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Laguncularia racemosa (L.) Gaertn. more than halophyte a halotolerant species

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ABSTRACT

Laguncularia racemosa grows on soils that vary widely in pore water salinity, and among Neotropical mangrove species, it is characterized by salt secretion and foliar succulence with higher Ca concentration. The objective of this article is to quantify the interactions between cation uptake and salinity on biomass allocation (roots, stems and leaves) as growth responses of *L. racemosa* seedlings. Propagules collected from a seasonal lagoon were grown in a saline gradient from 0 to 20 - 25 ‰ to evaluate their halophytic character. Culture was carried out in a greenhouse with 40% Hoagland solution and salinities of 0, 5, 10 and 20 ‰ for 9 months. Parameters evaluated (method): a) growth (allometry); b) soluble sugars (colorimetric); c) osmolality (Osmometry) and soluble cations (atomic absorption, AA) in leaf sap, d) Ca fractionation in leaves (AA), and e) concentrations of N (micro Kjeldahl), P (colorimetric), K, Mg, Ca, Na (AA) in seedling biomass. Results: 1) Maximum plant height and biomass decreased linearly with salinity, but the proportion of stems was reduced, whereas that of roots increased; 2) daily sugar accumulation and the concentrations of K, Mg, and Ca in leaf sap decreased with increasing salinity; 3) foliar Ca concentrations exceeded those of K and Mg, whereas in roots K increased, and Ca and Mg varied little with salinity; 4) oxalate constituted the largest fraction of Ca in all treatments. The results indicate that *L. racemosa* is halotolerant, whose growth is favored at low or zero salinities.

Key words: halophytic, halotolerant, *Laguncularia*, mangroves, osmolality.

Laguncularia racemosa (L.) Gaertn. más que halofita es una especie halotolerante

RESUMEN

Laguncularia racemosa crece en suelos que varían ampliamente en salinidad de agua intersticial, y entre las especies de mangle del Neotrópico, se caracteriza por secreción de sal y suculencia foliar y mayor concentración de Ca. El objetivo de este artículo es cuantificar las interacciones entre la absorción de cationes y salinidad en la asignación de biomasa (raíces, tallos y hojas) como respuestas de crecimiento de plántulas de *L. racemosa*. Propágulos colectados en una laguna estacional, se cultivaron en un gradiente salino de 0 hasta 20 - 25 ‰, para evaluar su carácter halófito. El cultivo se hizo en invernadero con solución Hoagland al 40 % y salinidades de 0, 5, 10 y 20 ‰, durante 9 meses. Parámetros evaluados (método): a) crecimiento (alometría); b) azúcares solubles (colorimétrico); c) osmolalidad (osmometría) y cationes solubles (absorción atómica, AA) en la savia de las hojas, d) fraccionamiento de Ca en hojas (AA) y e) concentraciones de N (micro Kjeldahl), P (colorimetría), K, Mg, Ca, Na (AA) en la biomasa de las plántulas. Resultados: 1) La altura máxima de las plantas y la biomasa total disminuyeron linealmente con la salinidad, pero la proporción de tallos se redujo, mientras que la de raíces incrementó; 2) la acumulación diaria de azúcar y las concentraciones de K, Mg y Ca en la savia de las hojas disminuyeron con la salinidad de las soluciones; 3) las concentraciones foliares de Ca superaron a las de K y Mg, mientras que en raíces K aumentó, y en Ca y Mg variaron poco con la salinidad; 4) oxalato constituyó la mayor fracción del Ca en todos los tratamientos. Los resultados indican que *L. racemosa* es halotolerante, cuyo crecimiento se favorece a salinidades bajas o nulas.

Palabras clave: halofitismo, halotolerante, *Laguncularia*, mangle, osmolalidad.

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INTRODUCCIÓN

Laguncularia racemosa (L.) Gaertn. is a salt-tolerant tree species that occurs as a main component of mangal ecosystems throughout the American tropics (Tomlinson 1986, Spalding *et al*. 2010). The species thrives on sediments varying widely in interstitial soil salinity and can sustain high salt concentrations in its cell sap

(Biebl and Kinzel 1965, Medina and Francisco 1997, Sobrado 2004, Méndez-Alonzo *et al*. 2016), reporting until 1650 mmol/Kg at 40 ‰ in coastal lagoons (Medina and Francisco 1997). Cardona Olarte *et al.* (2006), demonstrated that cultivated seedlings grow better at a salinity of 10 ‰, with alternating flooding, however Lonard *et al*. 2021, found that the optimal conditions for growth under natural environment are nutrient-rich soils and moderate salinity (15 – 20 ‰).

