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Will Avicennia Alba Thrive in Climate Change Induced Salinity Rise?

Microbiology

KEYWORDS : Avicennia alba, salinity, chlorophyll, carotenoid, proline, Indian Sundarbans

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ABSTRACT

The effect of salinity on chlorophyll a, chlorophyll b, total chlorophyll, carotenoid and proline content of hydroponically grown seedlings of Avicennia alba was studied to observe its tolerance to changing salinity. The selected seedlings were exposed to five different salinity levels (2, 5, 10, 15 and 20 psu) for a period of 30 days and observations were done at a regular interval of 7, 14, 21 and 30 days respectively. The concentrations of chlorophyll exhibited significant positive correlations with salinity ($p < 0.01$). The chlorophyll a:b ratio in the plant varied between 3.04 to 3.56 through out the period of investigation. The salinity fluctuation did not affect the carotenoid level and proline content in the leaves of the species as evidenced from the insignificant r values. The results show that Avicennia alba of Indian Sundarbans region can tolerate and adapt to high saline condition as witnessed in the central sector of the deltaic complex around the Matla River.

Introduction

Climate change has several components of varied nature and scale that affect the ecosystems of the planet Earth. For mangroves, however, the most relevant components include changes in sea level, high water events, storminess, precipitation, temperature, atmospheric CO_2 concentration, ocean circulation patterns, health of functionally linked neighbouring ecosystems, as well as human responses to climate change. Of all the outcomes from changes in the atmosphere's composition and alterations to land surfaces, relative sea level rise is the greatest threat to mangroves. The rising sea level (3.14mm/yr) increases the salinity of the ambient water and soil, which poses a negative impact on the growth and physiological set-up of mangroves. Mangroves are a taxonomically diverse group of salt-tolerant, mainly arboreal, flowering plants that grow primarily in tropical and subtropical regions (Ellison and Stoddart 1991). Generally, high latitude mangroves and mangroves found on arid coastlines have fewer species than tropical mangroves (UNEP 1994). The limiting factor for mangroves in higher latitudes is sea surface and/or atmospheric temperature (Saenger et al. 1977; Clusner and Breckle 1987). Salinity plays a crucial role in the growth and survival of mangroves. However saline condition is not a prerequisite for their development, rather mangroves choose saline condition to avoid the competition with the more vigorous terrestrial plants. Based on the physiological studies, Bowman (1917) and Davis (1940) concluded that mangroves are not salt lovers, rather salt tolerant. However, excessive saline conditions retard seed germination, impede growth and development of mangroves. Indian Sundarbans, the famous mangrove chunk of the tropics is gradually losing few mangroves species (like *Heritiera fomes, Nypa fruticans* etc.) owing to increase of salinity in the central sector of the deltaic complex around the Matla River. Reports on alteration of growth in mangroves due to difference in salinity between western and central sectors of Indian Sundarbans are available (Mitra et al. 2004). However no study has yet been carried out on the effect of salinity fluctuation on the biochemical components of mangroves under culture conditions from this part of the Indian subcontinent. The effects of salinity on mangroves have been studied in relation to antioxidative enzymes (Takemura et al. 2000; Parida et al. 2004b), leaf structure, rates of transpiration, stomatal conductance and rates of photosynthesis (Santiago et al. 2000; Parida et al. 2004a) and changes in chloroplast structure and function (Parida et al. 2003). Tanaka et al. (2000) reported that Na^+/H^+ anti-port catalyzed exchange of Na^+ for H^+ across the vacuolar membrane of the cells of *Bruguiera sexangula* offer tolerance to ionic stress imposed by NaCl and this mechanism is important for cellular salinity adjustments. Also, the mechanism of acclimation to salt in mangroves was suggested to be linked to the changes in the vacuolar size in *B. sexangula* (Hotta et al. 2000). Further, one of the biochemical mechanisms by which mangroves counter the high osmolarity of salt was accumulation of compatible solutes (Takemura et al. 2000). Proline has also been found to be an effective osmoregulating compounds that increase under high saline condition as a mechanism to combat salinity stress.

