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Research Article

Changes in photosynthesis with increasing salinity in mangrove ecosystem

A.K. Rajalakshmi*, P. Elavazhahan, P. Manivannan, R. Ranganathan

PG and Research Department of Botany, Government Arts College, C. Mutlur, Chidambaram, Tamil Nadu, India

*Corresponding Author, Email: akrajalakshmi2015@gmail.com

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ABSTRACT

The present study was carried out in *Lumnitzera racemosa* Willd. a dicotyledonous mangrove (family: Combretaceae). NaCl at the rate of 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM were given to 1 month old and fully established seedlings. Samplings were done on 60th 120th and 180th days after NaCl treatment. Chlorophyll, carotenoids, Net leaf photosynthesis, photosynthetic carboxylases and amylases were estimated from the samples. Salinity from sodium chloride encouraged the synthesis of carotenoid and chlorophyll in *L. racemosa* to the ideal concentration. When NaCl salinity is increased, photosynthetic properties such net leaf photosynthesis, stomatal conductance, transpiration, leaf chamber temperature, and intercellular CO_2 concentration decreased. This information can be used to assess the effect of increasing concentration of salinity in crop plants.

INTRODUCTION

In the tropics and subtropics, mangroves are salt-tolerant plants and shrubs that typically grow in the intertidal zone [1]. These plants are considered as model plants for studying increasing concentrations of salinity in water in coastal agroecosystem [2]. To better understand the adaptive mechanisms that enable different species to adapt to the unique environmental conditions of their habitat, physiological study on the photosynthetic process in mangroves is crucial. For the construction of more thorough and accurate models of the dynamics of the mangrove ecosystem, a better understanding of physiological processes is a requirement. For the construction of more thorough and accurate models of the dynamics of the mangrove ecosystem, a better understanding of physiological processes is a requirement [3].

Some research has been done on the impact of salinity on mangrove photosynthesis, primarily in relation to transpiration and stomatal conductance [4]. Similar ratios existed between the integrated photosynthetic rates at various salinities and the rates of transpiration during the day. The rate of photosynthesis was stimulated in Avicennia alba and A. marina at lower NaCl salinity and at higher concentrations, the rate was declined [5]. The rate of photosynthesis increased at lower concentration of NaCl in *Sesuvium portulacastrum* [6] and *Spartina townsendii* [7].

The rate of net photosynthesis greatly influences the rate of growth. The net photosynthesis of many plant species of saline habitat declined with increasing rhizosphere salinity [8,9]. The photosynthetic rates decreased with increasing salinity in *Avicennia marina* [10] and *Bruguiera gymnorrhiza* [11]. Joshi and Waghmode [12] reported that in the leaves of *Ceriops tagal* and *Lumnitzera racemosa*, phosphoenolpyruvate carboxylase (PEPcase) activity exceeded that of ribulose bisphosphate carboxylase (RuBPcase). They suggested that these two species followed C4 pathway.

There is a necessity to accumulate other ions to play a role in osmotic adjustment and in the same time, alleviate the Na^+ and Cl^- toxicity and consequently the continuity of biological process [13]. The osmotic adjustment required for maintenance of water movement into the plant was accomplished in halophytes by the accumulation of inorganic ions, generally K^+, Na^+ and Cl^- from the surrounding environment [14,15]. The leaf osmotic potentials of *Suaeda maritima* were lower in the higher salt concentration and were the lowest in the youngest leaves and stem of this species [16].

Based on the $14CO_2$ fixation pattern, photorespiratory and carboxylase enzymes, *Salvadora persica* was reported to follow C4 metabolism when grown in saline soil and behaved as a C3 species under non saline condition [17]. The most important anatomical feature that distinguishes C4 species from C3 species is the presence of 'kranz' anatomy in the leaves of C4 species and it is a prerequisite for C4 photosynthesis [18]. However, the 13C/14C carbon isotope ratios in leaves of a large number of mangrove species ranged from 24.6‰ to 32.2‰ indicating that all of them followed C3 photosynthesis. Salinity has also been implicated in promoting a shift from C3 to Crassulaecian Acid Metabolism (CAM) [19]. A study was conducted on the mangrove plant *Lumnitzera racemosa* to explore the changes in photosynthetic activity with increasing concentration of salinity.