Biebl and Kinzel (1965) early on they argued that salinity tolerance of this species is based on the development of leaf succulence, compensating ion accumulation in vacuoles through dilution, combined with and the active secretion of salt by leaf glands (Sobrado 2004, Francisco *et al*. 2009, Quadros *et al*. 2021).

Among mangrove species, *L. racemosa* is characterized by higher Ca leaf concentrations (Barboza *et al*. 2006, Madi *et al*. 2015, Medina *et al*. 2015 a, b). Calcium is an element essential for the maintenance of membrane permeability at the root level, and for the structural stability of shoots and roots through the formation of Ca pectates gluing together cell walls (Marschner 1995, White and Broadley 2003, Hirschi 2004). Nitrogen and phosphorous have been identified as limiting nutrients for structural development of mangrove ecosystems throughout the world (Feller 1995, Chen and Twilley 1999, McKee *et al*. 2002, Lovelock *et al*. 2006). It has been shown in non-halophytes that Na ions inhibit competitively the uptake of K, Mg, and Ca, whereas the high levels of Cl ions may reduce the uptake of PO_4 and NO_3 (Lu and Fricke 2023). These effects of salinity on nutrient uptake may differ in mangroves, as they display various mechanisms regulating Na accumulation such as reduced uptake through the roots, leaf succulence, and salt glands (Ball *et al.* 1987, Parida and Jha 2010). Several authors consider them as obligate halophytes (Wang *et al.* 2011), but in this study is prefer to describe as salt tolerant, along the lines discussed by Barbour (1970), Grigori *et al.* (2012) and Krauss and Ball (2013). However, the mangrove species show large differences in cations accumulation and salinity tolerance in the field (Medina *et al*. 2015b).

In the present study is quantified, under semi-controlled conditions, the effect of salinity on a) the biomas production and distribution into leaves, stems, and roots; b) the diurnal variations in osmolality and ionic composition of leaf sap; c) the fractionation of Ca in leaves; d) the nutrients uptake and allocation by cultivated

propagules of *L. racemosa*. The hypothesis is that the presence of high Na concentrations in the nutrient solution reduces the uptake of essential cations, thereby altering intracellular ionic balance, and leading to growth impairment, particularly in this species.

MATERIALS AND METHODS

Propagules of *L. racemosa* collected from natural communities (Unare Lagoon, Anzoátegui State, Venezuela. (0 - 5 m a. s. l.) (64º 35' W 10º 10' N) were sown in a glasshouse (temperature 20-30 day and 16- 22 night, and 45- 85 % RH day, and 90-60 % RH at night, depending on seasonality) in Venezuelan Institute of Scientific Research (IVIC) at 1550 m a. s. l.). They stayed there for three months, until cotyledons were fully opened, and the first leaf pair had developed. At this stage, seedlings were transplanted in sand-filled, 2.5 L pots that were located within plastic boxes. Pots were flushed once a week with the nutrient solution until it filled the external container to cover up one-third of the pot.

The stock Hoagland nutrient solution was prepared using desionized water following the formulation of Hoagland and Arnon (1938). The original concentration was diluted to 40% using tap water, due to the large quantities used, however the concentration of Ca in tap water, were considered. The treatments consisted in adding crystallized sea salt (from salt mines Las Cumaraguas, Falcón State, Venezuela) to the nutrient solutions until reaching salinity levels of 5, 10, and 20 ‰ as measured with a hand refractometer (Atago, Japan). There were 4 treatments and 12 plants per treatment, for a total of 48 plants.

The following set of measurements was conducted after 7 months under the salinity treatments:

a) Five plants per treatment were harvested, cleaned with distilled water, and separated into leaves, roots, stems, and twigs. Plant material was oven-dried at 60 ºC for 72 hours. Elemental composition of dry mass was conducted in acid-digested samples (sulfuric-perchloric acids 4:1) (Digestion Kjeltec system 40-1016. Foss Tecator, Sweden. Concentrations of Na, K, Mg and Ca were measured using atomic

absorption (SpectraAA 55B, Varian), those of P following Murphy and Riley (1962), and the organic N using the microKjeldahl method (Jackson1968).

b) A subsample of dried and ground leaves was used to determine Ca fractions extracted sequentially with hot water of dried leaves was used to determine Ca fractions extracted sequentially with hot water (inorganic and organic soluble Ca salts), 10 % NaCl (Ca pectates), 2 N acetic acid (Ca phosphates) and 2 N HCl (Ca oxalate) (Kinzel 1989).

c) Leaf sap was extracted from frozen 5-6 mature leaves insert in a descartable plastic syringes and squeezed with a mechanical winch, and analyzed for osmolality (dew point osmometer Wescor 5500X, Logan, USA), ionic composition (atomic absorption spectrophotometry, Varian SpectrAA 55B, Australia) and soluble sugars (fructose equivalents) (Hassid and Neufeld 1964). The osmolality and sugar concentrations of the leaf sap were measured in samples taken in the morning (7 to 8 h), at noon (12 to 13 h), and late afternoon (17 to 18 h).

