Research Article

Differential expression of physiological and biochemical characters of some Indian mangroves towards salt tolerance

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ABSTRACT

Mangroves are physiologically interesting as potential models for stress tolerance and as sources of alternative ideas about physiological strategies relevant at the ecosystem level. Variation in habitat has great impact on the physiological behavior and biochemical expression level of a particular plant species. Five species of mangroves, growing in saline and fresh water conditions were assessed for their ecological fitness in two different habitats. Assessments were based on some physiological and biochemical parameters measured from the fully exposed mature leaves under saline (15 - 27 PPT) and non-saline (1.2 - 2 PPT) conditions. Among the five species considered for investigation *Bruguiera gymnorrhiza*, *Excoecaria agallocha* and *Phoenix paludosa* grow luxuriously in the Sundarbans forest, while the rest two (*Heritiera fomes*, *Xylocarpus granatum*) are scanty. A comparative account of photosynthetic efficiency, chlorophyll content, mesophyll and stomatal conductance, specific leaf area, photosynthetic nitrogen use efficiency, total foliar free amino acids and differential expression of some antioxidant isoenzymes in leaf were estimated between the saline and non-saline plants. Elevated assimilation rate coupled with increased chlorophyll content, increased conductance and higher specific leaf area in non-saline condition indicates ability of these mangroves to grow even under minimal substrate salinity. The optimum PAR acquisition for photosynthesis in *B. gymnorrhiza*, *E. agallocha* and *P. paludosa* was higher under salt stress, while the maximum assimilation rate was lower in control plants. The opposite trend occurred in *H. fomes* and *X. granatum*, where the peak photosynthesis was lower under non-saline conditions even at a higher irradiance than in the saline forest. The isoform patterns of peroxidase, acid phosphatase and esterase indicated considerable difference in regulation of these enzymes due to salt stress and /or reverse adaptation. **[Physiol. Mol. Biol. Plants 2009; 15(2) : 151-160]** *E-mail : sauren@isical.ac.in*

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INTRODUCTION

A divergent species composition after a consequence of convergent evolution has got adapted to the saline environment and has similar physiognomy and structural adaptation (Yennce-Esinsine 1980). The uniqueness of mangroves is in their ability to colonies an ecosystem, which is under constant physiological stress (Chaudhuri 1996). Despite the extreme conditions, these plants have successfully colonized by developing some morphological, reproductive and physiological adaptation (Zimmermann 1983; Das 1999). Predictions for changes of this ecosystem due to of global warming indicate an increase in frequency and intensity of episodic natural calamities like cyclones, hurricanes, tsunami, storms and floods. Mangroves are the primary producers which shelter and feed many offshore populations of the topical coast.

It is still debatable whether these plants are 'halophilic' or 'salt -tolerant'. Due to regular tidal inundation, mangroves have to thrive in considerably high soil salinity and consequently, a physiologically dry substrate. Apart from this, the extreme microclimate (irradiance and temperature) associated with high salinity affects the rate of photosynthesis (Ball 1988) so that mangroves have to maintain low assimilation rate throughout the day (Cowan 1982; Bjorkman *et al*. 1988; Nandy and Ghose 2001). It was reported that optimum

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irradiance required for photosynthesis is as low as 800 to 1200 µmol $m^{-2}s^{-1}$ in some Rhizophoracean species (Nandy and Ghose 2005), whereas, more than 2000 µmol $m^{-2}s^{-1}$PAR is available in the forest canopy in a bright sunny day.

The features of mesophyll parenchyma and the packaging of chlorophylls into the chloroplasts determine the penetration and absorbance of light within the leaf (Sefton *et al*. 2002). Specific leaf area (SLA) represents the trade-offs between resource accumulation and the stress imposed by leaf structure (Dijkstra 1990). Low SLA is typical of plants grown in poorly resourced and high light environment where efficient water and nutrient management ensure the ecological success (Wright *et al*. 1994; Schulze *et al*. 1998). Constraints imposed by the need to conserve water, reduce CO_2 diffusion (Khan *et al*. 2000) and N-use efficiency of individual leaves (Sefton *et al*. 2002). Low CO_2 influx is generally attributed to stomatal closure and decrease in mesophyll conductance under drought and salt stress. Thus, leaf morphology affects the use of N and CO_2 and a high investment of photosynthetic tissue is associated with high leaf N concentration, chlorophyll content and photosynthetic efficiency per leaf area.

In mangroves, factors regulating photosynthesis are not yet well understood (Clough 1985; Ball *et al*. 1988; Kathiresan and Moorthy 1994). Kathiresan and Kannan (1985) reported that ratio of chlorophyll a:b acts as a regulating factor in photosynthetic efficiency of mangroves. The photosynthetic pigments are present in free forms or embedded in the protein complexes of chloroplast membranes (Thornber 1975). This pigments controls energy transfer and energy production through photosynthetic carbon assimilation. In *Bruguiera parviflora*, Parida *et al*. (2004) reported salt induced reduction of mesophyll and stomatal conductance that imposed restriction in assimilation rate. 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 (Das 1999; Nandy (Datta) *et al*. 2005).