MATERIALS AND METHODS

The present study was carried out in *Lumnitzera racemosa* Willd. a dicotyledonous mangrove (family: Combretaceae). 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. Later, they were transferred to the experimental yard roofed with transparent polythene sheet at a height of 3 m from the ground in order to protect the plants from rain. At a time, about 800 plants were maintained in the experimental yard. The plants had an approximate 12 h photoperiod and mean day temperature of 36°C and night temperature of 27°C.

Salinity induction

Sodium chloride (Laboratory AR grade assay 99.9% universal laboratories Pvt. Ltd., Mumbai) was used for the experiments. NaCl at the rate of 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM were given to 1 month old and fully established seedlings. Samplings were done on 60th 120th and 180th days after NaCl treatment.

Estimation of photosynthetic pigments

Estimation of chlorophyll a, b and total chlorophyll pigments were done by following standard method [20].

Net leaf photosynthesis

The net photosynthesis (CO2 uptake) was measured using a Li-Cor 6200 portable Infra Red Gas Analyser (Li-Cor Inc., USA).

Photosynthetic carboxylases

PEP carboxylase

PEP carboxylase was assayed by radiometric method. It was carried out according to Kanai and Edwards [21]. The reaction mixture in a volume of 1 ml contained 50 mM Tricine – KOH (pH 7.8), 100 mM $MgCl_2$, 5 mM DTT, 3 mM NaOH14 CO_3 (0.5 µ ci/µ mole),

1 mM PEP enzyme extract containing 5-10 µg equivalent chlorophyll. After 3 min. preincubation, the reaction was initiated by the addition of 1 mM PEP. Samples of 100 µl were taken after 2,5 and 10 min. incubation and the reaction was terminated by adding 100 µl of 20% trichloro acetic acid to each sample. The acid stable 14c radioactivity was determined using a Packard model 2425 liquid scintillation counter.

RuBP carboxylase enzyme assay was done by following Bowes and Ogren [22].

RESULTS

Photosynthetic pigments

Chlorophyll

Sodium chloride concentrations upto 500 mM, promoted the chlorophyll synthesis and at higher concentrations it was decreased in the leaves of *L. racemosa* and the data are shown in Table 1. The maximum chlorophyll synthesis was recorded at 500 mM sodium chloride on the 180th day samples and it was 2.96 mg/g fr. wt. Concentrations beyond 500 mM NaCl, had decreased the chlorophyll synthesis on all the sampling days.

Chlorophyll a content was found to increase with increasing concentrations upto the optimum level of 500 mM NaCl on all the 3 sampling days. Chlorophyll b also followed an increasing trend similar to that chlorophyll a. But, chlorophyll b content was always lesser in both the control and the experimental plants at all concentrations. The F values calculated for the difference between the sampling days and between the treatments were significant at 1% level.

Photosynthesis

Diurnal fluctuation of the organic acid in the leaf tissue of *L. racemosa* over 24 h period, after the different concentrations of sodium chloride treatment was studied on the 180th day and the results are given in Table 2. The results revealed that the NaCl did not produced any effect on the fluctuation of organic acid of the leaves between day and night hours. The small differences recorded in the acid content at various concentrations of salinity between different periods of day and night are possibly due to minor variations in the experimental procedure.

Along with the estimation of organic acid content of the leaves, the stomatal aperture measurements of the leaves were also studied. The stomatal aperture measurements did not show any variation between day and night time (data not given). The leaf stomata were opened throughout the day and completely closed during night hours.

Leaf net photosynthesis

The findings of the IRGA measurements of the effects of different NaCl concentrations on net leaf photosynthesis (CO2 uptake) are shown in Table 2. Up to 500 mM NaCl, net photosynthesis gradually increased; however, as concentrations rose above this point, CO2 uptake declined. On the 180th day, 500 mM NaCl was used to achieve the highest photosynthetic rate (17.5 mol/CO-2 S^{-1}), which was 82.29% higher than that of control plants.

Photosynthetic carboxylases

The results on the effect on the activity of photosynthetic carboxylases, PEPcase and RuBPcase in the leaves of salt treated plants of *L. racemosa* are showed in Table 3. Sodium chloride salinity stimulated considerable PEPcase activity with increasing salt concentration upto 500 mM. RuBPcase always showed less activity when compared with PEPcase. Besides, PEPcase: RuBPcase ratio increased with increasing salt concentration upto the optimum level and the ratio varied between 1 and 2 both in the salt treated and control plants. The F values calculated for difference between sampling days and between the treatments were significant at 1% level.