Biomass and nutrient concentrations both in leaf sap and biomass were submitted to an analysis of variance using parametric and non-parametric tests (Welch's test allowing for non-equal variances; Kruskall-Wallis's test). Thereafter, treatment averages were submitted to the Tukey-Kramer HSD test (JMP 14.2). Finally, interactions of element uptake with tissue Na concentration were explored using regression analysis.

RESULTS

Plant growth and biomass accumulation.

Plant height increased nearly exponentially during the measuring period, and the tallest plants were produced in the lower salinity treatments (0 and 5 %) (Fig. 1).

Total biomass per plant was higher at 0 ‰ and decreased by 42, 67, and 93 % as salinity increased to 5, 10, and 20 ‰ respectively (Table 1). The same pattern was fo-

und for leaves, stems, and roots. Stem biomass constituted the largest fraction at 0 and 5 ‰ treatments, followed by roots and leaves, whereas at salinities ≥ 10 ‰, biomass did not differ between the three organs. Within the range of salinities (0 to 20 ‰), the leaf fraction of total biomass remained within biomass remained within ≈ 30 %, whereas the stem fraction decreased from 46 to 29 % and the root fraction increased from 24 to 36 %. Dry mass per leaf was markedly affected even by the lowest salinity treatment. Compared to control, it decreased from control by 25 % at 5 ‰ to 80 % at 20 ‰ (Table 2).

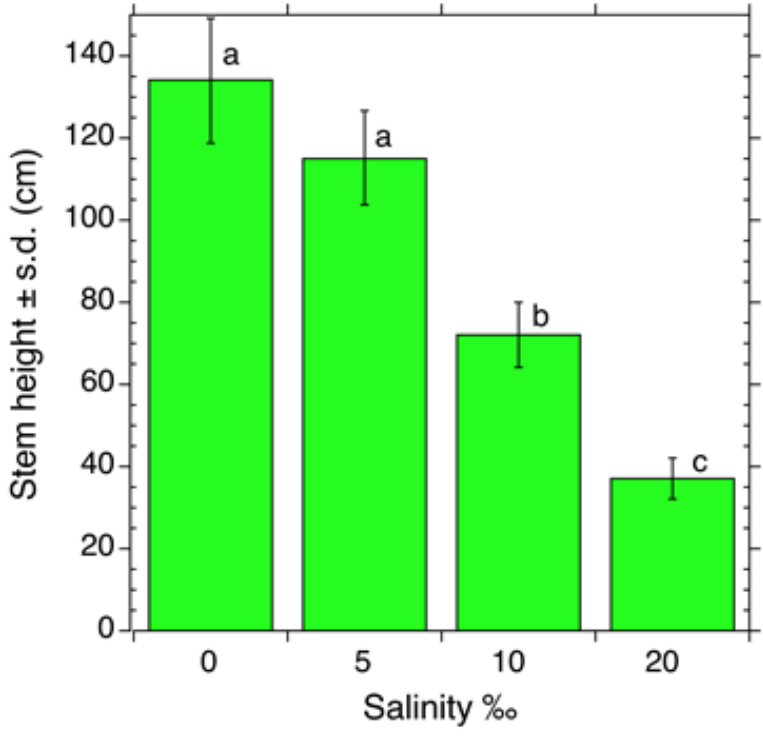

Figure 1. Plant height under salinity treatments (220 days under treatment (n = 5) ± *s. e*.

Table 1. Dry mass and biomass ratios (mean ± *s. e.*) of *L. racemosa* plants grown for 220 days under increasing salinity levels (A: 0 ‰; B: 5 ‰; C: 10 ‰; D: 20 ‰). (n= 5). Within columns, numbers followed by the same lowercase letter, and within rows followed by the same uppercase letter, do not differ statistically (all pair comparisons, Tukey-Kramer HSD, P = 0.05).

