorous propagules were significantly equal.

Herbivory appears to have little influence on early seedling recruitment patterns: *Avicennia marina* (Forssk.), because the propagules that showed some type of damage, when compared to healthy ones, were equally viable, showing that herbivory does not directly influence the life history of this species. (Minchinton 2001).

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The damage caused by insects had no significant effect on the survival of the species' propagules: *Bruguiera gymnorrhiza* (L.) Lamk. and Rhizophora stylosa Griff. and in its seedling growth; but in *Bruguiera parviflora* Wight, the viability of damaged propagules was significantly lower than that of the control (healthy), however, the growth of surviving seedlings was not influenced by damage to the propagules. The viability of propagules from *A. marina* e *Bruguiera exaristata* Ding Hou was not influenced by predator damage, but its growth was significantly hampered (Robertson 1990). Seedlings of the species *Rhizophora mangle,* their growth was not hampered by minor damage to their propagules (Rabinowitz 1977). When herbivory levels are more severe, this species presents impaired establishment (Onuf et al. 1977).

Pre-dispersal herbivory in propagules must be accounted for in estimates of offspring production, availability of propagules for forests, and restoration efforts (Farnsworth & Ellison 1997). When restoring areas of mangrove forests, it takes time to select the propagules to exclude those that are herbivorous (Robertson 1990).

Our results demonstrate that the germination performance of propagules from *L. racemosa* was not affected by the herbivory condition, on the contrary, it was sometimes more efficient than healthy propagules both in germination speed and in resistance to higher salinities. The successful germination of herbivorous propagules from *Laguncularia racemosa* suggests that they may be efficient in recovering degraded mangroves. It is suggested that further studies dealing with the germination phase of herbivorous propagules, including the stages of growth and establishment of seedlings, as well as addressing physiological, biochemical and histological aspects of the propagules be carried out.

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