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Research Article

Salinity as a determinant factor in morphology and anatomy of mangrove plant *Lumnitzera racemosa* Willd.

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ABSTRACT

Along with their ecological significance, the *Lumnitzera racemosa* Willd. mangrove is interesting for its abundance of natural compounds, some of which have the potential to be used in medicine. In this study the effect of different concentrations of sodium chloride on growth and development of seedlings of *L. racemosa* was analysed. The growth parameters such as shoot and root length, number of leaves, leaf area, fresh weight and dry mass production increased with increasing sodium chloride upto 500 mM and it can be concluded that, this plant can grow and survive in this salt level.

INTRODUCTION

Mangroves are one of the unique salt tolerant plant communities growing at intertidal zones at the river mouths. Mangroves exist under very hostile, inhospitable and adverse conditions [1]. The plants that flourish there must endure greater salinity, strong tidal movements, high winds, high temperatures, and muddy, anaerobic soil. The mangrove plants have great potential to adapt to the changes in climate (precipitation and temperature), increasing sea levels and solar UV radiation incidence [2].

Mangroves are a diverse group of woody trees and shrubs that make up the main flora in tidal, saltwater marshes along tropical and subtropical coasts [3]. Invariably mangrove distribution is controlled by the atmospheric temperatures which are dominant at 20°C summer isothermal within which mangroves grow well [4]. In cold climates where temperature drops down to 10°C, mangroves are marked by dwarf growth.

Mangrove species have been utilized in traditional remedies, and their extracts have demonstrated inhibitory efficacy against diseases that affect people, animals, and plants. Several mangrove species contain bioactive substances that might inhibit the growth of microorganisms [5]. Salinity of seawater is the number of grams of dissolved salts in 1000 g of seawater and is always expressed as parts per thousands (ppt - ‰). Normal seawater salinity ranges from 33 to 38 ppt. Salinity is a major seawater parameter that influence all marine organisms by the process of diffusion and osmosis [6].

This study was conducted to test the effects of increasing concentration of salinity on *Lumnitzera racemosa* Willd. a medicinal plant belongs to the category mangroves.

MATERIALS AND METHODS

Lumnitzera racemosa Willd. a dicotyledonous mangrove tree belonging to the family Combretaceae were collected from the mangrove belt of Pichavaram, on the east coast of Tamil Nadu, India. When the seedlings were about 10 cm, healthy plants of uniform height were screened for transplantation. The seedlings were uprooted carefully with intact root system from the mangrove belt and were washed thoroughly with fresh water. The seedlings then were planted into the individual polythene bags filled with fresh soil containing red earth, sand and farmyard manure (1:2:1).

The seedlings were allowed to stabilize for 15 days and were irrigated with fresh water. At a time, about 800 plants were maintained in the experimental yard.

Salinity induction

NaCl treatments were given to one month old and fully established seedlings at the rate of 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM NaCl. The control was maintained without any exogenous addition of salt. Both the control and experimental plants treated with varying concentrations of NaCl were regularly irrigated with tap water. As the objective of the present investigation was to study individually the effect of different concentrations of NaCl in *L. racemosa*. Seawater was not used for irrigation in the present study. The seawater besides containing NaCl had also other salts such as Na_2SO_4, $MgSO_4$, MgCl and Na_2CO_3. Sampling were done on the 60th, 120th and 180th days after NaCl treatment.

Leaf and stem anatomy

Leaf and stem anatomy were done by rotary microtome, stained and observed under light microscope.

Epidermal studies

Stomatal studies were made by the method of Stoddard [7]1965). In this method, nail polish was applied to the leaf surface and the nail polish peelings were examined under light microscope. Number of epidermal cells (E) and stomata (S) were counted in a specified field and stomatal index was arrived using the following formula.

Fresh weight and dry weight

For the estimation of fresh weight of leaf, stem and root portions were separated and weighed. To estimate the dry weight, the different plant organs were dried at 80°C for 48 h in an oven and weighed.

RESULTS

Growth

Lumnitzera racemosa is a salt marsh species and the seedlings could be grown with freshwater. The seedlings also could tolerate a wide range of exogenously added sodium chloride. Morphological observations were made on the 60th, 120th and 180th days after NaCl treatments. The effect of different concentrations of sodium chloride on the morphology of the seedlings is given in Fig. 1.