Salt	Leaves	Stems	Roots	TotalShoot/Root	Leaf/Root	
0 ‰ A	32.6 (3.1)a	49.2 (1.3)a	25.7 (1.7)a	107.6 (5.8)a	3.2 (0.1)a	1.3 (0.1)
5 ‰ B	17.5 (1.9)b	27.8 (2.3)b	17.1 (1.5)b	62.5 (5.5)b	2.7 (0.1)ab	1.0 (0.1)
10 ‰ C	10.3 (0.5)c	12.8 (0.5)c	12.7 (1.0)b	35.8 (0.9)c	1.9 (0.1)b	0.8 (0.1)
20 ‰ D	2.9 (0.5)d	2.3 (0.4)d	2.8 (0.5)c	8.0 (1.3)d	1.9 (0.3)b	1.0 (0.1)

Table 2. Leaf mass and area per leaf, and water content per unit leaf area of *Laguncularia racemosa* plants (218 days under treatment). Within columns numbers followed by the same letter do not differ statistically (all pairs comparison, Tukey-Kramer HSD, P=0.05).

Treatment	n	Fresh mass (g)	Dry mass (g)	Area (cm^2)	Water (g/m^2)
0 ‰ (A)	22	1.07 a	0.23 a	22.5 a	397
5 ‰ (B)	26	0.70 b	0.14 b	13.1 b	480
10 ‰ (C)	15	0.69 b	0.14 b	12.5 b	464
20 ‰ (D)	8	0.45 b	0.11 b	6.5 c	629

Diurnal variations in leaf sap osmolality and soluble sugars.

Leaf sap osmolalities increased significantly with the salinity of the nutrient solution, and within each treatment, they were higher at noon, revealing partial dehydration possible due to transpiration, recovering to morning values in the late afternoon (Fig. 2).

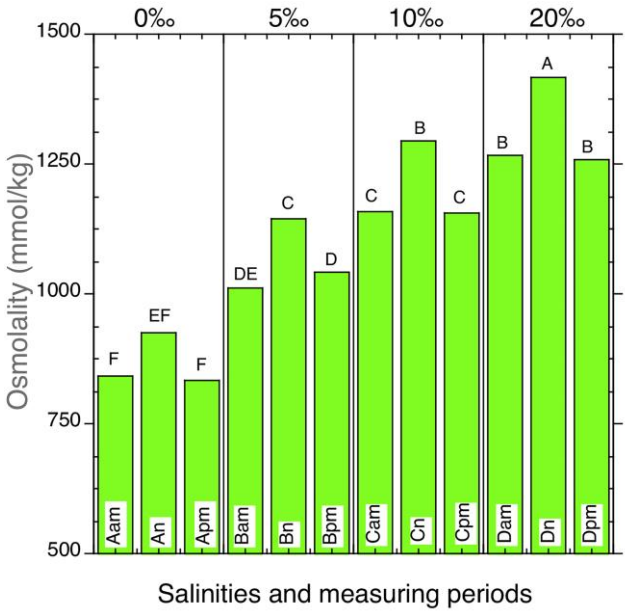

Figure 2. Variation of leaf sap osmolality with salinity treatment (A: 0 ‰; B: 5 ‰; C: 10 ‰; D: 20 ‰) and time of the day (morning: 7 to 8 h; noon: 12 to 13 h; late afternoon: 17 to 18 h). Differences tested with the Tukey-Kramer HSD test, $P = 0.05$).

The concentration of soluble sugars showed consistent differences between early morning and late afternoon leaf samples (Fig. 3). Under saline treatments, absolute concentrations decreased, but diurnal variations remained similar.

Cations concentrations and osmolality of leaf sap

In the leaf sap, osmolality and concentration of Na increased with salinity of the nutrient solution, whereas those of K, Mg, and Ca decreased in a non-linear fashion. The concentration of those ions did not differ significantly between 10 and 20 ‰ (Fig. 4). Concentrations of soluble Mg and K were higher than those of soluble Ca at all salinities. In addition, the Mg/Ca ratios were above 2 at 0 ‰ and converged to 1 as the salinity increased. The K/Ca molar ratio showed an opposite pattern, increasing from about 1.7 at 0 and 5 ‰ to 2 at 10 and 20 ‰.