Likewise, in salinity prone ecosystems, plants alter some biochemical reactions and many intermediate products are formed. Free amino acids are important markers that accumulate in plant tissues under stress without interfering the cell metabolism. In obligate halophytes, reverse adaptation often provoke significant metabolic shifts that can be partially characterized by isozyme study. Peroxidases (in different isoforms) are widely distributed throughout the growing phase and have great biological importance. In plants, peroxidase either bound to cell wall or located in the protoplast (Mader 1976). Cell wall bound peroxidases are probably involved in lignification while other isoenzymes have the regulatory role in plant senescence or in the destruction of auxins (Frenkel 1972; Stonier and Yang 1973). Due to changed ecological condition isoforms of this stress related enzymes are differentially expressed in different habitats. In view of these, five mangrove species were selected that grow well in estuarine as well as nonsaline habitats to compare their photosynthetic efficiency, water-use characteristics and some metabolic defense mechanisms in different salinity conditions.

MATERIALS AND METHODS

Five species of mangroves *viz. Bruguiera gymnorrhiza*, *Excoecaria agallocha*, *Heritiera fomes*, *Phoenix paludosa* and *Xylocarpus granatum* were investigated for their net assimilation rate, stomatal conductance, chlorophyll content, mesophyll ratio, specific leaf area (SLA), photosynthetic nitrogen use efficiency and three different isozymes (peroxidase. esterase and acid phosphate). Samples were collected from Sundarbans forest as well as from the garden of Indian Statistical Institute. Leaves were preserved in 0.1 M sodium phosphate buffer containing 2.5% (v/v) glutaraldehyde immediately after collection for anatomical work. For biochemical and isozyme analyses, the leves were collected in ice bucket. Photosynthesis data were collected from the required plants itself. The data furnished are average of 20 measurements from each of the three plants studied in each species from each habitat.

Measurement of photosynthesis and stomatal conductance

The rate of net photosynthesis and stomatal conductance in different PAR and leaf temperatures were measured. The instrument used is an infrared CO_2 gas analyser (PS 301 CID, USA) that uses an electronic mass flow meter to monitor airflow rate. Measurements were taken from the exposed surface of leaves from top, middle and bottom of each plant in a bright sunny day. The rate of net photosynthesis (*Pn*) was determined measuring the rate, at which a known leaf area assimilated CO_2 concentration at a given time.

$$Pn = - W \times (C_o - C_I)$$

$$= - 2005.39 \times \frac{(V \times P)}{(T_a \times A)} \times (C_o - C_I) \text{ .....}$$

[W = mass flow rate per leaf area (mmol $m^{-2}s^{-1}$; C_o (C_I) = outlet (inlet) CO_2 conc. (µmol $m^{-2}s^{-1}$); P = atm. pressure (bar); and T_a = air temp. (K)].

Stomatal conductance (C_{leaf}) was calculated from the rate of water efflux and leaf surface temperature (°C).

$$C_{leaf} = \frac{W}{\left(\frac{e_{leaf} - e_o}{e_o - e_I}\right)} \times \frac{P - e_o}{P} - RbW \times 1000$$

[e_{leaf} = saturated water vapour at leaf temperature (bar); Rb = leaf boundary layer resistance (m^2s / mol); P = atm. pressure (bar) and W = mass flow rate per leaf area (mmol $m^{-2}s^{-1}$)]. The data were downloaded and computed through RS 232 Port.

Chlorophyll Estimation

80% acetone extracts were prepared from the freshly collected leaves following Lichtenthaler and Wellburn (1983). Absorbance was measured at 645 nm and 663 nm using a Helios γ spectrophotometer and concentrations of chl a, chl b and the total chlorophylls were determined using the equations of Porra *et al*. (1989).

Measurement of Specific Leaf Area

Specific Leaf Area is a function of leaf thickness (T, mm) and leaf density (D, g mm^{-3}).

$$SLA = \frac{1}{(T \times D)} \text{ ..... (Sefton } et\ al. \text{ 2002)}$$

$$= \frac{\text{Leaf area}}{\text{Leaf (dry)}} \quad \text{wt.} = mm^2/g$$

Leaf area was measured from the leaf impression on graph paper and counting the small divisions covered by the leaf. Dry weight was measured after heating the leaf at 80°C for 48 hr. Average leaf thickness were taken from free hand sections of randomly preserved leaves.