In Indian Sundarbans, the *Avicennia alba* is one of the dominant mangroves in terms of relative abundance, and hence selected for the present study. In this paper, we present the effect of increasing salinity on pigments and proline content of hydroponically grown seedlings of *Avicennia alba* with an aim to obtain insights into the changes in these biochemical components with salt acclimation.

Materials and methods

Plant materials and culture conditions

Seeds of *Avicennia alba* were collected from Sundarbans mangrove ecosystem of India (22°16/40.6// N latitude and 88°38/18.4// E longitude). Seedlings were raised in the laboratory condition by diluting the source water with stored rain water. The source water was collected from high saline zones of Sundarbans (salinity = 30 psu). Two month old healthy seedlings were subjected to hydroponic culture in Hoagland's nutrient medium (pH = 5.8–6.0) under photosynthetically active radiation (PAR) of 1220–1236 µmol m^{-2} s^{-1}.The preliminary experiments were carried out in the selected species at five different salinities (2 psu, 5 psu, 10 psu, 15 psu and 20 psu respectively) in order to determine the optimum range of salinities in relation to photosynthetic pigments, carotenoids and proline. The cultures were aerated continuously with an air bubbler. The hydroponic cultures were maintained in a culture room under a 14 h photoperiod at PAR of 300 µmol m^{-2} s^{-1}, 26 ± 3°C and 80% RH. The culture medium was changed every 7 days. Leaves were harvested at 7, 14, 21 and 30 days intervals to measure the pigment and proline concentrations.

Extraction and estimation of pigments
Leaves (0.5 g) were homogenized in chilled N, N-dimethylformamide (DMF) in a mortar and pestle in dark at 4^0C and the homogenates were centrifuged at 8800×g for 10 min. The supernatants were collected and absorption spectra at 663.8 and 646.8 nm were recorded using Jasco V-530 UV–vis spectrophotometer for estimation of chlorophyll *a*, chlorophyll *b* and total chlorophyll following the procedure of Porra et al. (1989). For estimation of total carotenoids, leaf tissues (0.5 g) were homogenized in chilled 80% (v/v) acetone and the homogenates were centrifuged at 8800 × g for 10 min at 4^0C in the dark. The absorbance of the acetone extracts was measured at 663, 645 and 470 nm. Total carotenoids were calculated according to Arnon (1949).

Estimation of free proline
Free proline content was measured from leaf using 3% sulphosalicylic acid following the method of Bates et al. (1973) using L-proline (Sigma) as standard.

Statistical analysis
Statistical analysis of the results was carried out according to Duncan's multiple range tests. Data were also subjected to analysis of correlation coefficient (r) in order to evaluate the inter-relationship between salinity, selected pigments and proline content of the leaves of the selected species following the method of Sokal and Rohlf (1995).

Results
All the collected seedlings of *Avicennia alba* could tolerate salinity up to 20 psu and could be maintained for more than 30 days. The concentrations of chlorophyll increased significantly with salinity (Table 2). The total chlorophyll expressed on unit fresh weight basis increased by 10.58%, 12.50%, 12.98% and 9.87% at 7, 14, 21 and 30 days intervals respectively due to change of salinity from 2 psu to 20 psu (Table 1). The chlorophyll *a:b* ratio in the plant, however, remained almost constant for the species and varied only marginally during the period under observation. In our experiments with differential salinity exposure the chlorophyll *a: b* ratio yielded values ranging between 3.37 to 4.15 (Table 1). The increase of the photopigments with aquatic salinity is statistically significant (Table 2) and reflects the efficiency of photosynthetic machinery of the species even in high saline condition. As the chlorophyll *a:b* ratio remained unaffected at high saline condition in the selected species, it appears that the light harvesting complex (LHCs) of thylakoid membranes are little altered by salt exposure. The species thus seems to have a higher tolerance to increased salinity that may occur during climate change induced sea level rise in vulnerable islands of Sundarbans (Mitra et al. 2009).