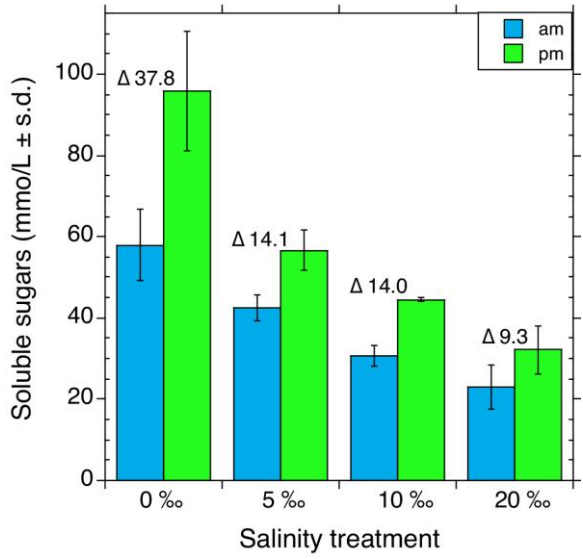

Figure 3. Diurnal changes in reducing sugars concentrations (glucose equivalents) (average ± *s. e.*) affected by salinity (three replicates per column). The Δ values indicate the net increase in soluble sugars during the day.

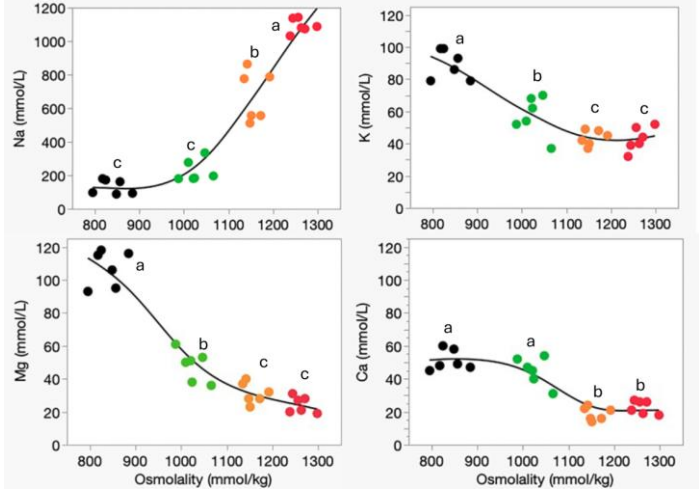

Figure 4. Changes in leaf sap ion concentration related to the corresponding osmolality in plants grown at different salinities for 220 days: 0 ‰ green, 5 ‰ blue, 10 ‰ orange, 20 ‰ red. Spline curves adjusted manually. By each curve, color groups aligned with the same letter do not differ statistically (Tukey-Kramer HSD test, P = 0.05).

Extractable fractions of leaf calcium

The water and HCl Ca fractions constituted 86 % of total extractable Ca from dried leaves in the 0 ‰ treatment, decreasing to 62 % at the highest salinity (Fig. 5). The HCl fraction (Ca-oxalate) was dominant in all treatments.

Element concentrations per plant organ

Mean element concentrations showed different patterns among organs and salinity treatments (Table 3). Nitrogen concentrations in leaves were higher in all treatments compared to roots and stems, whereas P concentrations overlapped throughout. Sodium concentration increased with the salinity of the nutrient solution in all organs and was similar in leaves and roots and higher than those of the stems. In addition, the concentration increases per salinity level relative to control was larger in leaves (factors: 3.2; 4.7; 5.7) than in roots (factors: 2.6; 2.9; 3.8).

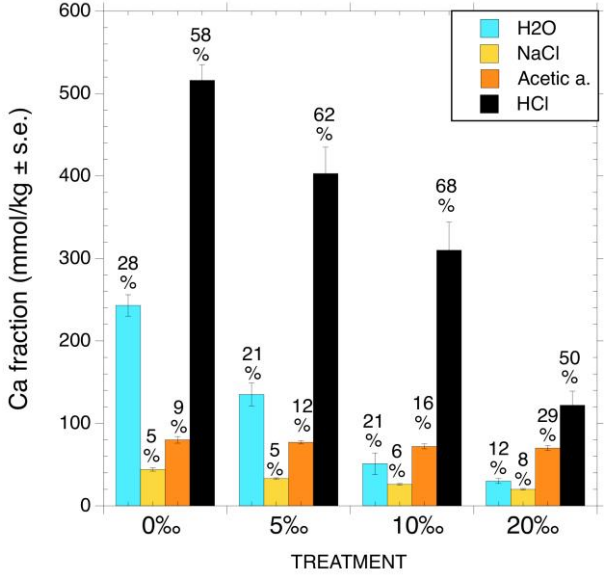

Figure 5. Leaf Ca fractions from plants grown for 220 days at different salinities of the nutrient solution. Fractions were obtained by sequential extraction of dried leaf tissue using water desionized, NaCl 10 %, acetic acid 2 N and HCl 2 N solutions.

