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EFFECTS OF SALINITY ON THE GROWTH, PHOTOSYNTHESIS AND MINERAL CONSTITUENTS OF THE MANGROVE *RHIZOPHORA APICULATA* L. SEEDLINGS

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Abstract

The effects of salinity on growth, chlorophyll content, photosynthetic rates, as measured by leaf stomatal conductance and leaf chlorophyll fluorescence induction, and ion accumulation in the mangrove plant *Rhizophora apiculata* were determined. The following questions were addressed: (1) What effect does salinity have on growth responses at different ages? (2) Is *R. apiculata an* ion accumulator? (3) Does *R. apiculata* accumulate chlorophyll, net photosynthesis and chlorophyll fluorescence in response to salinity? *Rhizophora apiculata* plants were grown in pots at 0, 25, 50, 75 and 100 % in sand culture in a plant growth chamber and plants were harvested after 30 and 90 d. Plant total fresh and dry weight and moisture content was significantly inhibited at 75 per cent seawater. Seawater salinity stimulated the chlorophyll contents and they were increased upto 75 per cent seawater. The net photosynthesis increased with increasing salinity upto the optimal level and the CO_2 uptake rate was identical in this species at various salt concentrations. Even at extreme salinity, the CO_2 uptake was comparable to that non-saline control plants and CO_2 uptake could be correlated with the chlorophyll content. The photochemical activities such as PSI and PSII of the isolated cells increased upto the optimal salinity. The result of the chlorophyll fluorescence kinetics provided with additional proof to the finding of CO_2 exchange rate and photochemical activities. The Na^+ and Cl^- content in both shoots and roots increased with increases in salinity. Increased treatment levels of NaCl induced decreases in Ca^+, P, K^+, Mg^+ and N in plants.

Key Words: Chlorophyll fluorescence; Photosynthesis; *Rhizophora*; Stomatal conductance; Salinity.

Introduction

Various species of mangroves form the dominant woody vegetation in the intertidal zones of tropical and subtropical coastlines around the world. The mangrove habitat exhibits many unique physical features, an important one of which is a salinity gradient from freshwater to seawater [25]. This salinity gradient has long been recognized as a potential stressor and an important factor that regulates physiological processes such as growth, height, survival, and zonation patterns in mangroves [22]. Mangroves may be found occurring naturally along a gradient of salinity, from riverine forests with salinities close to or at zero parts per thousand (ppt), to fringe forests with typical salinities around 35 ppt, and even in hypersaline areas where the salinity may reach 70 ppt [17]. Although mangroves are found growing over a wide range of salinities, some species have been found to grow ideally in low salinity of approximately two ppt [21] while other species tolerate much higher salinities before exhibiting signs of stress [31].

Photosynthetic rates are good indicators of physiological stress levels in all plants, including mangroves [20,28). Mangroves growing in areas of high salinity showed increasing stress as measured by decreased carbon dioxide assimilation, stomatal conductance, and growth rates [9]. In another study, Teas recalculated data collected by [5] and found that evapotranspiration, measured by stomatal conductance, by red mangrove (*R. mangle*) seedlings ceased at salinities above 65 ppt [27]. Changes in photosynthetic rate and stomatal conductance with changes in salinity and humidity were also measured by [3]. They found

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these two measures of carbon dioxide assimilation decreased with increasing salinity.

These studies all indicate the importance of high salinity stress on reduced photosynthesis and growth in mangrove plants, particularly seedlings.

Photosynthetic gas exchange through the leaf stomata can be determined using stomatal resistance to water vapor loss and is a sensitive indicator of the physiological state of the plant. As a plant becomes stressed the stomatal resistance often increases, and water vapor conductance decreases [3]. Another useful and rapid measure of photosynthesis is the induction of chlorophyll fluorescence (Kautsky effect), a sensitive indicator of photosynthetic energy conversion that occurs during the light reaction [29]. Chlorophyll fluorescence can be modified by any factor affecting the light reaction pathway of photosynthesis, including many environmental stressors [20].