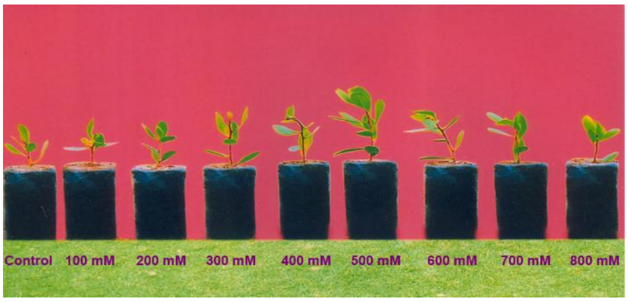

Fig. 1. Effect of sodium chloride on the growth of *Lumnitzera racemosa* seedlings on 60th day

Leaf anatomy

The leaf anatomy and stomatal studies of L. racemosa are given in Figs. 2 and 3. Leaves of this species is dorsiventral, succulent and small. The shape of the leaf is obovate and the apex is rounded or emarginated. The transverse section of the leaf showed well-developed epidermis with thick cuticle. Palisade parenchyma cells forms a continuous layer from one margin to the other on both epidermis and spongy parenchyma cells are compactly packed. The anatomy of this leaves with so-called 'kranz' cells differs fundamentally from that of C3 leaves. Midrib containing a crescent strand of xylem and phloem. In the matured leaf, many tannin containing cells are present in between the palisade cells.

In the epidermal peel of the leaves showed anamocytic type of stomata on both the upper and lower epidermis. Guard cells were somewhat thick walled, often with prominent edges. The beak like cuticular out growth covered the outer side of the stomatal pore. These structures provide resistance to stomatal transpiration.

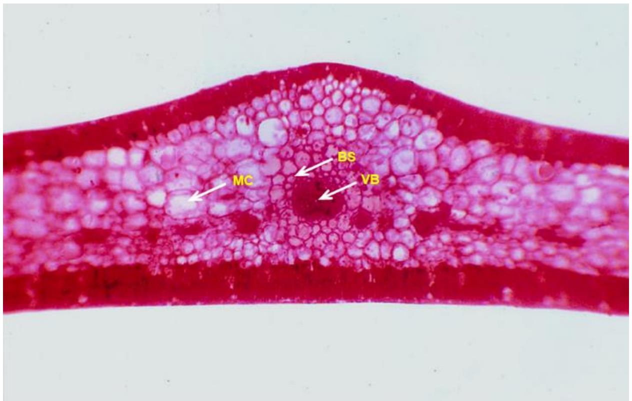

Fig. 2. Transverse section of *Lumnitzera racemosa* leaf showing Bundle sheath (BS), Vascular bundle (VB) and Mesophyll cells (MC)

Transverse section of stem

The transverse section of a young stem showed the outer thick cuticle layer and many gum canals and tannin containing cells (Fig. 4).

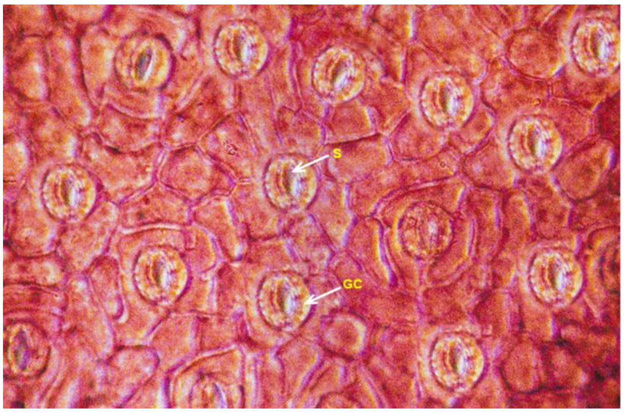

Fig. 3. Epidermal peel of Lumnitzera racemosa leaf showing stomata (S) and Guard cells (GC)

Cortex was made up of parenchymatous cells. Phloem consisting of very small cells and many tannin containing cells were interrupted between the cells of phloem. Gum canals were also found in the phloem cells. Xylem is in the form of a cylinder, transversed by rays. Vessles were mostly solitary, circular in outline. Pith was almost circular in shape and chiefly consisting of parenchymatous cells. Many tannin containing cells interrupted between the parenchyma cells.

Fig. 4. Transverse section of *Lumnitzera racemosa* stem showing Tannin containing cells and central pith (P)

Fresh weight

The results on the effect of NaCl salinity on the fresh weight of the leaves, stem and root of *L. racemosa* are presented in Table 1. There was a steady increase in the fresh weight of all the plant tissues on the three sampling days, upto the optimum level of 500 mM NaCl. The leaf fresh weight was higher than that of stem and root. The highest value of leaves fresh weight 8.12 g/plant was noticed at 500 mM NaCl on the 180th day samples and this was 24.92% higher than those of control plants. The F values calculated for the difference between the sampling days and between the treatments were significant at 1% level.