Free amino acids

Free amino acids were estimated from the leaves of the 5 mangrove taxa from two different ecological conditions. Two grams of freeze-dried leaf was homogenized in acetone and the extract was centrifuged at 3000 g (314.28 radian sec^{-1}) for 30 min. The supernatant was allowed to run in thin layer chromatogram using n-butanol: acetic acid: distilled water (4:1:1) as the running solvent. In the chromatogram, grayish purple, bluish purple and yellow spots appeared for respective amino acids, which were identified from the standard *Rf values*. The spots were eluted and the optical density measured in specific wavelengths (550 nm for proline and 400 nm for the other amino acids). Estimation was done on the basis of standard curves plotted for individual amino acids. In the case of tryptophan and tyrosine, where the *Rf* values are very close to each other, confirmatory tests (Hopkins-Cole test for tryptophan and Millon's reaction for tyrosine) were performed.

Isozyme analysis

Youngest leaves were collected in ice and extracted at 4°C following Gangopadhyay *et al*. (2002). Equimolar amounts of protein were loaded in each well and different isoforms were separated by native gel electrophoresis. Isozymes were stained and densitometric scanning of the gels was done with gel documentation system (Kodak MI software).

RESULTS

Photosynthesis and stomatal conductance: Mangroves grown native condition showed lower amount of net photosynthetic rate than that of their fresh water counterparts (Table 1), but the utilization of PAR for optimize photosynthesis were greater in most of the Sundarbans species except *H. fomes* and *X. granatum* In *B. gymnorrhiza*, the maximum photosynthesis (10.47 µmol $m^{-2}s^{-1}$) was achieved only at 873 µmol $m^{-2}s^{-1}$ PAR when grown in non-saline soil, but as high as 1078.5 µmol µmol $m^{-2}s^{-1}$ PAR was utilised to obtain the highest assimilation rate (9.19 µmol $m^{-2}s^{-1}$) under saline condition. In *E. agallocha* the optimum PAR required for maximum photosynthesis was 1445.8 µmol $m^{-2}s^{-1}$ in Sundarbans and 1402.6 µmol $m^{-2}s^{-1}$in garden, whereas the highest assimilation rates were 12.27 µmol $m^{-2}s^{-1}$ and 14.69 µmol $m^{-2}s^{-1}$ respectively. Similarly, in *P. paludosa*, the optimum PAR value was 1662.3 µmol $m^{-2}s^{-1}$ in Sundarbans forest beyond which photosynthesis started declining, whereas in garden, the highest rate of net photosynthesis (6.92 µmol m^{-2} s^{-1}) was recorded at a much lower PAR value (1012.6 µmol $m^{-2}s^{-1}$). On the contrary, under salt stress, the rate of assimilation in *X. granatum* dropped just beyond 827.7 µmol $m^{-2}s^{-1}$ of PAR, whereas in non-saline condition, the optimum PAR was as high as 1557.6 µmol $m^{-2}s^{-1}$. Among the studied species, photosynthesis rate was maximal in *H. fomes* under both the environmental conditions (10.63 µmol $m^{-2}s^{-1}$ in Sundarbans and 12.63

μmol $m^{-2}s^{-1}$ in garden plants). Considerable decrease in stomatal conductance was noticed under salt stress (Table 1). In *B. gymnorrhiza* and *E. agallocha* the salinity-imposed restriction on stomatal conductance was about 44%, in *P. paludosa* and *X. granatum* approximately 52% and in *H. fomes* it was 25%.

Chlorophyll content

In *Bruguiera gymnorrhiza*, *Excoecaria agallocha* and *Heritiera fomes*, chlorophyll content was higher in non-saline condition (Table 1). In the former two, increase in total chlorophyll occurred by 18% and 13% respectively whereas in *Heritiera*, insignificant increase (0.7%) was measured as salinity reduced in soil. In contrast, in *Phoenix paludosa* and *Xylocarpus granatum* elevated soil salinity induced the total chlorophyll content by 22% and 7% respectively. However, the ratio between Chlorophyll a and b is higher in plants grown in non-saline soil (Table 1).

Specific Leaf Area

In all the five species SLA was lower at higher salinity (Table 1). In *B. gymnorrhiza* and *H. fomes*, SLA reduced by 14% and 17% respectively in Sundarbans than in the non-saline habitat, whereas the reduction percentage was more in the other three species; in *E. agallocha* and *X. granatum* a moderate decrease by 25% and 35% was measured whereas in *P. paludosa* decrease in SLA was as high as 59%.

