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On the Physiological Responses of *Avicennia marina* (Forsk.) Vierh. From Sydney, Australia in Different Salinity Conditions

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Abstract: A comparative study was performed on *Avicennia marina* from two sites of New South Wales prevailed by different salinity conditions. In Kurnell, the average salinity was 17 PPT, whereas by the river Parramatta soil salinity was approximately 3 PPT. Assimilation rate was higher in saline condition due to salt induced increment in mesophyll and total chlorophyll, whereas, considerable restriction was imposed on transpiration, vapour pressure deficit and stomatal conductance in spite of higher stomatal frequency in sea-side plants. The significant positive relation between photosynthesis and water efflux in non-saline condition contradicted with the clustering of carbon assimilation within a low range of transpiration and stomatal conductance in enhanced salinity. Despite regulated water efflux in the saline habitat, leaf temperature did not rise as much as to cause photoinhibition, thus remained lower than that in the fresh water condition. However, in both the habitats, leaf temperature seemed to increase under controlled transpiration rate. Except the positive correlations between photosynthesis and water efflux, the other parameters viz., leaf temperature and vpd with assimilation, transpiration and leaf temperature with vpd followed inverse trends with their related variables. In elevated salinity, the relation of photosynthesis was insignificant with water efflux, stomatal behaviour, vpd and leaf temperature, thus pointed to the beneficial interference of saline environment on carbon assimilation of *A. marina*.

Key words: *Avicennia marina,* leaf temperature, photosynthesis, transpiration, salinity, stomatal conductance

INTRODUCTION

The mangrove ecosystems are fragile and dynamic, dependent on both biotic and abiotic environmental factors. Mangroves are not a single genetic group but represent genetic adaptation of a large variety of plant communities to a particular environment. Mangrove forests are best developed on tropical shorelines where there are large areas available between high and low tide points and are often associated with the formation of deltas at the mouth of a river system. The soil structure and soil salinity are the main agents that control the distribution of mangroves. Mangrove soils are quite different from normal terrestrial habitat. They are poorly drained, lacking in oxygen and rich in organic matter.

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True mangroves include about 54 species in 20 genera belonging to 16 families (Das *et al*., 2002). These are mostly tree or woody shrubs flourished in the estuarine zone along the coastline of the tropical world. The primary characteristic feature of this community is their tolerance to NaCl up to and above the salt concentration of seawater (500 mM) (Parida *et al*., 2004). On the basis of their salt management strategy, they are either secretors or non-secretors (Sugihara *et al*., 2000). The secretors have salt glands or glandular hairs on the leaf surface for excretion of excess salt, while the rest ones do not. *Avicennia marina*, the gray mangrove belonging to the family Avicenniaceae is considered as a secretor due to the presence of non-glandular hairs on both its abaxial and adaxial leaf surfaces and salt glands on the abaxial leaf surface (Das, 1999).

Owing to high salt concentration in the substrate, mangroves are always confronted with the problem of maintaining turgor pressure and protecting their metabolism from high NaCl concentration (Greenway and Munns, 1980). Restriction in water efflux is an obvious adaptation to cope with the physiologically dry substrate imposed by high salinity. Mencuccini and Grace (1995) reported variation in stomatal conductance within a species in response to site differences in vapour pressure deficit (vpd) and soil water status. Leaf anatomy is closely associated with assimilation rate through its effects on light and CO_2 acquisition (Nandy *et al*., 2005). The proportion and arrangement of mesophyll parenchyma and the packaging of chlorophylls determine the penetration and absorbance of light within the leaf (Sefton *et al*., 2002). Salt induced increase in leaf succulence was observed in *A. germinans* (Suarez and Sobrado, 2000), while reduction in mesophyll and stomatal conductance was reported in *Bruguiera parviflora* (Parida *et al*., 2004) that, in turn, imposed restriction on CO_2 assimilation rate. Mangrove photosynthesis indeed is a function of some micromorphological parameters (e.g., frequency and size of stomata, relative thickness of mesophyll parenchyma, abundance of intercellular space within mesophyll tissue and has significant correlations with them (Nandy *et al*., 2005)). In *Avicennia marina*, leaves accumulate salt in two subsequent phases (Cram *et al*., 2002); in the first phase rapid increase in leaf salt concentration occurs as it grows from bud to maturity followed by a slower but continuous change in salt content via changes in ion concentration and/or increase in leaf thickness.

