Estimation of osmotic potential and free amino acids in some mangroves of the Sundarbans, India

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Osmotic potential (OP) of root and leaf was measured in 19 species of mangroves collected from the Sundarbans, India. Leaf OP was estimated in 11 taxa from among them, grown in fresh water and compared with that deriving from taxa grown in saline water. Free amino acids were estimated from leaves of 16 mangrove species of the Sundarbans. The osmotic potential in leaves collected from their natural habitat was more negative than that from those grown in fresh water conditions. Seedlings grown without salt provided less negative leaf OP than those treated with $0.1 \, mol \, dm^{-3}$ NaCl. The more negative OP was found in more saline soil, which might be related to adaptation to facilitate water uptake from a highly saline and frequently waterlogged anaerobic substratum. Aspartic acid, alanine, proline, tryptophan, tyrosine and phenylalanine are the major free amino acids detected in the leaves. Leucine was recorded only in *Avicennia* spp., where phenylalanine content was negligible. Proline content was estimated in seven species. The positive linear trend obtained between leaf OP and proline content points to its role as an osmoticum in mangrove leaves. Cysteine, aspartic acid, alanine and phenylalanine were determined in seedlings, while proline could be estimated only in *X. mekongensis*. Cysteine and proline content in seedling leaves were gradually reduced with increasing soil salinity. Lack of cysteine in mature leaves and its decrease in more saline soil probably indicates salinity as a photorespiration restricting factor. A more or less inverse relationship was found between proline and alanine content both in seedlings and mature plants.

Key words: Mangroves, osmotic potential, osmoticum, soil salinity, water uptake.

Introduction

Mangroves are a group of halophytes, which grow in the intertidal saline swamps of tropical and subtropical coasts. These forests are subjected to varying degrees of water-logging owing to regular tidal inundation. Despite apparently effective systems of root ventilation in mangroves (TOMLINSON 1986) their growth and functioning may be adversely affected by the waterlogged substrate. Flow of water from soil into plant root depends on the difference in water potential between the soil solution and plant cell sap. Due to the pres-

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ence of excessive dissolved salts, the water potential of littoral soil is highly negative, and this prevents water uptake. Tropical rainforest trees may rarely experience water potential more negative than –2 MPa, whereas a mangrove would never have a water potential less negative than that of sea–water, i.e. –2.5 MPa (Sperry et al. 1988). Naidoo (1985) reported that the rate of water uptake is lower in waterlogged mangroves than in those grown under well-drained condition. To cope with the situation, mangroves have to maintain a high negative osmotic potential in their leaf cells to absorb water and to raise the waterstream through the xylem vessels. Typically, the leaf water potential in mangroves varies between –2.5 to –6 MPa (Scholander et al. 1964, Scholander 1968, Naidoo 1989, Rada et al. 1989, Smith et al. 1989, Sternberg et al. 1991).

In order to maintain such a high negative OP, mangrove leaves have to deposit some solutes in their cytosol. Excessive salt accumulation is deleterious for living cell metabolism, as it dehydrates the cytosol and denatures several essential metabolic enzymes. Leaf salt concentrations are regulated in mangroves through dilution (succulence) or excretion through salt glands (Tomlinson 1986). Therefore, 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). Proline is such an osmoticum that is 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. Joshi et al. (1962) reported that salinity enhances amino acid biosynthesis and inhibits organic acid synthesis.

In the present article, some mangrove taxa from the Sundarbans, India, have been investigated from the point of view of their osmotic potential and the free amino acid contents in the leaves. An attempt has been made to determine the difference between root and leaf OP and to compare the leaf OP values in saline and fresh water conditions. Free amino acids were estimated in leaves of mature plants grown in their natural habitat as well as in some seedlings treated with different NaCl gradients.

Materials and methods

Roots and leaves of 19 species belonging to 11 families of mangroves were collected from different islands of the Sundarbans forest (Tab. 1). Leaves were also collected from 11 mangrove taxa, grown to maturity under fresh water conditions and two sets of potted seedlings (twelve weeks old) treated with 0 and 0.1 mol dm^{-3} NaCl respectively.

One gram of root / leaf sample was immediately crushed after collection and the extract was preserved in airtight tubes. Another 1g was kept in a hot air oven in order to record the dry weight. The extract was homogenised with deionised water and filtered. The filtrate was centrifuged at 6000 g (628.57 radian s^{-1}), for 30 minutes and the soup volume was made up to 25 mL adding deionised water. Electrical conductance of the solution was measured with a specific conductivity meter (Systronics, Direct Reading Conductivity Meter 304) at room temperature (23 °C) and the value was converted to osmotic potential (OP) following the formula:

$$OP \text{ (–bars)} = (EC \times 0.36 \times df) \quad 0.987$$

EC = Electrical conductance (dS m^{-1}) of the plant extract at 23 °C; df (dilution factor) = 25 mL (filtrate) × 1g (fresh weight) ¸ moisture content (fresh weight – dry weight) g^{-1} leaf tissue; 0.987 = factor for converting atmospheric pressure into bars (JANARDHAN et al.1975). OP values obtained thereby were discussed in relation to the ratio between leaf thickness and colourless zone (T_l:C_z) given by DAS and GHOSE (1996) (Fig. 2).