Table 3. Element concentration (mmol/kg dry mass ± s.d.) of leaves, stems, and roots of *Laguncularia racemosa* plants grown for 220 days under increasing salinity levels (A: 0 ‰; B: 5 ‰; C: 10 ‰; D: 20 ‰). Within column numbers followed by the same letter are not statistically different (Tukey-Kramer HSD test P=0.05) (n=5).

Organ Treatment	N		P		Na		K		Mg		Ca	
LEAVES												
A	1023 (44)	b	31 (5)	c	375 (90)	g	276 (94)	de	283 (79)	a	1073 (167)	a
B	863 (47)	c	38 (5)	bc	1200 (128)	de	169 (32)	ef	54 (32)	b	791 (126)	b
C	837 (71)	c	35 (7)	bc	1751 (362)	bc	125 (53)	f	140 (29)	bc	595 (189)	b
D	1203 (96)	a	52 (11)	ab	2304 (273)	a	141 (24)	ef	111 (29)	bcd	413 (104)	c
STEMS												
A	300 (24)	gh	27 (3)	c	252 (63)	g	239 (15)	def	32 (3)	e	292 (24)	c
B	239 (15)	h	35 (3)	bc	584 (168)	fg	163 (57)	ef	24 (3)	e	279 (33)	c
C	265 (32)	h	38 (3)	cd	618 (171)	fg	198 (44)	def	28 (9)	e	267 (52)	c
D	404 (54)	fg	61 (7)	a	1025 (346)	ef	352 (38)	cd	48 (4)	de	294 (40)	c
ROOTS												
A	619 (65)	de	31 (12)	c	543 (125)	fg	442 (106)	c	101 (12)	bcd	331 (73)	c
B	512 (21)	ef	38 (15)	bc	1390 (278)	cde	691 (108)	b	77 (13)	cde	344 (21)	c
C	497 (35)	f	42 (11)	abc	1565 (242)	bcd	781 (72)	ab	71 (12)	de	330 (42)	c
D	690 (80)	d	48 (13)	abc	1993 (165)	ab	918 (70)	a	62 (14)	de	316 (34)	c

Potassium averages were higher in roots in all treatments but were similar within treatments by each organ. Instead, Mg and Ca concentrations were higher in leaves and decreased significantly with salinity. In contrast with the proportions of ions measured in leaf sap, Ca was the dominant cation because of the amount of Ca in the form of insoluble oxalate. In addition, the K/Ca ratios were always below 0.4 in the leaves, whereas in the roots it increased from 1.4 in the control to 2.9 in the 20 ‰ treatment.

Sodium correlations with biomass and element concentrations

The elemental composition patterns induced by salinity emerging from Tables 1 and 3 can be visualized by drawing the correlations between Na concentrations, biomass, and element concentrations.

Tissue Na concentration is linearly correlated with the mass of leaves and roots accumulated at the end of the experiment (Fig. 6). This relationship suggests that Na is a major factor causing the reduction in biomass production.

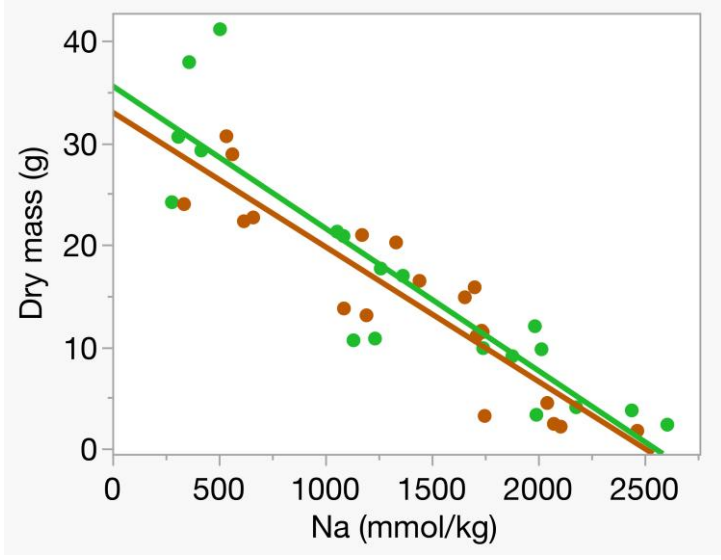

Figure 6. Relationship between biomass of roots and leaves produced after 220 days under salinity treatments, and the corresponding Na concentrations. R^2 (n= 19) brown) roots 0.80; (green) leaves 0.81.