In Australia, *Avicennia marina* grows well in fresh water condition and the luxuriant growth points to its adaptability in wide range of soil salinity. In Sydney, the summer temperature rises as high as 44°C, while in winter it drops below 0°C. The gray mangrove, *A. marina* seems to withstand such extreme climatic conditions and is one of the two mangrove species available in New South Wales. Experimental studies on physiological responses of mangroves have been brought forward under both field condition (Aniwill and Clough, 1980; Andrews and Muller, 1985; Carter *et al*., 1990; Cheeseman *et al*., 1991; Martin and Loeschen, 1993; Nandy and Ghose, 2001, 2005) and differentially graded *in vitro* saline conditions (Clough *et al*., 1982; Ball and Frequhar, 1984; Ball *et al*., 1987; Ball, 1988; Parida *et al*., 2004), but hardly any information is available on the anatomical and metabolic shifts of 8-10 years old mangroves grown in non-saline condition. In view of this, the present study dealt with a comparative approach towards photosynthesis, water use characteristics, leaf temperature and leaf anatomy of *A. marina* plants grown under the saline environment of Kurnell (151°12' E, 34°01' S) by the Botany Bay and in the non-saline habitat of Parramatta River bank (151°02' E, 33°49' S).

MATERIALS AND METHODS

Avicennia marina plants were investigated for the net assimilation rate, transpiration, stomatal conductance, vapour pressure deficit, leaf temperature, chlorophyll content, mesophyll ratio and stomatal frequency from the Kurnell swamp as well as from the Parramatta riverside vegetation in NSW, Australia (Fig. 1). In both the habitats, only plants grown to maturity were considered and the data furnished are average of ten measurements from each of the three plants studied in each species.

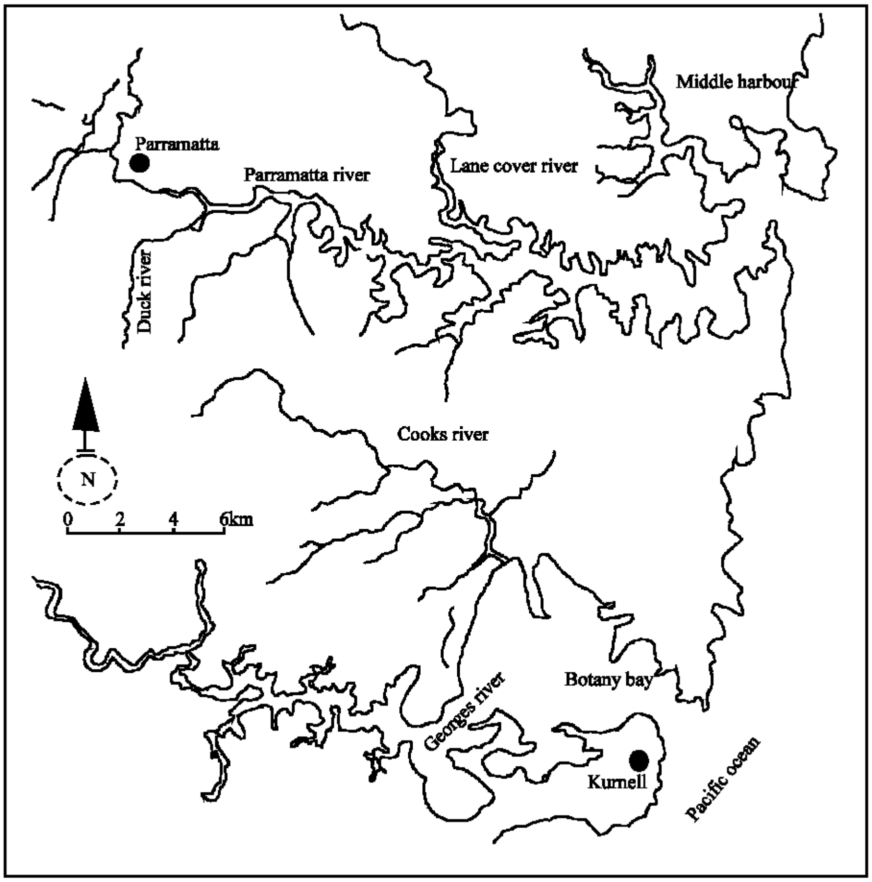

Fig. 1: Map of the study sites

Studies of Physiological Parameters

The rate of net photosynthesis, transpiration, stomatal conductance, vpd and leaf temperature were recorded keeping the PAR value constant at 1500 µmol m^{-2} s^{-1} in both the study sites. The instrument used is basically an Infrared Gas Analyzer (IRGA-LI-6200). It is a closed system instrument and the measurements were taken from the exposed surface of leaves from top, middle and bottom of each plant. The rate of net photosynthesis (P_n) was determined measuring the rate, at which a known leaf area assimilated CO_2 at a given time.