Free amino acids were estimated in leaves of 16 mangrove taxa from the Sundarbans as well as in three species of seedling leaves nourished with 0, 0.1 and 0.3 mol dm^{-3} NaCl.

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 subjected to paper chromatography (JAYARAMAN 1999) using a mixture of 4:1:1 n-butanol, acetic acid and distilled water as the running solution. In the chromatogram, greyish purple, bluish purple and yellow spots appeared for different amino acids, which were identified from their standard R_f values (JAYARAMAN 1999). These spots were eluted by 80 % ethanol and optical density was 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 R_f values were very close to each other, confirmatory tests (Hopkins-Cole test for tryptophan and Millon's reaction for tyrosine) were performed following JAYARAMAN (1999). The value for each species (Tabs. 1–3) is the average of nine observations, *i.e.* three replica from each plant and three plants of each species.

Results and Discussion

Leaf and root OP of the investigated taxa (natural habitat) ranged between –7.65 MPa (*Suaeda maritima*) to –1.22 MPa (*Sonnerata apetala*) and –3.41 MPa (*S. maritima*) to –0.61 MPa (*Exoecaria agallocha*) respectively (Tab. 1). In most mangrove leaves, the hypodermis is composed of one or more layers of colourless water storage cells below the adaxial epidermis (TOMLINSON 1986). DAS and GHOSE (1996) measured the leaf thickness and the thickness of this non-chlorophyllous zone in leaves of some mangrove taxa of the Sundarbans. Osmotic potential was remarkably high (> –3 MPa) mostly in the leaves where water storage tissue (C_z) content is much less with respect to the leaf thickness (T_l), *i.e.* (T_l: Cz > 6) as found in *Aegialitis rotundifolia, Bruguiera gymnorrhiza, B. sexangula, Ceriops decandra, C. tagal, Exoecaria agallocha* and *Suaeda maritima* (Fig. 2). In contrast, –1.2 MPa to –2 MPa OP were recorded in leaves of *Avicennia alba, A. officinalis, Nypa fruticans, Sonneratia apetala, Xylocarpus granatum* and *X. mekongensis*, which have a relatively higher amount of water storage tissue compared to leaf thickness (T_l: Cz < 5) (Fig. 2). In the latter plants, the high solvent concentration in leaves is probably due to the less negative leaf OP, while it increases as water content decreases in the former species. However, a reverse trend was noticed in *Acanthus ilicifolius, Aegiceras corniculatum, Heritiera fomes, Phoenix paludosa* and *Rhizophora mucronata* (Fig. 2).

Considerable differences between leaf and root OP were recorded in most of the Sundarbans mangroves, creating the tensile strength that maintains a constant upward flow of water through the xylem vessel, except in *N. fruticans* and *S. apetala* (Fig. 1). Interestingly, in *S. apetala*, the difference was lowest (–0.16 MPa), though it is a tall tree, while the maximum difference was recorded in the herbaceous species *S. maritima* (–4.24 MPa).

Tab.1. Root and leaf osmotic potential of some mangroves in different soil salinities.

Name of the species	Family	Leaf OP (MPa)			
		S	**G**	**Seedling**	
		0.25–0.4 [#]	0.03 [#]	0 [#]	0.1 [#]
1. *Acanthus ilicifolius* L.	Acanthaceae	−2.33 (0.79)	−1.02 (1.55)	−0.68 (0.11)	–
2. *Aegialitis rotundifolia* Roxb.	Plumbaginaceae	−4.02 (1.82)	–	–	–
3. *Aegiceras corniculatum (L)Blanco.*	Myrsinaceae	−3.50 (0.66)	–	–	–
4. *Avicennia alba Blume.*	Avicenniaceae	−1.95 (0.68)	–	–	–
5. *A. marina (Frosk) Vierh.*	Avicenniaceae	−3.64 (1.90)	−1.83 (2.93)	–	–
6. *A. officinalis* L.	Avicenniaceae	−2.16 (0.09)	–	−1.24 (0.59)	–
7. *Bruguiera gymnorrhiza* (L) Lam.	Rhizophoraceae	−3.77 (2.53)	−1.83 (0.41)	−1.40 (0.81)	−2.10 (0.08)
8. *B. sexangula* W & A	Rhizophoraceae	−5.16 (2.37)	−2.13 (1.17)	−1.03 (0.55)	–
9. *Ceriops decandra* (Grif.) D.H.	Rhizophoraceae	−4.30 (2.10)	−2.74 (1.38)	–	–
10. *C. tagal* (Pierr.) Robins.	Rhizophoraceae	−3.44 (1.19)	−2.37 (0.65)	–	–
11. *Exoecaria agallocha* L.	Euphorbiaceae	−3.24 (2.06)	−1.62 (0.76)	−1.10 (1.02)	–
12. *Heritiera fomes* (Buch.) Ham.	Sterculiaceae	−2.58 (0.88)	−1.48 (0.83)	−1.19 (0.68)	−2.38 (1.07)
13. *Nypa fruticans** (Thunb.) Wurmb.	Arecaceae	−1.89 (1.48)	–	–	–
14. *Phoenix paludosa** Roxb.	Arecaceae	−4.05 (2.97)	−1.43 (1.19)	–	–
15. *Rhizophora mucronata* Lam.	Rhizophoraceae	−2.25 (1.71)	−1.48 (0.58)	–	–
16. *Sonneratia apetala* Buch. Ham.	Sonneratiaceae	−1.21 (0.36)	–	–	–
17. *Suaeda maritima* Dumort.	Chenopodiacee	−7.65 (2.79)	–	–	–
18. *Xylocarpus granatum* Koenig.	Meliaceae	−1.45 (0.46)	–	–	–
19. *X. mekongensis* Pierree.	Meliaceae	−1.96 (1.32)	−0.93 (0.81)	−1.49 (1.05)	−2.11 (0.59)