The correlations between leaf tissue concentrations of Na and those of N, P, and major cations revealed substantial differences in element uptake and allocation between leaves and roots (Fig. 7). By increasing concentrations of Na, the concentrations of N remained constant, and those of P increased in both leaves ($P >$ t ratio 0.0006) and roots ($P >$ t ratio 0.0039). The concentrations of Mg decreased in both tissues ($P >$ t ratio 0.0006 for leaves, and 0.00015 for roots). In the case of Ca, the leaves showed a strong reduction in concentration ($P >$ t ratio 0.0002), but it remained constant in the roots. Potassium concentrations showed a contrasting behavior, decreasing in the leaves ($P >$ t ratio 0.0015) and increasing strongly in the roots ($P >$ t ratio < 0.0001).

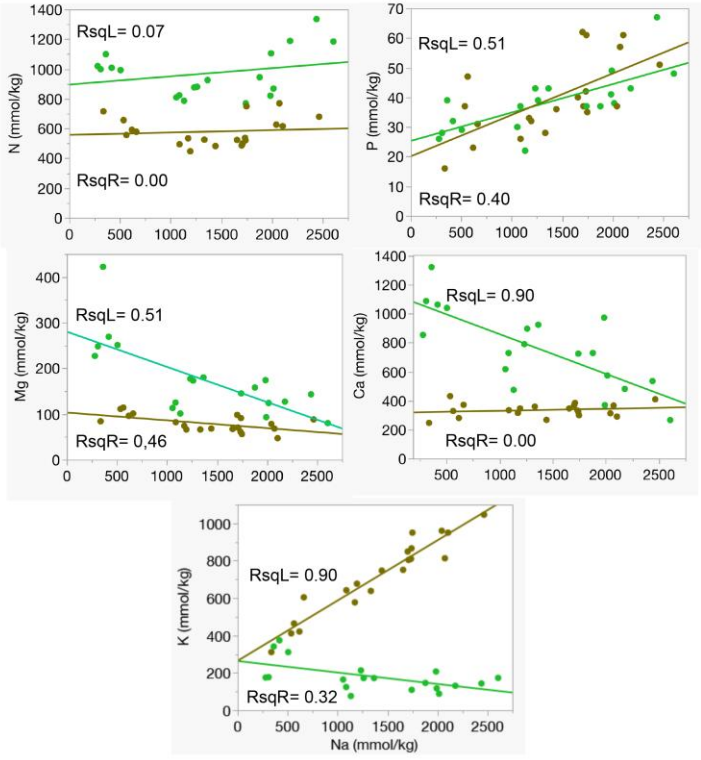

Figure 7. Correlation between the concentrations (mmol/kg dry mass) of Na and those of N, P, Mg, Ca, and K in leaves (L green) and roots (R brown) from plants grown at different salinity for 220 days.

DISCUSSION

The main objective of this research was to evaluate the salinity tolerance of *Laguncularia racemosa* seedlings by measuring the growth and allocation of organic matter into different organs during prolonged exposure to different salinity levels. In this experiment, we measured the interaction of salinity, determined by the Na concentration of the nutrient solutions, with the uptake of N, and P and the cations K, Mg, and Ca. Indicating the Na ion is preferentially transported to the leaves.

The salinity treatments applied were moderate, as they did not reach the level of average seawater (35 ‰). Under field conditions, salinities between 10–20 ‰ are considered optimal for developing *L. racemosa* (Lugo *et al.* 2007, Lonard *et al.* 2021).

In this study with the greenhouse experiments it was shown that *L. racemosa* plants were taller and produced more biomass in the nutrient solution without added marine salt.

Using this approach, also showed that K^+ and N uptake was impaired in roots exposed to NaCl. Concerning Ca^{2+}, there was no indication of uptake inhibition by NaCl. However, restriction of K^+ uptake by roots was compensated by an increase in the K^+-use efficiency, so that growth was not inhibited.

Area and mass per leaf decreased with salinity, whereas the opposite tendency was observed in the leaf water content per unit area (succulence). The same responses for *L. racemosa* were reported by Biebl and Kinzel (1965) in natural communities and by Sobrado (2007) with cultivated plants.