$P_n = A_eC_e\text{-}A_oC_o / L\Delta t$ ……. [A_e= Air entering (mol); C_e= CO_2 entering (mol); A_o= Air coming out (mol); C_o= CO_2 coming out (mol); L= Leaf area measured (m^2); Δt= Time taken for measurement (s)].

The data stored in the instrument was dumped out and computed with the RS 232 Port.

Stomatal Count and Measurement

The abaxial surface of leaf is wrapped by a number of non-glandular hairs, which were removed by scrapping with a blade. On the surface, a thin film of clear nail polish was applied and allowed to dry. To remove the cast and view it, a small piece of sticky tape was stuck first on the dried film of nail polish and then on a slide. The number of stomatal apertures was counted under the 10x objective of fluorescence BX60 microscope (Olympus).

Study of Leaf Anatomy

Fresh leaves were fixed in 0.1 M sodium phosphate buffer containing 2.5% (v/v) glutaraldehyde for 24 h. Samples were dehydrated through an alcohol series (30%-absolute), followed by resin-alcohol grades and embedded in Durcupan resin. Semi-thin sections of 1 μm thickness were obtained using a LKB Ultratome V with a glasscutter and photomicrographs were taken under 10x and 20x objectives of the fluorescence BX60 microscope (Olympus) with a Prog Res C14 digital camera (Jenoptik). Thickness of mesophyll tissue with respect to the entire leaf was measured from the photographs using the software Image pro Plus (version 5.1).

Statistical Analysis

The estimated parameters were analyzed in view of their correlation and significance in intra specific difference using the BMDP 7.1 software.

RESULTS

The study sites considered were distinctly different in soil salinity, in Kurnell the average salinity being 17 PPT due to regular tidal inundation through the Botany Bay, whereas by the Paramatta riverbed, soil salinity was as low as 2.8 PPT. The river is tidal to Parramatta, some 19 km upstream of the commencement of the river at Balmain or approx. 30 km from Sydney heads. The PAR value was fixed to 1500 μmol m^{-2} s^{-1} that is usual in the tropical mangrove forests in a bright sunny day (Nandy and Ghose, 2005).

The average rate of net photosynthesis was higher in saline condition than in the riverside plants (Table 1). In contrast, both transpiration and stomatal conductance were restricted by 8 and 84%, respectively as salinity enhanced in the substrate (Table 1). Vapour pressure deficit also reduced by 78% and the average leaf temperature by 8% in saline soil (Table 1). The total chlorophyll content and the mesophyll ratio (T_l:T_m) however, increased about 27% and 32% respectively under saline condition (Table 1), the latter indicating the relative thickness of mesophyll tissue with respect to the whole leaf. In sea-side plants, stomatal frequency increased by 9% than that in the non-saline ones (Table 1).

Photosynthesis estimated under salt stress, mostly clustered within 15.5 mmol m^{-2} s^{-1} stomatal conductance (Fig. 2A) and 0.47 mmol m^{-2} s^{-1} transpiration (Fig. 2B), the highest rate (16.7 μmol m^{-2} s^{-1}), however was measured at 61.7 and 1.98 mmol m^{-2} s^{-1}, respectively. In riverside plants assimilation rate increased almost linearly with stomatal conductance and transpiration (Fig. 2A and B), the maximal value (10.3 μmol m^{-2} s^{-1}) being measured at 127 and 4.35 mmol m^{-2} s^{-1}, respectively. The break-even leaf temperature was 27°C in saline condition and in non-saline riverbank 29°C, beyond which photosynthesis declined (Fig. 2C). The trends of assimilation with vpd were almost similar in both the substrates; the potency deceased as vpd exceeded 3.06 mmol m^{-2} s^{-1} in enhanced salinity and 3.51 mmol m^{-2} s^{-1} in the riverside *A. marina* (Fig. 2D). In seaside plants, like photosynthesis vapour pressure deficit clustered within a low range of transpiration (0.47 mmol m^{-2} s^{-1}), the maximum value (3.27 mmol m^{-2} s^{-1}) however, being measured when water efflux was 1.98 mmol m^{-2} s^{-1} (Fig. 2E). As salinity ceased, vpd initially declined with increase in transpiration rate till 3.21 mmol m^{-2} s^{-1} and then followed almost a straight trend at least till

Table 1: Physiological and anatomical parameters of *A. marina*, a comparative estimation