Table 1. Effect of NaCl on the Chlorophyll a, Chlorophyll b and Total Chlorophyll contents in the leaves of *Lumnitzera racemosa* (mg/g fresh weight).wt).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60			120			180		
	Chl-'a'	Chl-'b'	Total chl	Chl-'a'	Chl-'b'	Total chl	Chl-'a'	Chl-'b'	Total chl
Control	0.86	0.72	1.58	1.00	0.80	1.80	1.15	0.97	2.12
100	0.90 (+4.65)	0.78 (+8.33)	1.68 (+6.32)	1.08 (+8.00)	0.87 (+8.75)	1.95 (+8.33)	1.23 (+6.95)	1.05 (+8.24)	2.28 (+7.54)
200	0.97 (+12.79)	0.89 (+23.61)	1.86 (+17.72)	1.15 (+15.00)	0.95 (+18.75)	210 (+16.66)	1.32 (+14.78)	1.12 (+15.46)	2.44 (+15.09)
300	1.06 (+23.25)	0.96 (+33.33)	2.02 (+27.84)	1.24 (+24.00)	1.04 (+30.00)	2.28 (+26.66)	1.42 (+23.47)	1.18 (+21.64)	2.60 (+22.64)
400	1.15 (+33.72)	1.05 (+45.83)	2.20 (+39.24)	1.36 (+36.00)	1.12 (+40.00)	2.48 (+37.77)	1.53 (+33.04)	1.25 (+28.86)	2.78 (+31.13)
500	1.26 (+46.51)	1.12 (+55.55)	2.38 (+50.63)	1.48 (+48.00)	1.33 (+66.25)	2.81 (+56.11)	1.64 (+42.60)	1.32 (+36.08)	2.96 (+39.62)
600	1.12 (+30.23)	1.00 (+38.88)	2.12 (+34.17)	1.20 (+20.00)	1.02 (+27.50)	2.22 (+23.33)	1.50 (+30.43)	1.15 (+18.55)	2.65 (+25.00)
700	0.80 (-6.97)	0.70 (-2.77)	1.55 (-1.89)	0.90 (-10.00)	0.78 (-2.50)	1.68 (-6.66)	1.10 (-4.34)	0.95 (-2.06)	2.05 (-3.30)
800	0.70 (-18.60)	0.66 (-8.33)	1.36 (-13.92)	0.75 (-25.00)	0.72 (-10.00)	1.47 (-18.33)	1.08 (-6.08)	0.90 (-7.21)	1.98 (-6.60)
		Leaves :	F^1 = 76.1458* F^2 = 174.6362*		Leaves:	F^1 = 65.7483* F^2 = 91.0993*		Leaves :	F^1 = 110.4586* F^2 = 202.7508*

Table 2. Effect of NaCl on the net leaf photosynthesis, stomatal conductance, transpiration, leaf chamber temperature and intercellular CO_2 concentration of *Lumnitzera racemosa* (µmol/ CO2-2S-1).