In this study the physiological state of the photosynthetic apparatus was determined by measuring growth, chlorophyll, photosynthesis, stomatal conductance, the chlorophyll fluorescence yield and mineral ions contents in seedlings of the red mangrove, *R. apiculata* subject to five different salinity levels: 0, 25, 50, 75, and 100 % seawater. The null hypothesis (H0) was that both stomatal conductance and the chlorophyll fluorescence yield are not statistically different across all salinity levels in these sensitive early life-stages of the red mangrove. The present study addresses the following questions: (1) what effect does salinity have on growth responses at different ages? (2) is *R. apiculata* an ion accumulator? (3) Does *R. apiculata* accumulate chlorophyll, net photosynthesis and chlorophyll fluorescence in response to salinity?

Materials and Methods

Study Area

The study area, Pichavaram mangrove wetland (lat 11°22N to 11°30N and long 79°45E to 79°52E), is located in the northernmost end of the Cauvery delta (Fig.1).

The total area of this mangrove wetland is about 1470 ha, consisting of about 50 small islands which are colonized by mangrove vegetation. The climate is sub-humid with very warm summer (< 30°C). The annual average rainfall (70 years) is 1310 mm and annual average number of rainy days is 56. Most of the rainfall occurs during the northeast monsoon season (October to December) and nearly 70% of the rainfall occurs between November and December. The tide is micro and diurnal, and amplitude during the spring and the neap tide is about 42 and 20 cm, respectively. Recent survey indicates the presence of a total number of 13 mangrove species in the Pichavaram mangrove wetlands [19, 30].

The community-structure studies show that *Avicennia marina* (Forsk.) Vierh is monospecifically dominant. Other species are represented by limited number of individuals. Regarding distribution pattern of the flora, two distinct zones, namely *Rhizophora* zone and *Avicennia* zone are identified. The *Rhizophora* zone is found along the fringes of the tidal creeks and channels and is about 5 to 12 m in breadth. Distribution of 11 species is restricted to this narrow zone. *Avicennia* zone starts just behind the *Rhizophora* zone and *A. marina* forms pure stands in this zone3. According to [6], a total quantity of 245 tons of fish, prawn and crab is harvested annually from the Pichavaram mangrove wetland, which gives livelihood security to about 3000 traditional poor fishers living in 14 hamlets.

Fig.1. Study Area Location Map

Plant culture

One month old healthy seedlings of *Rhizophora apiculata* of uniform size were uprooted from the mangrove soil without damaging the root system. This seedling were washed thoroughly with tap water and brought to the botanical garden of the Annamalai University. Polythene sleeves (7"X5") were filled with

homogenous mixture of garden soil, comprising of red earth, sand and farm-yard manure in the ration of 1:2:1. Healthy seedlings were selected and planted in the polythene sleeves. These were irrigated with tap water and allowed to establish well. The seedlings established well within a month and then were transferred to the experimental site roofed with transparent polythene sheet for protection from rainwater. The plants had an approximate 12 h photoperiod, a mean day temperature of 36 °C and night temperature 27 °C.

Salinity treatment

Seedling (Forty five days old healthy seedlings) were selected and kept in 5 plots, each consisting of 100 plants for seawater treatments. Different concentrations of seawater solution were prepared in distilled water. The treatment constituted 0 (control), 25, 50, 75 and 100 per cent of seawater. The control plants were maintained without the addition of seawater. After the completion of seawater treatment, the seedlings were irrigated with tap water. Samples were collected periodically at bimonthly intervals for different analyses.

Fresh and dry matter production

The test seedlings were harvested and separated into roots and shoots. Each part was then weighed for the determination of fresh matter mass. They were then dried at 80°C for 48 hours for the measurement of dry matter mass.