Dry weight

The effect of sodium chloride salinity on the dry weight of leaves, stem and root of *L. racemosa* was studied and the data are given in Table 2. The dry weight of the leaves, stem and root increased with increasing NaCl salinity upto 500 mM. The stem dry weight was higher than that of leaves and root. The highest percentage of stem dry weight (71.56%) was noticed on the 60th day samples. The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

Table 1. Effect of NaCl on the fresh weight of leaves, stem and root of *Lumnitzera racemosa* (g/plant).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	3.00	5.02	6.50	2.25	3.75	5.78	1.60	2.62	3.50
100	3.15 (+ 5.00)	5.18 (+ 3.18)	6.67 (+ 2.61)	2.30 (+ 2.22)	3.85 (+ 2.66)	5.90 (+ 2.07)	1.70 (+ 6.25)	2.80 (+ 6.87)	3.70 (+ 5.71)
200	3.40 (+ 13.33)	5.47 (+ 8.96)	6.98 (+ 7.38)	2.50 (+ 11.11)	4.20 (+ 12.00)	6.32 (+ 9.34)	1.92 (+ 20.00)	3.13 (+ 19.46)	4.00 (+ 14.28)
300	3.65 (+ 21.66)	5.70 (+ 13.54)	7.20 (+ 10.76)	2.90 (+ 28.88)	4.85 (+ 29.33)	6.90 (+ 19.37)	2.18 (+ 36.25)	3.36 (+ 28.24)	4.25 (+ 21.42)
400	3.90 (+ 30.00)	6.08 (+ 21.11)	7.55 (+ 16.15)	3.35 (+ 48.88)	5.25 (+ 40.00)	7.36 (+ 27.33)	2.50 (+ 56.25)	3.70 (+ 41.22)	4.60 (+ 31.42)
500	4.60 (+ 53.33)	6.50 (+ 29.48)	8.12 (+ 24.92)	4.00 (+ 77.77)	6.00 (+ 60.00)	8.05 (+ 39.27)	3.12 (+ 95.00)	4.33 (+ 65.26)	5.20 (+ 48.57)
600	3.72 (+ 24.00)	5.60 (+ 11.55)	7.00 (+ 7.69)	2.70 (+ 20.00)	4.30 (+ 14.66)	6.75 (+ 16.78)	2.40 (+ 50.00)	3.50 (+ 33.58)	4.20 (+ 20.00)
700	2.90 (- 3.33)	4.60 (- 8.36)	6.36 (- 2.15)	2.15 (- 4.44)	3.62 (- 3.46)	5.50 (- 4.84)	1.50 (- 6.25)	2.50 (- 4.58)	3.40 (- 2.85)
800	2.50 (- 16.66)	4.25 (- 15.33)	6.00 (- 7.69)	2.00 (- 11.11)	3.38 (- 9.86)	5.03 (- 12.97)	1.36 (- 15.00)	2.35 (- 10.30)	3.20 (- 8.57)
	Leaves : F1 = 150.1666* F2 = 3248.429*			Stem: F1 = 37.79195* F2 = 608.2401*			Root : F1 = 263.7335*F2 = 2043.513*		

Table 2. Effect of NaCl on the dry weight of leaves, stem and root of *Lumnitzera racemosa* (g/plant)