Free amino acids

The major free amino acids obtained in the leaves of the Sundarbans mangroves are aspartic acid, alanine, proline, tryptophan, tyrosine and phenylalanine (Nandy Datta and Ghose 2003). Five mangrove taxa from in situ condition and as well as their counterpart from sweet water condition were estimated. Considerable amounts of aspartic acid, phenylalanine and alanine were detected in all the investigated taxa. But the quantitative analysis shows a considerable lower amount was detected from the plants grown in non-saline environment. Free proline was detected in, *B. gymnorrhiza*, *P. paludosa* and *Xylocarpus granatum*, where as it is absent in *Heritiera* and *Excoecaria*. In *P. paludosa*, proline content was relatively high while alanine concentration is low, whereas a lower concentration of proline was estimated in *B. gymnorrhiza* and *Xylocarpus granatum*, with relatively high alanine content. In *Excoecaria* and *Heritiera*, a considerable high amount of aspertate was detected. For easy comparison, all detected free amino acids were expressed in lucine equivalent (Table 1). The

Table 1. Leaf anatomy, carbon assimilation and free amino acid in the studied taxa in two different habitats.

Name of the Species	Pn (μmol $m^{-2}s^{-1}$)		Chl a/b		Chl_{total} (μg g^{-1} fw)		SLA (mm^2 g^{-1})		T_m:T_l		St. Cond. (mmol $m^{-2}s^{-1}$)		Free amino acid# (%)	
	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
Bruguiera gymnorrhiza	6.75 (±0.67)	7.05 (±0.14)	0.65★ (±0.32)	0.92★ (±0.73)	44.86★ (±0.6)	52.88★ (±0.19)	22.45★★ (±0.76)	26.09★★ (±0.43)	0.83★ (±0.3)	0.94★ (±0.51)	45.47★★ (±0.18)	81.54★★ (±0.6)	9.74 (±0.38)	6.48 (±0.76)
Excoecaria agallocha	7.66 (±0.19)	10.34 (±0.24)	0.55★★ (±0.21)	0.76★ (±0.3)	26.6★ (±0.52)	30.04★ (±0.28)	29.99★★ (±0.36)	46.37★ (±0.45)	0.88★ (±0.15)	0.91★ (±0.24)	49.19★★ (±0.27)	87.96★★ (±0.46)	8.74 (±0.43)	7.98 (±0.27)
Heritiera fomes	10.63 (±0.54)	12.63 (±0.41)	0.54★★ (±0.62)	0.56★★ (±0.7)	17.11★★ (±0.29)	17.23★★ (±0.42)	31.15★★ (±0.18)	37.58★★ (±0.26)	0.77★★ (±0.37)	0.82★★ (±0.5)	89.06★★ (±0.22)	119★★ (±0.36)	9.26 (±0.77)	4.13 (±0.33)
Phoenix paludosa	4.59 (±0.5)	5.69 (±0.37)	0.83★ (±0.7)	0.89★ (±0.29)	38.02 (±0.48)	31.26★★ (±0.61)	25.93 (±0.39)	63.63★ (±0.31)	0.92★ (±0.45)	0.94★ (±0.33)	30.23★★ (±0.5)	63.86★★ (±0.28)	11.38 (±0.64)	7.60 (±0.57)
Xylocarpus granatum	5.29 (±0.4)	5.86 (±0.41)	0.69★★ (±0.53)	0.87★ (±0.36)	42.13 (±0.55)	39.47 (±0.52)	24.86★★ (±0.37)	33.29★★ (±0.44)	0.72★ (±0.6)	0.85★ (±0.62)	41.56 (±0.36)	86.88★★ (±0.47)	12.07 (±0.88)	9.16 (±0.61)

N.B. : P_n – Rate of net photosynthesis, **T_m:T_l –** Mesophyll ratio, **S –** Saline soil, **NS –** Non-saline soil. #% equivalent to leucine, ★indicate significance levels of the correlation coefficient (r) with photosynthesis. ★Significance level 1%; ★★Significance level 5%. Values within parenthesis denote the standard error.

percentage of occurrence of free amino acids were reduced in the plants grown in non saline condition; such as 33.47% in *Bruguiera*, 9.21% in *Excoecaria*, 55.4% in *Heritiera*, 33.22% in *Pheonix* and 24.1% in *Xylocarpus*.

Isozyme analysis

Peroxidase

Experimental data shows that in *Bruguiera gymnorrhiza* at saline condition, two distinct bands appear (more than 200 OD) at 25 and 35 mm from the gel front whereas in non-saline condition shows the first peak (above 200 OD) appeared at 15 mm distance and the second one (170 OD) at 30 mm distance. In *Excoecaria agallocha* in garden plants only one sharp peak above 200 OD and few smaller peaks were found within a distance of 10 to 20 mm from gel front, but in native plants the first peak (above 220 OD) occurred at 25 mm, the second one (200 OD) at 40 mm and a third one (200 OD) at 60 mm distance. In *Heritiera fomes* the fresh water plant showed a sharp peak (above 200 OD) at 20 mm and some auxiliary bands at 35 mm distance and the saline one have two major isoforms (above 220 OD and 180 OD) at 25 and 35 mm distance respectively. In *Phoenix paludosa* the fresh water plant showed two isoforms (200 and 220 OD) at 20 and 25 mm that were distinctly different from the saline plants where the 260 and 180 OD peaks occurred at 25 and 40 mm distance respectively. In *Xylocarpus granatum* fresh water plant had few small isoforms (< 150 mm) before 20 mm and a sharp peak of 260 OD at 38 mm but in the saline plant the bands were of about 200 OD at 20 mm, 150 OD at 40 mm, 240 OD at 35 mm and last one of about 200 OD at 60 mm distance from the gel front (Figs. 1A, 2).