Measurement of chlorophyll contents

All leaves were excised from the test seedlings for the measurement of chlorophyll content. Chlorophyll contents were determined according to [7]. Chlorophyll was extracted by soaking excised intact leaves (1 g) into 5 ml of dimethyl sulfoxide (DMSO) at 30°C for 1 day in darkness. Absorbance at 648 and 664 nm was recorded using a spectrophotometer (DU640, Beckman Instruments Inc., Fullerton, CA, USA). The obtained absorbance values were used to calculate the chlorophyll contents using equations as described below;

Chlorophylla=12.25A664nm − 2.79A648nm

Chlorophyllb=21.50A648nm − 5.10A664nm

Measurement of chlorophyll fluorescence

Chlorophyll fluorescence was determined with field portable, pulse amplitude, modulated fluorometer (PAM- 2100, Walz, Effeltrich, Germany). Fluorometer operation and data processing were conducted with a Hewlett Packard palmtop computer (HP 200LX). All measurements were taken on the lamina, midway between the base and tip of mature leaves. Quantum yield of photosystem II (PSII) electron transport (F/Fm') was calculated as (Fm' – F)/ Fm' [15] where F is the light-adapted fluorescence when a saturating light pulse of 7500 mmol m^{-2} s^{-1} PPFD for 700 ms duration is superimposed on the prevailing environmental irradiance level [29]. Electron transport rate (ETR) through PSII was calculated as 0.5 x 0.84 x PPFD x F/Fm' assuming that 84% of incidental light is absorbed by leaves and that photons are equally distributed between PSII and PSI [29]. Measurements of chlorophyll fluorescence were taken under ambient conditions at saturating light on the same day and on similar leaves on which gas exchange measurements were made. On each of six days, five representative trees were selected at each site and six measurements taken per tree. Different trees were randomly selected on each of the six measurement days. The intrinsic efficiency of light energy conversion of PSII (Fv/Fm) was measured after 30-min dark adaptation with a dark leaf clip (Walz, Effeltrich, Germany). For Fv/Fm, five measurements were taken on each tree over the six measurement days as described for the other fluorescence measurements.

Measurement of mineral analyses

Leaves were collected for ion analyses twice in September 2006, twice in December 2006 and once in June 2007 during two spring and three neap tides. Fifty fully expanded mature leaves were removed from each of five representative trees at each site, rinsed for10 s in distilled water to remove surface salt and dried at 70 °C to constant mass. Samples were milled through a 1-mm screen and stored in plastic vials. Subsamples were dryashed at 450 °C and dissolved in 1 M HCl. Concentrations of Na^+, Cl^-, K^+, Ca^{2+} and Mg^{2+} were determined by atomic absorption (Varian Spectra AA-10, Mulgrave, Australia), P by the molybdenum blue procedure, and N by the automated Dumas dry combustion method, using a LECO CNS 2000, Leco Corporation, Michigan, USA [23]. All chemical analyses were verified by the use of tissue standards.

Results

A two-way ANOVA showed a significant individual effect of salinity and time of harvest and their interaction in affecting the leaf fresh weight and dry weight of *R. apiculata* (Fig.2). Salinity and the interaction of salinity and time of harvest did not significantly affect leaf fresh and dry weight (Fig.2). Total fresh and dry weight accumulation of plants was not inhibited at low salinities, but fresh and dry weight production was significantly inhibited at 75 per cent of seawater. Moisture content increased upto optimal level of 75% seawater (Fig.2). Leaf fresh and dry weight did not show a significant effect

of salinity in any of the harvests, whereas leaf fresh and dry weight progressively declined with an increase in salinity. Seawater salinity stimulated the chlorophyll synthesis in the leaves of *R. apiculata* (Fig. 3). The total chlorophyll increased with increasing salinity upto 75% and this was 43.16 % higher than that of control on 90th day after saline treatment. At higher chlorophyll 'a' and chlorophyll 'b' increased at the optimum seawater salinity of 75%. Chlorophyll 'a' synthesis was always higher than the chlorophyll 'b' at all concentrations and both the sampling days. The seawater salinity stimulated photosynthetic CO_2 uptake upto the optimum level of 75 per cent and this was 52.3 per cent higher than that of control. Concentration beyond 75 per cent, reduced the photosynthetic rate and stomatal conductance (Fig. 4).