	Physiological parameters					Anatomical parameters		
	Photosynth	Transp	St.Cond	VPD			St. freq	Total Chl.
Site	μmol m^{-2} s^{-1}	---------------	mmol m^{-2} s^{-1}	--------------	Leaf temp °C	T_l : T_m	mm^{-2}	μg g^{-1} fw
Saline	12.89±1.91	0.3038±0.013	9.829±0.41*	3.055±0.11	27.621±0.51	4.9±0.45	52.3±0.73	12.22±0.82
NonSaline	6.028±2.56	2.2719±0.037	63.125±1.24*	13.19±0.33	30.179±0.61	6.3±1.02	48.0±1.26	9.66±0.63

N.B. *significant at 1% level

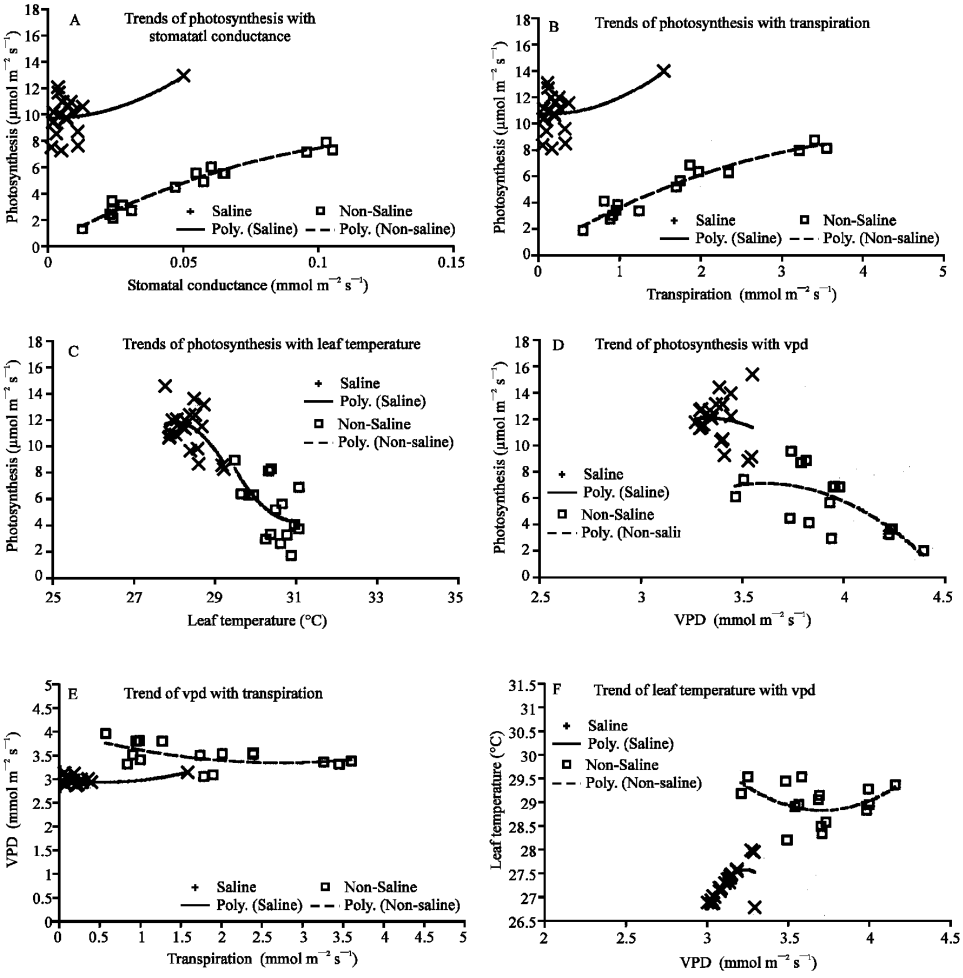

Fig. 2: Graphical representation of different physiological parameters of *Avicennia marina* in two different ecological conditions

4.54 mmol m^{-2} s^{-1} (Fig. 2E). Under high salinity, leaf temperature arose sharply between 2.94-3.13 mmol m^{-2} s^{-1} vpd whereas when substrate salinity is low, leaf temperature retrieved after an initial decline as vpd elevated to 3.81 mmol m^{-2} s^{-1} (Fig. 2F).

Amongst the parameters studied stomatal conductance differed significantly ($p<0.01$) with change in habitat. Stomatal conductance and transpiration followed positive correlation with assimilation rate, the values being highly significant (1% level) only in non-saline condition (R = 0.97 and 0.95, respectively) where as under salt stress it was insignificant. Leaf temperature and vpd were inversely related in both the habitats (R = -0.9 in saline condition and -0.98 in non-saline condition), the values being highly significant (1%). Photosynthesis was inversely correlated with leaf temperature and vpd, but the R- values were insignificant in seaside plants, whereas in fresh water condition, 5 and 1% significance was noticed with leaf temperature and vpd respectively. VPD however, related inversely to water efflux, the correlation values being 5 and 1% significant in saline and non-saline conditions, respectively.