Esterase

Bruguiera gymnorrhiza grown in saline condition showed single peak of 1.6 OD values at 50mm gel distance. But in the *ex situ* sample a smaller peak of 0.3 OD appeared at 50mm gel distance. In saline condition *Excoecaria agallocha* showed single peak (0.19 OD) at 20mm gel distance, in the garden sample a similar peak appeared at the same distance. In *Heritiera fomes* from saline condition a peak of 1.5 OD appeared at 50mm gel distance, whereas in the *ex situ* sample three peaks were observed in the range of 0.4 to 0.5 OD values at 30mm, 60mm, and 80mm distance respectively. In *Phoenix paludosa* a single large peak (0.6 OD) appeared at 60mm distance. But in the garden sample two peaks appeared of 0.4 and 0.7 OD at 40 and 60mm distance respectively. In *Xylocarpus granatum* two peaks (0.6 and 0.3 OD) appeared at 40 mm and 60mm gel distance respectively, whereas in *ex situ* sample a peak (1.5 OD) appeared at 35mm gel distance (Figs. 1B, 3).

Acid phosphatase: In *Bruguiera gymnorrhiza,* when collected from Sundarbans, two consecutive peaks (0.4 OD) appeared at 15 mm distance, and two smaller peaks (0.2 OD) at 30 mm and 40 mm distance. The *ex situ* plant showed single large peak of 0.8 OD at 50mm of gel distance. In *Excoecaria agallocha* two peaks (0.6 and 1.2 OD) appeared in native plants at 20mm and 35mm respectively, whereas in non-saline condition single peak of 1.4 OD appeared at 35mm distance. In *Heritiera fomes*,

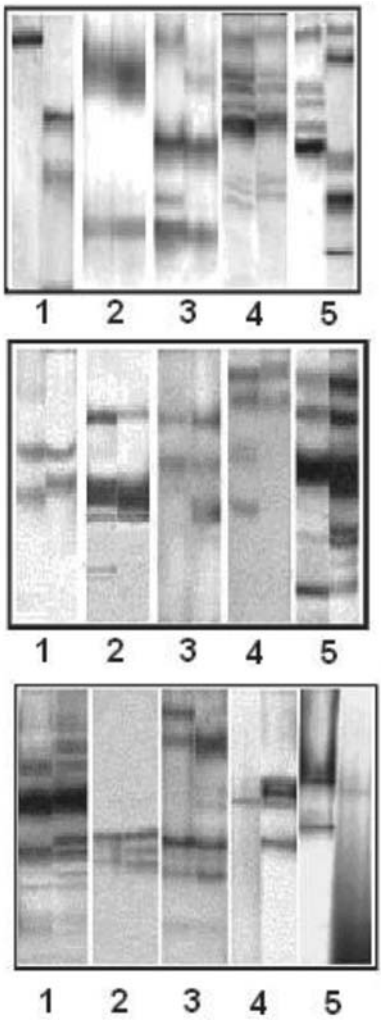

Fig. 1A – C. Photographs of gel electrophoresis. Bands in left side of each pare of gel indicate sample from saline habitat and in right side from non-saline habitat. 1. *Bruguiera gymnorrhiza*, 2. *Excoecaria agallocha*, 3. *Heritiera fomes*, 4. *Phoenix paludosa* and 5. *Xylocarpus granatum*. A. Peroxydase; B. Esterase; C. Acid Phosphatase.

a small peak (0.3 OD) appeared at 30mm distance but for the garden sample two peaks appeared (0.3 and 0.25 OD) at 25mm and 35mm distance respectively. In *Phoenix paludosa* a small peak of 0.1 OD appeared at 45mm gel distance but the fresh water sample showed two peaks of 0.17 OD at 10mm distance. In *Xylocarpus granatum*, a distinct peak (200 OD) appeared at 35mm distance but in the garden sample there was no distinguishable peak in the densitogram (Figs. 1C, 4).