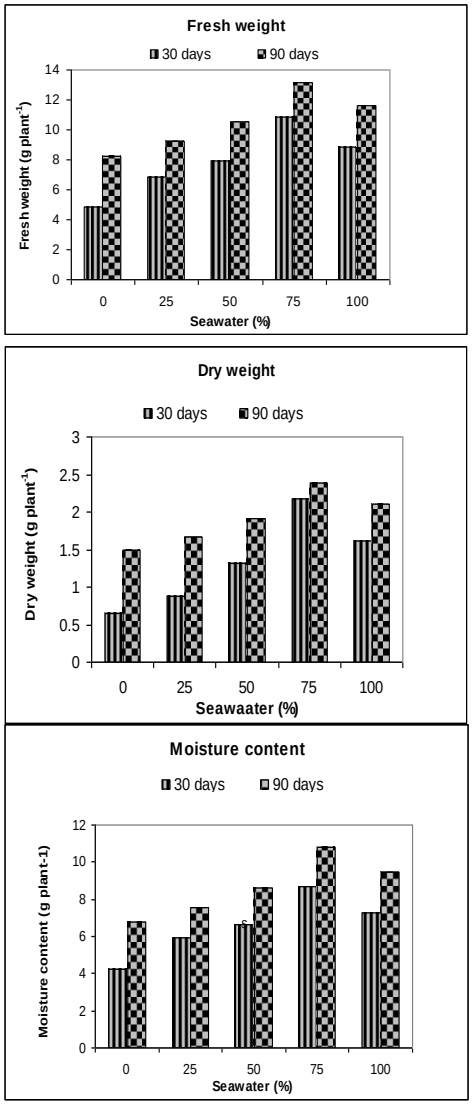

Fig.2. Effect of seawater on fresh weight, dry weight and moisture content of leaf of Rhizophora apiculata at 30 and 90 days of treatment

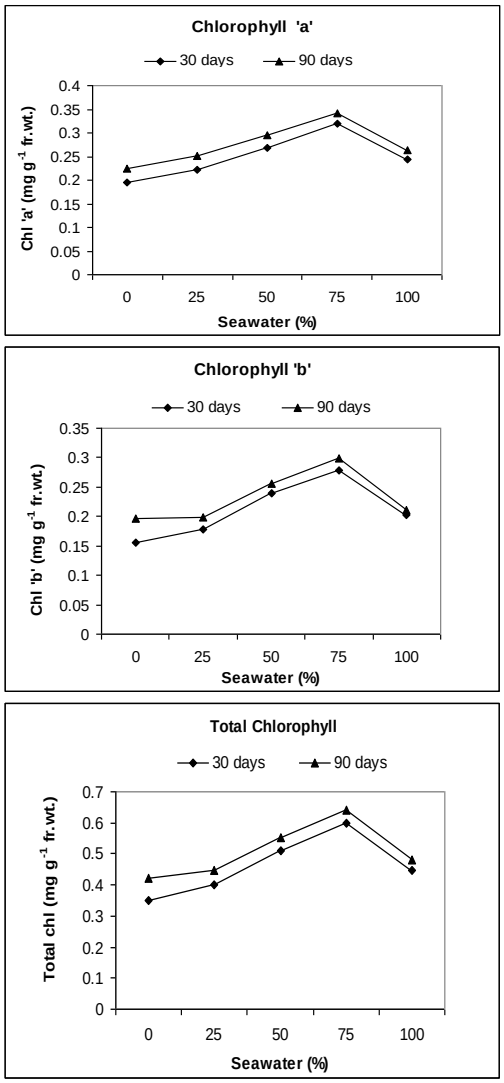

Fig.3. Effect of seawater on chlorophyll 'a ' , chlorophyll ' b' and total chlorophyll in the leaves of Rhizophora apiculata at 30 and 90 days of treatment

The polarographic measurement of PSI activity by the isolated cells from the leaf of control and saline treated plants were made on the 30th and 90th after saline treatment (Fig. 5). The rate of PSI activity (O_2 uptake) increased with increasing seawater salinity upto 75% and thereafter it considerably decreased. There was 42.60% increase in PSI activity on the 30th day and 115.38% increase on the 90th day when compared to that of control. At extreme salinity of 75 per cent, there was only 14.78% reduction in PSI activity on the 30th day. The PSI activity decreased with age of plant and on 90th day, the activity was considerably less at all concentration when compared to the values of 30th day samples. The spectrophotometer measurement of PSII activity by the isolated cells is given in Fig. 5.