DISCUSSION

The elevated assimilation rate in saline soil can be explained by the increase in total chlorophyll content and mesophyll ratio. Mesophyll ratio is an effective index for abundance of intercellular spaces in leaf, hence points to the mesophyll conductance (Parida *et al*., 2004). At a constant PAR of 1500 μmol m^{-2} s^{-1} the maximal peak of photosynthesis was higher in Kurnell (16.7 μmol m^{-2} s^{-1}) than that measured from Parramatta riverside (10.3 μmol m^{-2} s^{-1}). This points to better photosynthetic efficiency of *A. marina* when grown in saline substrate. Notably, the average rate of photosynthesis (12.89 μmol m^{-2} s^{-1}) in Kurnell swamp was almost similar to that in the Sundarbans forest during winter (11.8 μmol m^{-2} s^{-1}) probably due to similar air temperature (~26°C) and PAR (1300-1700 μmol m^{-2} s^{-1}) (Nandy and Ghose, 2001). On the other hand, during summer, increased temperature and higher irradiance in Sundarbans forest attributed to the decreased assimilation rate of its gray mangroves.

In spite of regular tidal inundation, elevated salt concentration makes the substrate physiologically dry. The reduction in water efflux and controlled stomatal conductance in saline condition points to adaptation of *A. marina* against desiccation and xylem embolism under salt stress. This accords well with the stomatal behaviour and water efflux of gray mangroves in the Sundarbans forest (Nandy and Ghose, 2001, 2005), but contradicts with the earlier studies on photosynthesis of *Avicennia* in Australia that reported stomata to remain open throughout the day (Saenger, 1979). In elevated salinity, *A. marina* seemed more comfortable to photosynthesise within lower range of stomatal conductance (15.5 mmol m^{-2} s^{-1}) so that water efflux did not exceed 0.47 mmol m^{-2} s^{-1}. This has also been reflected by the insignificant correlation of photosynthesis with transpiration and stomatal conductance that also indicated the interference of salinity on their metabolism. Although salinity induced stomatal closure restricted CO_2 influx, but might elevate the CO_2 partial pressure inside leaf cells due to respiration. Thus, a higher assimilation rate coupled with a higher peak value in saline condition than in the riverside plants.

Despite the substrate salinity did not seem to affect the trends of photosynthesis and vpd, it restricted the estimated data within a low range of stomatal conductance and transpiration, thus sustained the insignificant relation between assimilation rate and water efflux. Vapour pressure deficit increases as the evaporative demand decreases in elevated atmospheric moisture content (McClenahan *et al*., 2004). In both the habitats, the negative correlation between transpiration and vpd can explain the slow rise of vpd as water efflux increased in the seaside *A. marina* and the descending trend of vpd at least up to certain extent in the riverside plants.

In spite of the significantly inverse relation between leaf temperature and vapor pressure deficit, leaf temperature in Kurnell plants initially elevated up to 28.1°C till the vpd was 3.13 mmol m^{-2} s^{-1} while in fresh water ones, leaf temperature regained as vpd exceeded 3.81 mmol m^{-2} s^{-1}. Thus in both the habitats, leaf temperature tend to increase under controlled transpiration rate. Excess heat production may denature the photosynthetic enzymes and disrupt several membrane processes (Heldt, 1999) thus, leading to photoinhibition. It is noteworthy that despite lower transpiration and stomatal conductance in saline condition, leaf temperature did not rise more than that recorded from the non-saline site. This, in turn, contributed to the elevated assimilation rate even under salinity stress. In seaside plants, however, the highest assimilation rate (16.7 μmol m^{-2} s^{-1}) coupled with the minimum leaf temperature recorded (26.91°C) and the following temperatures led to decreased photosynthesis rates.

Thus the present study infers that even at the same PAR, ambient CO_2 and temperature, *A. marina* is better suited for high salinity regimes where high humidity prevails in the atmosphere. In addition to the presence of salt glands (Das, 1999) other micromorphological adaptations like higher amount of chlorophyll and photosynthetic tissue contributes to better assimilation potency in saline condition with simultaneous regulation towards water-use management in the physiologically dry substrate. The trends of data suited well with the earlier studies on gray mangrove from the Sundarbans forest (Nandy and Ghose, 2001, 2005) that sustains its affinity towards salinity.

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