DISCUSSION

The carbon assimilation rate and chlorophyll a: b ratio was higher in non-saline samples than in the saline ones. Increase in leaf nitrogen attributes to increased chlorophyll concentration and the amount of palisade tissue and chloroplasts contain up to 75% of leaf organic N (Poorter and Evans 1998). Feller *et al*. (2003) experimentally proved that higher accumulation of N affects the internal dynamics of N and cause increase in net photosynthesis. The higher chlorophyll contents in leaves of garden mangroves may be due to both the greater proportion of mesophyll (T_m: T_l value in Table 1) and/or the inherently higher chlorophyll concentration of palisade tissue compared with spongy tissue (James *et al*. 1999). Under high salinity, leaf photosynthetic efficiency is controlled by the electron transport capacity of thylakoid proteins, the activity of Rubisco and the mesophyll resistance (Searson *et al*. 2004). In *B. gymnorrhiza*, *E. agallocha* and *H. fomes* the total chlorophyll count was higher in non-saline condition than in Sundarbans plants which effectively enhanced the rate of energy transfer and energy production, thereby increased the rate of assimilation. The reduction

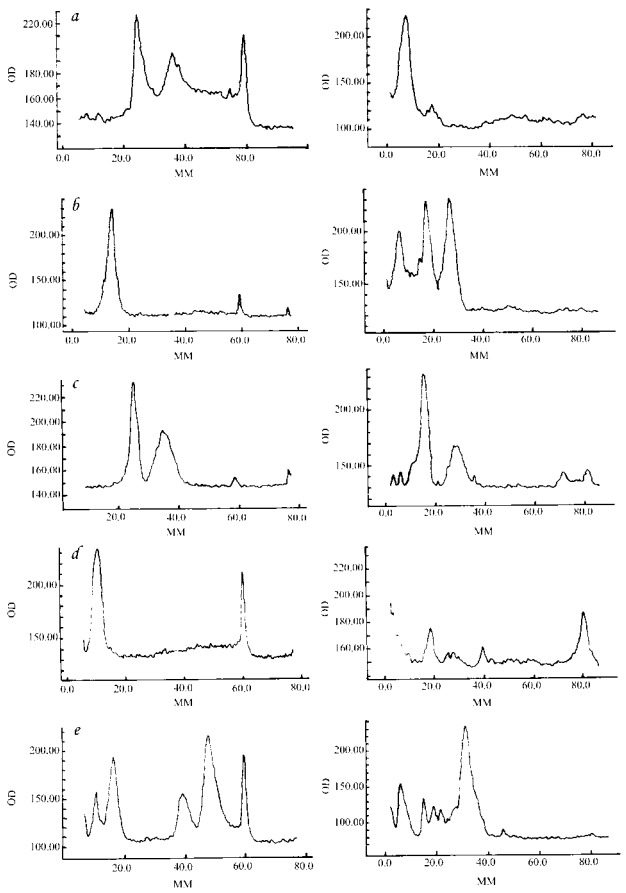

Fig. 2. Densitometric analysis of PAGE – Peroxydase. Graphs in left side of each pare indicate sample from saline habitat and in right side from non-saline habitat. 1. *B. gymnorrhiza*, 2. *E. agallocha*, 3. *H. fomes*, 4. *P. paludosa* and 5. *X. granatum*.

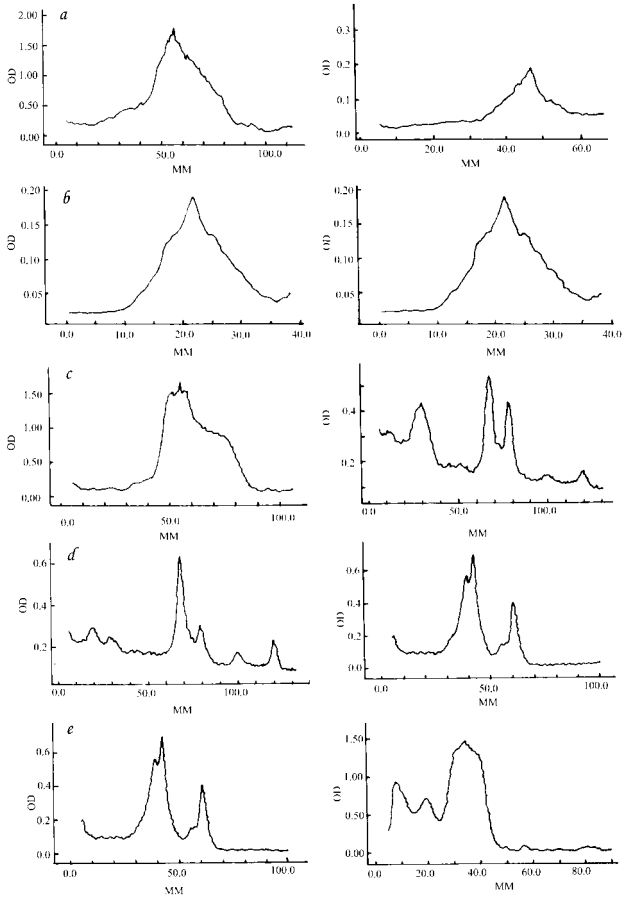

Fig. 3. Densitometric analysis of PAGE – Esterase. Graphs in left side of each pare indicate sample from saline habitat and in right side from non-saline habitat. 1. *B. gymnorrhiza*, 2. *E. agallocha*, 3. *H. fomes*, 4. *P. paludosa* and 5. *X. granatum*.