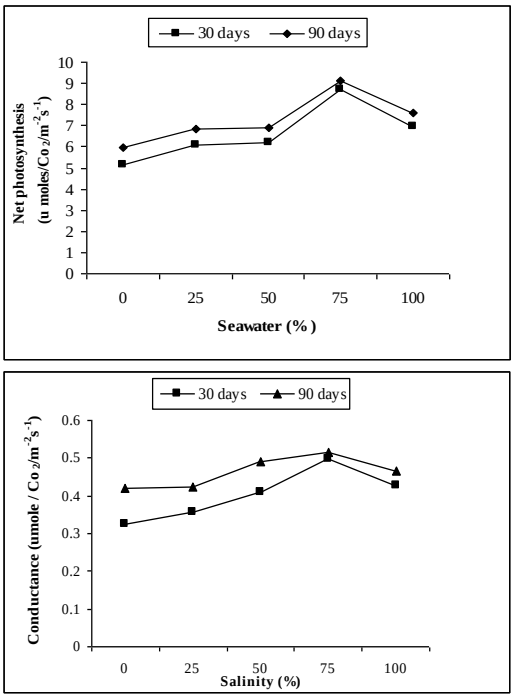

Fig. 4. Effect of seawater on net photosynthesis and stomatal conductance in the leaves of Rhizophora apiculata at 30 and 90 days of treatment

The results showed similar trends to that of PSI activity. The maximum activity was obtained at 75 per cent seawater on the 30th day and this was 156.14 % higher than that of control. Even at 100% seawater, the PSII activity was equal to that of control plants. However, between the two sampling days, 90th day plants showed lesser PSII activity at all concentration than the 30th day plants. The effect of seawater at various concentrations on the photosynthetic efficiency as studied by the following the chlorophyll fluorescence induction kinetics and the results are presented in Fig. 5. The fast and slow chlorophyll fluorescence's transients were recorded using intact leaf obtained from control and saline treated plants on 30th and 90th days of samplings. The leaf of control plants showed a slow O-P rise when compared to saline treated samples. No significant changes were found in F_O levels at different concentrations of seawater. The F_V/F_M ration increased upto 75% seawater and this was 44.44% higher when compared to that of control on 30th day. At higher concentrations the value declined gradually (Fig. 5).

The accumulation of sodium and chloride ions increased upto the extreme level of 75 % seawater. Ca^+, P, K and Mg^+ contents increased upto the optimum concentrations and thereafter declined gradually. Total Nitrogen content also increased with increasing salinity upto the optimal level and declined at higher salinity (Fig. 6).