Concentration of NaCl (mM)	Days after NaCl treatment														
	60	120	180	60	120	180	60	120	180	60	120	180	60	120	180
	Net photosynthesis			Stomatal conductance			Transpiration			Leaf chamber temperature			Intercellular CO_2 concentration		
Control	8.5	9.0	9.6	0.18	0.20	0.24	5.0	6.0	6.8	30.2	32.0	33.8	0315	0327	0332
100	9.4 (+10.58)	9.9 (10.00)	10.5 (+9.37)	0.20 (+11.11)	0.24 (+20.00)	0.30 (+25.00)	5.4 (+8.00)	6.7 (+11.66)	7.5 (+10.29)	30.8 (+1.98)	33.4 (+4.37)	34.6 (+2.36)	0321 (+1.90)	0340 (+3.97)	0365 (+9.93)
200	10.7 (+25.88)	11.0 (+22.22)	11.6 (+20.83)	0.23 (+27.77)	0.30 (+50.00)	0.37 (+54.16)	6.0 (+20.00)	7.3 (+21.66)	8.4 (+23.52)	31.7 (+4.96)	34.2 (+6.87)	35.7 (+5.62)	0335 (+6.34)	0355 (+8.56)	0387 (+16.56)
300	12.0 (+41.17)	12.7 (+41.11)	13.6 (+41.66)	0.27 (+50.00)	0.36 (+80.00)	0.44 (+83.33)	6.7 (+34.00)	8.0 (+33.33)	8.9 (+30.88)	32.8 (+8.60)	35.3 (+10.31)	36.9 (+9.17)	0356 (+13.01)	0374 (+14.37)	0399 (+20.18)
400	13.5 (+58.82)	14.0 (+55.55)	15.0 (+56.25)	0.30 (+66.66)	0.44 (+120.00)	0.53 (+120.83)	7.5 (+50.00)	8.9 (+48.33)	10.0 (+47.05)	33.8 (+11.92)	36.5 (+14.06)	38.0 (+12.42)	0380 (+20.63)	0396 (+21.10)	0420 (+26.50)
500	16.3 (+91.76)	16.8 (+86.66)	17.5 (+82.29)	0.36 (+100.00)	0.51 (+155.00)	0.65 (+170.83)	8.7 (+74.00)	9.6 (+60.00)	11.4 (+67.64)	35.0 (+15.89)	38.0 (+18.75)	39.3 (+16.27)	0395 (+25.39)	0421 (+28.74)	0443 (+33.43)
600	13.5 (+58.82)	13.0 (+44.44)	14.5 (+51.04)	0.25 (+38.88)	0.30 (+50.00)	0.40 (+66.66)	5.5 (+10.00)	6.5 (+8.33)	8.6 (+26.47)	31.0 (+2.64)	34.0 (+6.25)	36.0 (+6.50)	0350 (+11.11)	0363 (+11.00)	0390 (+17.46)
700	8.0 (-5.88)	8.6 (-4.44)	9.0 (-6.25)	0.17 (-5.55)	0.20 (+0.00)	0.24 (+0.00)	4.8 (-4.00)	5.6 (-6.66)	6.5 (-4.41)	28.3 (-6.29)	30.3 (-5.31)	32.8 (-2.95)	0308 (-2.22)	0325 (-0.61)	0330 (-0.60)
800	7.2 (-15.29)	8.0 (-11.11)	8.4 (-12.5)	0.15 (-16.66)	0.18 (-10.00)	0.20 (-16.66)	4.2 (-16.00)	5.0 (-16.66)	5.8 (-14.70)	26.5 (-12.25)	28.6 (-10.62)	30.5 (-9.76)	0300 (-4.76)	0314 (-3.97)	0324 (-2.40)
	Leaves :	F^1 = 517.0053*			F^1 = 21.3803*			F^1 = 83.5157*			F^1 = 269.7044*			F^1 = 87.4564*	
		F^2 = 60.4063*			F^2 = 25.8238*			F^2 = 127.3403*			F^2 = 467.0345*			F^2 = 66.1476*	

Table 3. Effect of NaCl on the photosynthetic carboxylases [PEPcase, RUBPcase and PEP:RUBPcase] of *L. racemosa* (µmol CO_2 fixed/mg protein/h)

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Phosphoenolpyruvate carboxylase			Rebulose bisphosphate carboxylase			PEP:RUBPcase		
Control	068	082	090	044	050	065	1.54	1.64	1.38
100	093 (36.76)	121 (47.56)	137 (52.22)	0.056 (27.27)	0.72 (44.00)	090 (38.46)	1.66 (7.79)	1.68 (2.43)	1.52 (10.14)
200	105 (54.41)	146 (78.04)	156 (73.33)	060 (36.36)	080 (60.00)	100 (53.86)	1.75 (13.62)	1.82 (10.97)	1.56 (13.04)
300	120 (76.47)	167 (103.65)	178 (97.77)	066 (50.00)	090 (80.00)	112 (72.30)	1.81 (17.35)	1.85 (12.80)	1.58 (14.49)
400	138 (102.94)	192 (134.14)	206 (128.88)	075 (70.45)	100 (100.00)	125 (92.30)	1.84 (19.48)	1.92 (17.07)	1.64 (18.84)
500	180 (164.70)	210 (156.09)	225 (150.00)	090 (104.54)	108 (116.00)	133 (104.61)	2.00 (29.87)	1.94 (18.29)	1.69 (22.46)
600	104 (52.94)	143 (74.39)	154 (71.11)	060 (36.36)	092 (84.00)	115 (76.92)	1.73 (12.33)	1.55 (-5.48)	1.33 (-3.62)
700	090 (32.35)	108 (31.70)	120 (33.33)	054 (22.72)	070 (40.00)	090 (38.46)	1.66 (7.79)	1.54 (-6.09)	1.33 (-3.62)
800	060 (-11.76)	075 (-8.53)	085 (-5.55)	040 (-15.00)	049 (-2.00)	065 (0.00)	1.50 (-2.59)	1.53 (-6.70)	1.32 (-4.34)
	Leaves :	F^1 = 75.0601*			F^1 = 35.2021*			F^1 = 21.2856*	
		F^2 = 60.8136*			F^2 = 100.9535*			F^2 = 54.0867*	