in the total chlorophyll content is related to the effect of salinity on stomatal closure (Kawasaki *et al*. 2001). In all the five species, stomatal conductance was reduced by 25% to 52% under salinity stress that effectively limited CO_2 influx. In the non-saline soil, increased mesophyll thickness provided the leaf with more intercellular spaces and thereby more mesophyll conductance. This accord well with earlier studies in olive (Bongi and Loreto 1989), cotton (Brugnoli and Bjorkman 1992), and *B. parviflora* (Parida *et al*. 2004). Thus, the higher stomatal conductance coupled with increased mesophyll conductance enhances CO_2 diffusion inside leaf cells, which, in turn, aids to higher photosynthesis rate in garden mangroves. In *B. gymnorrhiza*, *E. agallocha* and *P. paludosa* the higher peak of photosynthesis in the garden plants can also be attributed to increased nitrogen use efficiency. Moreover, reduction in K+ content under elevated salinity may cause damage to the photosynthetic apparatus (Chow *et al*. 1990), thereby reducing the rate of assimilation under salt stress. Another possible factor contributing to decreased photosynthesis in Sundarbans mangroves is the inhibitory effect of salt on the efficiency of translocation and assimilation of photosynthetic products (Demiral *et al*. 2005).

On the contrary, in *B. gymnorrhiza*, *E. agallocha* and *P. paludosa*, the optimum PAR acquisition was higher in Sundarbans than that in the garden plants, whereas, the peak photosynthesis rates were higher in the non-saline soil. In Sundarbans however, despite tidal ebb and flow, high salinity makes the substrate physiologically dry. In order to check desiccation and xylem embolism, mangrove leaves reduce the rate of water efflux (Nandy and Ghose 2001) that may enhance the tendency to elevate leaf temperature with subsequent decline in photosynthesis. However, *B. gymnorrhiza*, *E. agallocha* and *P. paludosa* grown under salt stress, are better adapted to withstand restricted water efflux and considerably high irradiance for a long period and leaf temperature does not rise high enough to such an extent that inhibits photosynthesis. This accords well with the optimum irradiance for photosynthesis measured in *Bruguiera gymnorrhiza* at Durban Bay site (around 1000 ìmol $m^{-2}s^{-1}$) (Naidoo *et al*. 2002). In contrast, *H. fomes* and *X. granatum* exhibited just an opposite trend that can explain less affinity of these species towards high salinity, irradiance and temperature prevailing in the Sundarbans forest. Kiato *et al*. (2003) placed *B. gymnorrhiza* as the shade tolerant, intermediately adaptive species to strong light and *X. granatum* as the climax one. High photosynthetic capacity can theoretically increase water use efficiency as more carbon is assimilated per unit water transpired. Though Grassi *et al.* (2002) and Sefton *et al.* (2002) denied any relationship between net carbon assimilation and water use efficiency, in mangroves, a positive relation was reported between photosynthesis and stomatal conductance, which is an important determinant of water use efficiency (Nandy *et al*. 2005).

In the studied mangroves, specific leaf area decreased by 14% to 59% under salinity stress. Restricted water efflux and the necessity to conserve water renders the leaves succulent thus, increase leaf thickness. Increase in leaf succulence is a salt regulatory mode achieved by salt sequestration into hypodermal tissue (Werner and Stelzer 1990). The T_m: T_l values obtained clearly indicate that under high salinity, the increased leaf thickness