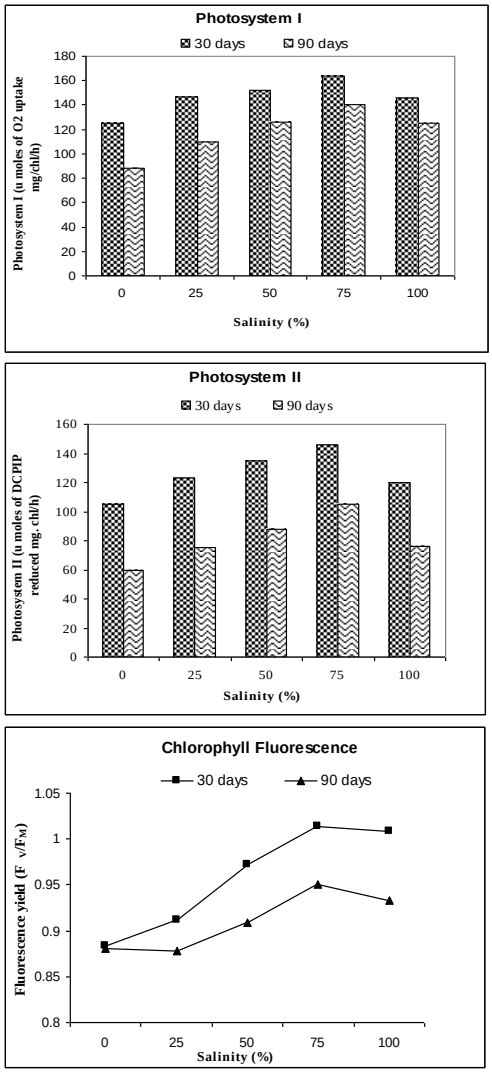

Fig. 5. Effect of seawater on photosystem I and photosystem II in the isolated cells and fluorescence induction kinetics of Rhizophora apiculata at 30 and 90 days of treatment

Discussion

Growth of many mangroves species is maximal/optimal under relatively low salinities [14]. Generally mangroves are facultative halophytes and may survive and grow well in freshwater conditions. However, there are reports indicating the importance of salt for some mangroves, as well as evidence that different species exhibit different tolerances and salinity optima [26].

The *Avicenniacaeae* appear to be more tolerant to salinity stress than the *Rhizophoraceae* [17]. For instance, *Avicennia marina* trees seem to grow well up to 75 % seawater, with a hypothesized growth inhibition at higher salinities due to high sodium chloride (NaCl) concentrations in the tissues [10]. However, seedlings of *A.marina* exhibited various growth responses under

different salinity regimes, with highest growth recorded at 50% seawater (17 ppt). Lowest overall growth was found in seedlings of *A. marina* raised under 0 ppt salinity, which was even lower than growth of seedlings raised in 100% seawater (35 ppt) [11, 16]. These studies suggest that even within a species, salt tolerance changes depending on life stage, with seedlings being potentially more sensitive to salt stress than mature trees.

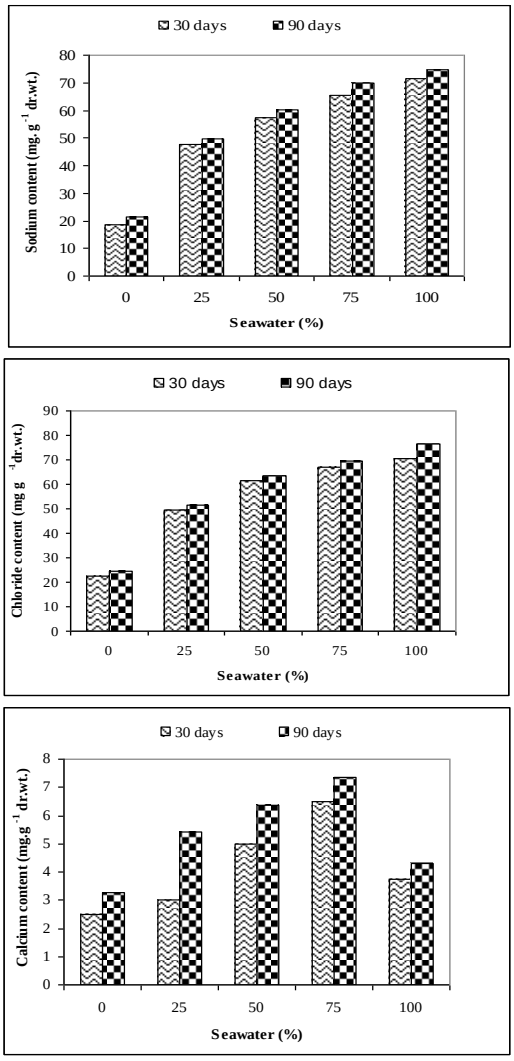

Fig. 6. Effect of seawater (0, 25, 50,75 and 100 %) on the leaf and mineral nutrient in sodium, chloride and calcium content in Rhizophora apiculata at 60th and 90th days after treatment