At the highest salinity applied (20 ‰) the reduction in biomass accumulation was about 91% below the control (Table 1). This strong effect might be due to the experimental conditions with approximately constant salinity, and no daily flooding cycle. Cardona-Olarte *et al.* (2008) showed that permanent flooding reduced stem length and net primary productivity in *L. racemosa* seedlings grown at moderate (10 ‰) and high (40 ‰) salinities. Their results for the 10 ‰ treatment with permanent flooding are similar to those reported here, however, the relative reduction in growth induced by the high salinity is small compared to our results. A long the same line, Gu *et al.* (2019) showed that moderate shading and low salinity (0–10 ‰) increase survival and growth of *L. racemosa* seedlings.

Experiments of Nandy *et al*. (2009) with several other mangrove species (*Bruguiera gymnorrhiza, Excoecaria agalocha, Heritiera fomes* and *Xylocarpus granatum* show that growth is maximized in low salinity solutions. Similarly, Burchett *et al.* (1989) found that mangrove species with salt secreting glands (*Avicennia marina* and *Aegyceras corniculatum*), grow best at 9 ‰. Ye *et al.* (2005) compared salt-secreting mangrove species and reported maximum relative growth rates by 0 ‰ in *A. corniculatum* and *Acanthus ilicifolius* and by 5 ‰ in *A. marina*.

Leaf sap osmolality and ion concentration

The osmotic concentration of leaf sap increased as expected with the salinity of the nutrient solution. The range of values was like those reported for natural communi-

ties growing in riverine and coastal sites (Medina and Francisco 1997). Leaf sap osmolality increased at noon time, by all treatments, revealing stomata opening and partial dehydration because of transpiration. The noon osmolalities returned to morning values at the end of the day, indicating the capacity of the plants to compensate for water losses. Sugar accumulation was always lower in salt treatments compared to the marine salt-free control, indicating the reduction in diurnal net CO_2 fixation in all salt treatments.

The analysis of the ionic composition of leaf sap revealed that higher osmolality was mostly related to larger Na concentration, accompanied by moderate reductions in K and Mg concentrations. Leaf sap osmolality increased 1.5 times between the control and the 20 ‰ treatment, and Na increased 6 times in the same range of salinity. Meanwhile, K, Mg and Ca decreased by 52, 77 and 55 %, respectively. Sodium in the nutrient solution inhibits the uptake of essential cations. Interestingly, the K/Ca was >1 and showed a tendency to increase with salinity, whereas the Mg/Ca followed the opposite trend. Salinity-induced K deficiency has been indeed shown in other mangrove species, *Avicennia marina* (Ball *et al*. 1987). *Laguncularia racemosa* does not behave as a calciotrophic plant (Kinzel 1989) because its soluble molar K/Ca and Mg/Ca ratios are above one, indicating the exclusion of Ca from the physiologically active ion set.

Ca fractions in leaves

Plants sensitive to high levels of Ca synthesize oxalic acid and accumulate water-insoluble Ca-oxalate in the vacuoles. Once the wall, membrane, cytosol, endoplasmic reticulum, and vacuoles are filled, the rest can be transported to the shoots through the xylem. The concentration of Ca in cytoplasm must remain at the submicromolar level, to preserve the role of Ca as a cellular messenger regulating membrane permeability (White and Broadley 2003). This is attained by pumping Ca into the vacuole, where it is rendered insoluble by combination with oxalate anions. *Laguncularia racemosa* keeps precipitating a large fraction of the available Ca as Ca oxalate in the vacuoles. This mechanism may enable plants to maintain low cytosolic Ca in natural habitats with high Ca availability (Lee and Liu 1999). Salinity decreases both the concentration of water-soluble and HCl-soluble Ca fractions. However, in all treatments, the HCl-soluble fraction constitutes 50 % or more of the total Ca in leaves (Fig. 5). It appears that *L. racemosa* takes up Ca exceeding its physiological requirements, and its concen-

tration as free ion is regulated by the synthesis of oxalic acid. Free oxalic acid has been reported in *Lumnitzera racemosa*, a mangrove genus included with *L. racemosa* within the tribe Laguncullariae (Combretaceae) (Popp 1984, Manohar 2021). Salt toxicity includes osmotic and ionic components that can seriously affect root growth and sprouts. Absorption of Na^+ through the plasma membrane is very rapid, resulting in physiological effects at both extracellular and intracellular sites. Sodium reduces the binding of Ca^{2+} to the plasma membrane, inhibits the inflow while increasing the outflow of Ca^{2+}, and depletes the internal reserves of Ca^{2+} in the endomembrans (Rengel 1992). However, the accumulation of considerable calcium concentration in the form of oxalate could be a reserve source of CO_2 under water stress conditions, with stomatal closure thus avoiding water loss by transpiration, and use it in a process of phytomineralization (Tooulakon *et al*. 2016).

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