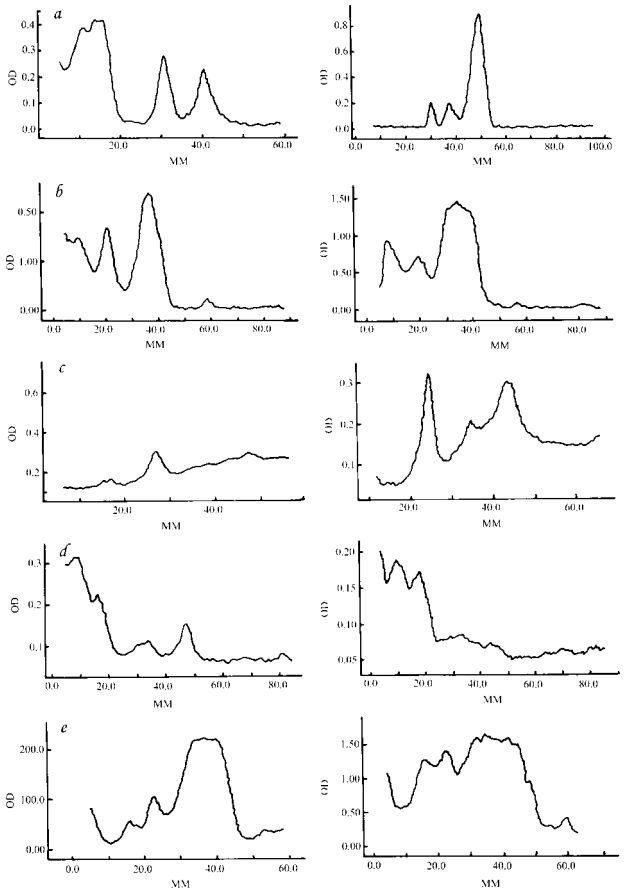

Fig. 4. Densitometric analysis of PAGE – Acid Phosphatase. Graphs in left side of each pare indicate sample from saline habitat and in right side from non-saline habitat. 1. *B. gymnorrhiza*, 2. *E. agallocha*, 3. *H. fomes*, 4. *P. paludosa* and 5. *X. granatum*.

can be attributed to the water storage hypodermal tissue rather than the mesophyll bulk. Thus, salinity induced increase in leaf thickness and fresh weight due to water storage probably contribute to the decrease in SLA. In Sundarbans, the various constraints imposed by salinity upon water acquisition and CO_2 availability leads to reduction in SLA that accord well with Schulze *et al.* (1998). This in turn, can explain the decrease in net assimilation rate in Sundarbans mangroves as compared to their non-saline counterparts. SLA in the present study varied with leaf thickness that can explain a high leaf N concentration in thick leaves of Sundarbans mangroves instead of their low specific leaf area.

During photosynthesis, pyruvate and oxaloacetate are transaminated to form alanine and aspartic acid respectively to serve as the carbon source for other metabolites, thereby increasing the concentration of these amino acids in leaf cells. The abundance of these amino acids in mangrove leaves grown under *in situ* condition supports the view of Bhosale (1985) that the maximum percentage of foliar nitrogen in most mangroves remains coupled with aspartate and alanine. In mangroves, osmotic adjustment is apparently achieved by synthesis of some compatible solutes, which can exist in the cell sap at high concentration without interfering cell metabolism (Flowers *et al*. 1977). Accumulation of such osmoprotectants, especially in the cytosol, chloroplasts and mitochondria minimizes water loss from the leaf cells under salinity stress (Heldt 1999). Free amino acids are such osmoticum that are accumulated in leaves of mangroves in order to maintain high negative leaf OP. Free amino acid contents vary widely in leaf cells, depending on the species and the metabolic conditions. Present result revealed that salinity enhances amino acid biosynthesis in plants which was supported the earlier work (Joshi *et al* 1962).

As salinity increased, considerable intraspecific difference was observed in isoforms patterns of peroxidase and acid phosphatase. In the both habitats, *B. gymnorrhiza* and *P. paludosa* exhibited two isoforms of peroxidase, but their distribution patterns were different. In *E. agallocha*, a single band of peroxidase was detected in non-saline samples whereas two more isoforms were induced in saline condition. Likewise in *H. fomes*, number of peroxidase isoforms increased under stress. The situation was more prominent in *X. granatum* collected from Sundarbans where three more isoforms were added to the single one obtained in the garden sample. Reverse adaptation is prominent in acid phosphatase profiles of *H. fomes* and *P. paludosa*; shift to non-saline substrate cost synthesis of an extra isoforms in both the species. In *B. gymnorrhiza* and *E. agallocha* however, salinity induced one more isoforms in each species. Intraspecific esterase profile differed significantly in *H. fomes* and *P. paludosa* owing to reverse adaptation; instead of a single band as obtained in the saline sample, extra bands appeared in non-saline condition. Interestingly, in *E. agallocha* and *B. gymnorrhiza* esterase profiles were similar in both the habitats though in the latter one band intensity decreased in non-saline condition. In *X. granatum* however, single band of higher intensity appeared in nonsaline condition in contrast to two separate peaks of lower intensity under salinity stress. To summarise, some isoforms are induced or stimulated under salt stress, whereas some are suppressed. Isoform, where induction or stimulation occurs in exposure to salt can be notified as a stress marker. For further inference, molecular weight of the isoforms and other characterizations are yet to be determined.

Thus, the present investigation indicates that all the five mangroves studied can survive well in non-saline soil, though efficiency to utilize high irradiance coupled with high soil salinity and limited CO_2 availability is more in *B. gymnorrhiza*, *E. agallocha* and *P. paludosa.* This study points to the gradual decline of *H. fomes* in highly saline substrate of the Indian Sundarbans, as also previously reported by Khan (1977) and Zabala (1990). Depending on the other parameters studied e.g. total chlorophyll, mesophyll conductance, specific leaf area and stomatal conductance it can also be inferred that at least these mangroves are not salt loving, but salt tolerant.

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