In comparison, salinity of 15 ppt was found to be optimal for growth of seedlings of *Rhizophora apiculata* [18]. *Rhizophora stylosa* was found to exhibit poor growth at salinities greater than 25-50% seawater. Water stress may be the main effect of high salinity on growth of the *Rhizophoraceae* [10]. However, *R. stylosa* appears to be less tolerant of high salinity than *R. mangle*. The red mangrove was shown to be tolerant of salinities up to 35 ppt, from the initial propagule stage to the early seedling stage, with no observed adverse effects on growth [31]. A fluctuating salinity regime on the other hand had a significant negative effect on photosynthesis (as measured by leaf stomatal conductance) and plant growth rates in *R. mangle* relative to constant salinities with the same mean [22]. It is apparent that different species of mangroves exhibit different tolerances to salinity stress and the ability to cope with salinity stress may change over the course of development. One of the major problems associated with the use of growth as a parameter to measure salinity stress is the necessity for long-term studies to achieve results. The use of non-invasive, *in-vivo* techniques for monitoring stress by looking at physiological parameters allows a more rapid quantification of short-term acute effects, as well as long-term chronic effects of salinity stress in mangroves.

It has been observed that increasing salinity is often accompanied by a decrease in turgor of the leaves [4], a factor that was also noted in this study in plants in the 45 and 60 ppt treatments. High salinities associated with intertidal mangrove habitats impose two potential restrictions on the photosynthetic rate of mangrove leaves: high leaf water deficits (i.e., a loss of turgor) and low stomatal conductance rates [5]. Leaf stomatal conductance rates in mangroves have been found to be lower than for nonhalophytic C_3 plants [17]. [5] proposed that low stomatal conductance is a requisite for a low ratio of transpiration to C fixation (i.e., high water use efficiency), which may be required for the maintenance of a physiologically acceptable salt/carbon balance within the leaves [1]. Rates of photosynthetic CO_2 fixation were found to decline with increasing salinity and this was attributed to stomatal limitations on CO_2 uptake [3, 5]. The use of chlorophyll fluorescence is a more recent technological advance and allows rapid assessments of light reaction kinetics associated with PSII [29]. These kinetics can be related to CO_2 uptake and O_2 production [29], therefore, this technique provides rapid assessment of plant photosynthesis. All Fv/Fm values that were observed in our study indicated that leaves were photosynthetically active [8]. Ratios near 0.83 are indicative of healthy photosystem function, whereas ratios less than 0.75 were associated with unhealthy trees [12].

In other studies using chlorophyll fluorescence, the quantum yield of PSII photochemistry in the dark-adapted state (Fv/Fm) was significantly higher in *Lumnitzera racemosa* seedlings grown in both 7.5 and 15 ppt compared to those at 0 and 30 ppt salinities [13]. The non-photochemical fluorescence quench-ing (qN) of

seedlings grown in 30 ppt salinity increased indicating that the reduction in Fv/Fm was due to increased heat dissipation, whereas the photochemical quenching (qP) was lower at 0 ppt reflecting the higher capacity of P680 reaction centers [13]. These results help explain the better growth and physiological performance of seedlings of *L. racemosa* when grown at intermediate salinities. Other species, *Avicennia marina* and *Bruguiera gymnorrhiza*, were found to have higher Fv/Fm and electron transport rate (ETR) at a high salinity site of 35 ppt than at 12 ppt, the low salinity site [24]. Photochemical (qP) and nonphotochemical quenching (qN) were corres-pondingly lower in plants at the high salinity site. A possible mechanism for these observed reductions in Fv/Fm is a salinity-induced potassium deficiency causing loss of photosystem II (P680) function through depletion of the atrazine-binding polypeptide [2].

Conclusion

The results of this study are in accordance with these previously published findings, in that photosynthetic gas exchange rates (measured by stomatal conductance), as well as photosynthetic light reaction performance (measured by chlorophyll fluorescence) decreased as salinity stress increased. The use of leaf stomatal conductance and chlorophyll fluorescence as a measure of photosynthesis allowed a rapid and reliable quantification of a known stressor, salinity, in seedlings of *R. apiculata*. It is suggested that these techniques can be applied to rapidly assess the health of mangrove plants in forestry, nursery, and restoration activities.

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