Enzymes - Amylases

The α-and β-amylase activity was studied in the seedlings of *L. racemosa* grown under various concentrations of sodium chloride and the results are given in Table 4 and 5. Both the amylases activity were increased upto 500 mM NaCl and at higher salinity concentrations the α-amylase and β-amylase activity were decreased at all the sampling days. The highest α-amylase activity (6.38 µg/min. mg/protein) was noticed in the leaf tissue at 500 mM NaCl on the 180th day samples. Compared with α-amylase activity, β-amylase activity was slightly higher in all plant tissues on all the sampling days. The F values calculated for difference between the sampling days and between the treatments were significant at 1% level.

DISCUSSION

Photosynthetic pigments

Up to 500 mM of sodium chloride salinity enhanced the synthesis of chlorophyll in *L. racemosa* leaves. Chlorophyll content decreased with increasing concentrations. A similar positive trend of NaCl salinity on the chlorophyll synthesis has been reported in several halophytes such as *Ceriops roxburghiana* [23], *Rhizophora apiculata* [24] and in *Kandelia candel* [25]. The increased chlorophyll synthesis can be correlated with increase in the photosynthetic activity.

Table 4. Effect of NaCl on α-amylase activity in the leaves, stem and root of *Lumnitzera racemosa* (µg/min. mg/protein).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	3.80	4.15	4.33	3.23	3.45	3.58	2.35	3.00	3.18
100	3.96 (+ 4.21)	4.68 (+ 12.77)	4.87 (+ 12.47)	3.35 (+ 3.71)	3.88 (+ 12.46)	4.03 (+ 12.56)	2.43 (+ 3.40)	3.14 (+ 4.66)	3.32 (+ 4.40)
200	4.15 (+ 9.21)	4.85 (+ 16.86)	5.18 (+ 19.63)	3.54 (+ 9.59)	4.10 (+ 18.84)	4.34 (+ 21.22)	2.56 (+ 8.93)	3.36 (+ 12.00)	3.55 (+ 11.63)
300	4.35 (+ 14.47)	5.27 (+ 26.98)	5.48 (+ 26.55)	3.73 (+ 15.47)	4.66 (+ 35.07)	4.89 (+ 36.59)	2.78 (+ 18.29)	3.53 (+ 17.66)	3.76 (+ 18.23)
400	4.58 (+ 20.52)	5.44 (+ 31.08)	5.76 (+ 33.02)	3.97 (+ 22.91)	4.98 (+ 44.34)	5.16 (+ 44.13)	2.96 (+ 25.95)	3.75 (+ 25.00)	3.96 (+ 24.52)
500	4.93 (+ 29.73)	6.00 (+ 44.57)	6.38 (+ 47.34)	4.21 (+ 30.34)	5.50 (+ 59.42)	5.66 (+ 58.10)	3.17 (+ 34.89)	3.90 (+ 30.00)	4.13 (+ 29.87)
600	4.45 (+ 17.10)	4.77 (+ 14.93)	4.93 (+ 13.85)	3.60 (+ 11.45)	4.60 (+ 33.33)	4.75 (+ 32.68)	2.88 (+ 22.55)	3.27 (+ 9.00)	3.61 (+ 13.52)
700	3.70 (- 2.63)	4.00 (- 3.61)	4.25 (- 1.84)	3.20 (- 0.92)	3.40 (- 1.44)	3.51 (- 1.95)	2.35 (- 0.00)	2.90 (- 3.33)	3.07 (- 3.45)
800	3.54 (- 6.84)	3.86 (- 6.98)	4.03 (- 6.92)	3.00 (- 7.12)	3.31 (- 4.05)	3.44 (- 3.91)	2.07 (- 11.91)	2.60 (- 13.33)	2.84 (- 10.69)
	Leaves :	F^1 = 32.3753*		Stem :	F^1 = 22.8114*		Root :	F^1 = 79.6502*	
		F^2 = 46.8977*			F^2 = 32.1625*			F^2 = 318.392*	

Table 5. Effect of NaCl on β-amylase activity in the leaves, stem and root of *Lumnitzera racemosa* (µg/min. mg protein).