Clough (1985) stated in his communication that the rate of light saturated photosynthesis decreases with increasing salinity of ambient media, attributing this to co-limitation of assimilation rate by stomatal conductance and photosynthetic capacity in response to differences in water status induced by the various salinity treatments. Thus, on the evidences available so far it is most likely that salinity exerts its effect on photosynthesis mainly through changes in leaf water status and this study reveals that the photosynthetic process may be affected at high saline condition due to decrease in chlorophyll *a* and *b* concentrations in mangroves. The present study is different from several works as the salinity of water has been altered naturally (through dilution with rain water) keeping the all the constituent salts of brackish water constant unlike several previous studies where the plants were exposed to different NaCl concentrations (Mishra and Das 2003; Netondo et al. 2004) that are not the real image of ambient seawater. Various studies have shown that a number of mangrove species grow best at salinities between 4 psu and 15 psu (Connor 1969; Clough 1985; Downton 1982; Burchett et al. 1984 and Clough 1984) and for *Heritiera fomes*, the preferred salinity range is much lower. In this context, *Avicennia alba* seems to be a befitted species for arid, high saline zone.

The carotenoid pigment of the species showed a mosaic nature on exposure to different salinity. At the end of 7 and 21 days there was no decrease, but after 14 days the carotenoid content in the leaf decreased by 5 %. Again the value remained constant at the end of 30 days. Overall trend however, indicates no effect of salinity on carotenoid level of the species (Table 2). The result implies that the species does not alter the synthesis of carotenoid under stress condition and is ideal for high saline environment. Our results is contradictory to several reports of decrease content of chlorophyll and carotenoids by salinity as observed in a number of glycophytes (Gadallah 1999; Agastian et al. 2000).

Proline accumulation is a common phenomenon in halophytes. As *Avicennia alba* is a true halophyte and a salt excreting species, it is of interest to study proline accumulation in response to salinity in this plant. It is well known that proline content in leaves of many plants gets enhanced by several stresses including salt stress (Lee and Liu 1999; Hernandez et al. 2000). Thus, we monitored the proline levels in leaves of the species treated with 2, 5, 10, 15, 20 psu saline water for 7, 14, 21 and 30 days. Our results exhibited almost uniform proline content in leaves of the species which is contrary to several reports of accumulation of proline as compatible osmolyte during NaCl exposure (Lee and Liu 1999; Hernandez et al. 2000; Parida et al. 2002). The constancy of proline value confirms the unique tolerance power of the species even under salinity stress. This uniformity of proline level may be attributed to the activity of proline dehydrogenase, a catabolic enzyme of proline (Lee and Liu 1999). It appears that the enzyme remain unaffected in *Avicennia alba* even under high saline condition which is a unique adaptive mechanism.

Our results show that *Avicennia alba* of Indian Sundarbans region can easily be propagated in saline zone around the Matla River. Even at 15 and 20 psu salinity the chlorophyll pigments showed an increase. The high salinity could not affect the carotenoid and proline content of the species that usually increase under stressful situation.

Concluding remarks
Indian Sundarbans and its adjacent estuaries situated in the lower Gangetic region at the apex of Bay of Bengal are one of the less studied regions of the world ocean in context to impact of rising salinity fluctuation on mangrove floral community, although the region sustains the 5th largest mangrove chunk in the world (2120 km^2 in the Indian part and 4500 km^2 in the Bangladesh part). The present study is extremely important from the point of view of rising salinity in the central sector of Indian Sundarbans over a period of two decades (Mitra et al. 2009a) due to complete obstruction of the freshwater supply of Ganga-Bhagirathi-Hooghly River as a result of heavy siltation since the late 15th century (Chaudhuri and Choudhury 1994) and rising sea level (Hazra et al. 2002) at the rate of 3.14 mm/yr, which is higher than the global average sea level rise of 2.12 mm/yr and 2.50 mm/yr along the Indian coastline (Lal and Aggarwal 2000). Increased salinity and lack of freshwater is likely to result in a decrease in mangrove productivity, growth and seedling survival, and may change species composition favoring more salt tolerant species (Ellison 2000, 2004).