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	0.68	0.95	1.43	1.02	1.69	2.12	0.65	0.90	1.37
100	0.80 (+ 26.47)	1.13 (+ 18.94)	1.55 (+ 8.39)	1.10 (+ 7.84)	1.75 (+ 3.55)	2.28 (+ 7.54)	0.74 (+ 13.84)	1.08 (+ 20.00)	1.50 (+ 9.48)
200	0.93 (+ 36.76)	1.36 (+ 43.15)	1.78 (+ 24.47)	1.17 (+ 14.70)	1.84 (+ 8.87)	2.40 (+ 13.20)	0.82 (+ 26.15)	1.20 (+ 33.33)	1.74 (+ 27.00)
300	1.00 (+ 47.05)	1.64 (+ 72.63)	1.95 (+ 36.36)	1.24 (+ 21.56)	1.98 (+ 17.15)	2.61 (+ 23.11)	0.90 (+ 38.46)	1.36 (+ 51.11)	1.86 (+ 35.76)
400	1.14 (+ 67.64)	1.85 (+ 94.73)	2.10 (+ 46.85)	1.35 (+ 32.35)	2.07 (+ 22.48)	2.75 (+ 29.71)	1.02 (+ 56.92)	1.65 (+ 83.33)	1.95 (+ 42.33)
500	1.40 (+ 105.88)	2.05 (+ 115.78)	2.32 (+ 62.23)	1.75 (+ 71.56)	2.60 (+ 53.84)	2.90 (+ 36.79)	1.18 (+ 81.53)	1.92 (+ 113.33)	2.10 (+ 53.28)
600	1.06 (+ 55.88)	1.30 (+ 36.84)	2.00 (+ 39.86)	1.25 (+ 22.54)	1.92 (+ 13.60)	2.45 (+ 15.56)	0.88 (+ 35.38)	1.20 (+ 33.33)	1.80 (+ 31.38)
700	0.70 (+ 2.94)	1.08 (+ 13.68)	1.40 (- 2.09)	0.94 (- 7.84)	1.60 (- 5.32)	2.10 (- 0.94)	0.60 (- 7.69)	0.88 (- 2.22)	1.33 (- 2.91)
800	0.58 (- 14.70)	0.90 (- 5.26)	1.26 (- 11.88)	0.81 (- 20.58)	1.43 (- 15.38)	1.96 (- 7.54)	0.50 (- 23.07)	0.81 (- 10.00)	1.24 (- 9.48)
	Leaves F1 = 32.0345* F2 = 146.5824*			Stem : F1 = 60.7559* F2 = 735.1727*			Root : F1 = 28.1988* F2 = 175.8892*		

The study on the effect of salinity on plant growth is fundamental to any investigation for the physiological basis of salt tolerance. The seedlings survived NaCl concentrations upto 800 mM with an approximate reduction of 14% growth in shoot length and 40% in root length when compared to that of control plants. This shows that growth appears depressed both in the absence of salt and at high salinity levels. A stimulation of growth in response to low to moderate levels of sodium chloride salinity has been reported in number of halophytes such as *Atriplex amnicola* [8] and *Stenotaphrum secundatum* [9]. At low salinity level, stimulation of growth was also reported for *Acanthus ilicifolius* and *Halopyrum mucronatum* [10], *Bruguiera gymnorhiza* [11] and *Plantago coronopus* [12,13]. Significant reduction of growth at high salinity has been reported in certain halophytes and mangroves such as *Atriplex patula* [14], *A. prostrata* [15] and in *Avicennia germinans* [16].

The results on the growth of the seedlings definitely indicated that the NaCl concentrations at 500 mM was found to be optimum level and stimulated maximum growth in this species. Moreover, the increase in the plant height in response to NaCl was greater during the second 60 days of salt treatment when compared to that of the other sampling days. Sodium chloride salinity stimulated the leaf production and leaf area upto the optimum level of 500 mM (Table 4). At higher concentrations, there was a reduction in the leaf number and leaf area. The decrease in the leaf number at high salinity level may be attributed to senescence followed by leaf fall with the ageing of the leaves [17]. When the salinity exceeds the optimum limit, it would be metabolically advantageous and less costly to drop the leaves than to overcome this stress.

Sodium chloride salinity has been reported to promote succulence in certain halophytes such as *Sesuvium portulacastrum* [18] and *Bruguiera gymnorhiza* [11]. The leaves of *L. racemosa* developed greater succulence due to the accumulation of available salts in the vacuoles of the cell and maintained a lower ion concentration in the cytoplasm than in the vacuoles as a means of salt regulation and hence, this species is appropriately called “salt accumulating mangrove”.

The increase in the root fresh weight was less pronounced at all concentrations. The results of the fresh mass production obtained in the present study in *L. racemosa* agree with the previous studies on halophytes such as *Salicornia bigelovii* [19], and in *Suaeda salsa* [20].

NaCl salinity also increased the dry weight of all the plant tissues with increasing concentrations of salt upto the optimum level (Table 2). Similar results were observed in a few other halophytes. High salinity greatly reduced dry matter accumulation in shoots and roots of *Kadelia candel* [21]. In Suaeda salsa [20], the dry mass production decreased at higher NaCl concentrations (400 m mol/l). The present study revealed that NaCl supplied externally to this species had many effects on growth and development, leaf number and leaf area.

CONCLUSION

In this mangrove species, *Lumnitzera racemosa* Willd., the growth parameters increased with increasing sodium chloride upto 500 mM and it can be concluded that, this plant can grow and survive in this salt level.

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