Concentration of NaCl (mM)	Days after NaCl treatment								
	60	120	180	60	120	180	60	120	180
	Leaves			Stem			Root		
Control	4.20	4.37	4.51	3.34	3.55	3.67	2.70	3.10	3.30
100	4.28 (+ 1.90)	4.80 (+ 9.83)	5.20 (+ 15.29)	3.48 (+ 4.19)	3.95 (+ 11.26)	4.23 (+ 15.25)	2.77 (+ 2.59)	3.21 (+ 3.54)	3.45 (+ 4.54)
200	4.40 (+ 4.76)	4.90 (+ 12.12)	5.32 (+ 17.96)	3.70 (+ 10.77)	4.37 (+ 23.09)	4.65 (+ 26.70)	2.85 (+ 5.55)	3.45 (+ 11.29)	3.70 (+ 12.12)
300	4.57 (+ 8.80)	5.34 (+ 22.19)	5.77 (+ 27.93)	3.91 (+ 17.06)	4.85 (+ 36.61)	5.14 (+ 40.05)	2.97 (+ 10.00)	3.66 (+ 18.06)	3.93 (+ 19.09)
400	4.79 (+ 14.04)	5.60 (+ 28.14)	5.89 (+ 30.59)	4.16 (+ 24.55)	5.13 (+ 44.50)	5.67 (+ 54.49)	3.13 (+ 15.92)	3.94 (+ 27.09)	4.18 (+ 26.66)
500	5.08 (+ 20.95)	6.27 (+ 43.47)	6.60 (+ 46.34)	4.40 (+ 31.73)	5.78 (+ 62.81)	6.00 (+ 63.48)	3.28 (+ 21.48)	4.14 (+ 33.54)	4.41 (+ 33.63)
600	4.50 (+ 7.14)	5.13 (+ 17.39)	5.70 (+ 26.38)	3.95 (+ 18.26)	5.05 (+ 42.25)	5.30 (+ 44.41)	2.95 (+ 9.25)	3.53 (+ 13.87)	4.00 (+ 21.21)
700	4.18 (- 0.47)	4.22 (- 3.43)	4.45 (- 1.33)	3.34 (- 0.00)	3.50 (- 1.40)	3.62 (- 1.36)	2.60 (- 3.70)	3.06 (- 1.29)	3.28 (- 0.60)
800	4.00 (- 4.76)	4.25 (- 2.74)	4.42 (- 1.99)	3.15 (- 5.68)	3.41 (- 3.94)	3.54 (- 3.54)	2.46 (- 8.88)	2.80 (- 9.67)	3.05 (- 7.57)
	Leaves :	F^1 = 17.9479*		Stem :	F^1 = 20.1963*		Root :	F^1 = 31.2884*	
		F^2 = 30.4547*			F^2 = 26.3326*			F^2 = 118.7443*	

Numerous halophytes, as well as agricultural plants like Plantago, have reported decreases in chlorophyll concentration when exposed to salinity. [26], *Phaseolus vulgaris* [27] and in *Brassica nigra* [28] and in a few other halophytes [29,30]. A reduction in chlorophyll content might be due to inhibition of biosynthesis or degradation of chlorophyll and their precursors [31], inhibition of the cap genes, which codes for chlorophyll proteins [32] or accumulation of chlorophyllides a and b [33]. The contents of pigments of chlorophyll a, chlorophyll b and total chlorophylls, which changed uniformly, showed a 'U' shaped response. The chlorophyll contents were changed due to water logging. There was increase of pigment contents of *Kandelia candel* seedlings under long inundation [25,34].