In summary, results show that the mangrove *Avicennia alba* can easily be propagated under high salinity conditions and may be a better suited species for central sector of Indian Sundarbans where the Ganges lost its flow on account of heavy siltation.

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Table 1 Effects of different salinities on pigment level and proline concentration in *Avicennia alba*

Duration of treatment (d)	Salinity (psu)	Chl a	Chl b	Total Chlorophyll	Chl a:b	Carotenoid	Proline
7	2	0.62±0.02ab	0.17±0.007ab	0.79±0.03ab	3.65	0.17±0.03a	1.1±0.03a
	5	0.59±0.02a	0.15±0.004a	0.74±0.03a	3.93	0.14±0.03a	1.2±0.03a
	10	0.64±0.02ab	0.18±0.006ab	0.82±0.03ab	3.55	0.19±0.03a	1.0±0.03a
	15	0.66±0.01ab	0.19±0.008bc	0.85±0.03ab	3.47	0.16±0.03a	1.3±0.03a
	20	0.68±0.02b	0.20±0.006c	0.88±0.03b	3.40	0.17±0.03a	0.9±0.03a
14	2	0.59±0.02ab	0.15±0.005ab	0.74±0.03ab	3.93	0.18±0.03a	1.0±0.03a
	5	0.62±0.04a	0.16±0.005a	0.78±0.03a	3.87	0.14±0.03a	0.8±0.03a
	10	0.57±0.02ab	0.16±0.004ab	0.73±0.03ab	3.56	0.16±0.03a	1.1±0.03a
	15	0.61±0.03ab	0.18±0.007bc	0.79±0.03ab	3.38	0.15±0.03a	0.7±0.03a
	20	0.65±0.04b	0.19±0.009c	0.84±0.03b	3.42	0.17±0.03a	1.0±0.03a
21	2	0.56±0.01ab	0.15±0.003ab	0.71±0.03ab	3.73	0.14±0.03a	1.1±0.03a
	5	0.54±0.01a	0.14±0.004a	0.68±0.03a	3.86	0.14±0.03a	0.6±0.03a
	10	0.59±0.02ab	0.15±0.003ab	0.74±0.03ab	3.93	0.16±0.03a	1.4±0.03a
	15	0.58±0.02ab	0.16±0.005bc	0.74±0.03ab	3.62	0.13±0.03a	0.8±0.03a
	20	0.63±0.04b	0.18±0.005c	0.81±0.03b	3.50	0.16±0.03a	0.8±0.03a
30	2	0.60±0.04ab	0.15±0.002ab	0.75±0.03ab	4.00	0.18±0.03a	0.9±0.03a
	5	0.54±0.01a	0.13±0.002a	0.67±0.03a	4.15	0.15±0.03a	1.1±0.03a
	10	0.58±0.02ab	0.15±0.004ab	0.73±0.03ab	3.86	0.17±0.03a	0.7±0.03a
	15	0.59±0.04ab	0.17±0.007bc	0.76±0.03ab	3.47	0.13±0.03a	1.0±0.03a
	20	0.64±0.04b	0.19±0.009c	0.83±0.03b	3.37	0.18±0.03a	0.8±0.03a

Units of all pigments are mg/gm fresh weight; unit of proline is nmol/gm fresh weight. Different letters besides figures indicate statistically different means as at p ≤0.01.

Table 2 Inter-relationships between salinity and selected pigments in *Avicennia alba*

Combination	'r' value	'p' value
Salinity × Chl a	0.6145	<0.01
Salinity × Chl b	0.7703	<0.01
Salinity × Total Chl	0.6864	<0.01
Salinity × Carotenoid	0.0711	IS
Salinity × Proline	- 0.1898	IS

IS means insignificant

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