Net leaf photosynthesis

Changes in the net leaf photosynthesis, stomatal conductance, transpiration, leaf chamber temperature and intercellular CO2 concentration were studied in the leaves of *L. racemosa* at different concentrations of sodium chloride and these activities were maximum at the optimum level of 500 mM. The increase in the photosynthetic rate on the 180th day samples was with increase in the age of the seedlings. The changes in the net photosynthesis were parallel with changes in the chlorophyll synthesis of the leaves at various concentrations of NaCl and the changes also could be correlated with starch synthesis. On the other hand, the reduction of net photosynthesis, transpiration and stomatal conductance at higher salinity level has been reported in a few halophytes such as *Scaevloa serica* [35], *Rhizophora apiculata* [24], and Rhizophora mangle [36]. Photosynthetic capacity was not affected by high salinity in *Suaeda salsa* [37,38]. High salinities retarded CO_2 fixation in mangroves and enhanced the reduction of molecular oxygen to generate high toxic superoxide radicals [39, 40].

Photosynthesis

All the dominant species of mangroves were C3 plants exhibiting low rates of gas exchange [41]. Salinity and irradiance are the two key

environmental factors regulating photosynthesis in halophytes. Based on the carboxylases activities and several other biochemical evidences, many mangrove species have been suggested to follow C4 photosynthesis. Findings of the present study in the mangrove species *Lumnitzera racemosa* agree with the earlier reports for the operation of C4 metabolism in mangroves such as *Avicennia marina*, *A. officinalis* and *Rhizophora mucronata* [42], *Salsola kali, Suaeda aegyptiaca* and *S. monoica* [43]. Aspartate dominates as the initial products of light $14CO_2$ assimilation. PEPcase is a key enzyme of C4 plants and is essential for synthesis of aspartate pyruvate pidikinase, an enzyme which plays a confirmatory role of C4 pathway which is also seen from several mangrove species [44].

The crassulacean acid metabolism (CAM) species are characteristic of night time stomatal opening and diurnal fluctuation of organic acid and CO_2 fixation in the dark leading to the formation of free malic acid [45]. The possibility of CAM operation in *L. racemosa* treated with various concentrations of NaCl was investigated by following the diurnal fluctuation and stomatal aperture movements in the leaves over 24 hours. The stomata of this species were predominantly opened during day time and completely closed during night, at all concentrations. The organic acid content of the leaves remained unchanged over 24 hours period.

In view of the above facts, CAM pathway was not likely to operate in *L. racemosa*. Presence of 'Kranz' syndrome and higher phosphoenol pyruvate carboxylase activity than Rebulose bisphosphate carboxylase activity in this species should be considered in common with C4 pathway and NaCl salinity had no effect on the carbon metabolism [46].

Enzymes

Enzymes which occupy a key position in plant metabolism, more so, in the plants subjected to a water stress or a salinity stress. Greenway and Osmond [47] reported that the enzyme response to salinity in certain halophytes was similar to glycophytes. While the salinity stress inhibited the starch metabolism in glycophytes, halophytes were only slightly affected.

Amylases

Sodium chloride enhanced both α- and β-amylase activity with increasing salinity upto the optimum level in *L. racemosa*. At higher concentrations, amylases activities were declined. Amylases are the starch degrading enzymes universally distributed among higher plants [48]. The activity of these enzymes was reported in many plant species under salinity stress.

Sheoran [49] pointed out that Na+ and Cl- were the most effective ions on the amylase activity which delayed and inhibited the synthesis as well as activation of amylases in Mungbean cotyledons. The increase in amylase activity was positively correlated with salt tolerance of cultivars in Sugar beet exposed to sodium chloride salinity [50].

CONCLUSION

The present study was carried out in *Lumnitzera racemosa* Willd. to estimate the effect of NaCl at the rate of 0 (control), 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 mM. Samplings were done on 60th 120th and 180th days after NaCl treatment. Chlorophyll, Net leaf photosynthesis, photosynthetic carboxylases and amylases were estimated from the samples. Salinity from sodium chloride encouraged the synthesis of carotenoid and chlorophyll in *L. racemosa* to the ideal concentration. When NaCl salinity is increased, photosynthetic properties such net leaf photosynthesis, stomatal conductance, transpiration, leaf chamber temperature, and intercellular CO2 concentration decrease. It should be taken into consideration that *L. racemosa* shares the C4 pathway since it exhibits the "kranz" condition. Sodium chloride had no impact on how carbon was metabolized. These results will be useful in future researches with saline water irrigation in crop plants.

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