N.B. S = Sundarbans; G = Garden; figures within parenthesis indicates +/– standard deviation; * Monocot species; [#] Soil salinity (mol dm^{-3}). Each value presented in the table is the average of nine observations (three replica from each plant and three plants from each species). Corresponding data for garden plants and seedlings for all species could not be estimated due to the unavailability of materials.

Soil salinity in the creeks of the Sundarbans generally ranges from 0.25 to 0.4 mol dm^{-3} during tidal fluctuations, while that of garden soil is 0.03 mol dm^{-3}. Due to the enhanced salinity level in the soil, leaf OP was more negative in plants collected from their natural habitats than in those grown under freshwater conditions (Tab. 1). A similar trend was also noticed in the seedlings. Those treated with 0.1 mol dm^{-3} NaCl measured more negative leaf OP than that of control (0 mol dm^{-3}) (Tab. 1). Thus, the higher negative osmotic potential points to an adaptation to salt stress and the prevailing anaerobic condition of the substratum.
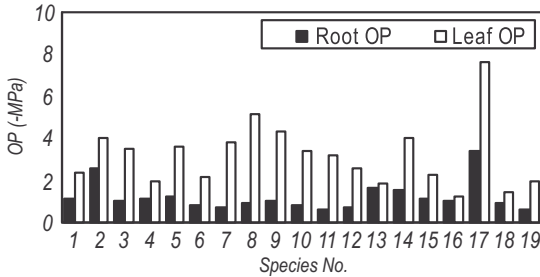

Fig. 1. Difference between root and leaf osmotic potential (OP). Species numbers follow Tab. 1.
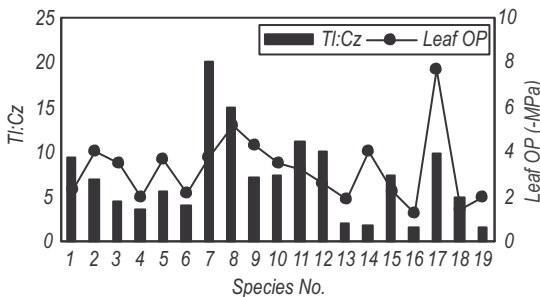

Fig. 2. Leaf osmotic potential (OP) vs Tl:Cz in some mangroves. Species numbers follow Tab. 1.

The major free amino acids obtained in the leaves of the Sundarbans mangroves are aspartic acid, alanine, proline, tryptophan, tyrosine, leucine and phenylalanine (Tab. 2). Considerable amounts of aspartate and alanine were detected in all the investigated taxa. 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 (Tab. 2) supports the view of BHOSALE (1985) that the maximum percentage of foliar nitrogen in most mangroves remains coupled with aspartate and alanine. Phenylalanine was obtained almost in all the species except *Avicennia* spp., where leucine is detected in stead (Tab. 2). Tryptophan was present in most of the species, except *Avicennia* spp., *E. agallocha* and *R. mucronata,* while tyrosine was detected only in *Bruguiera* spp, *C. decandra* and *E. agallocha*. In the course of photosynthesis, phenylalanine, tryptophan and tyrosine are produced from enolpyruvylshikimate-3-phosphate (EPSP), a condensation product of shikimate and phosphoenol pyruvate. Free proline was

Tab. 2. Free amino acids (g g^{-1} FW) in some Sundarbans mangroves

Species	Asp	Ala	Pro	Trp	Tyr	Leu	Phe
Acanthus ilicifolius	12.2 (2.05)	14 (4.02)		1.72 (0.70)	–	–	1.44 (0.77)
Aegialitis rotundifolia	32 (1.97)	20 (1.69)	–	2.70 (0.19)	–	–	2.71 (1.15)
Aegiceras corniculatum	17 (1.33)	13 (0.06)		1.83 (1.08)	–	–	2.50 (1.32)
Avicennia alba	13.5 (4.74)	16 (1.73)	7 (1.59)	–	–	1.35 (0.31)	–
A. marina	24 (2.07)	12.5 (3.14)	5.5 (3.04)	–	–	2 (1.02)	–
A. officinalis	18.1 (3.63)	14.3 (1.97)	7.5 (0.63)	–	–	1.54 (0.41)	–
Bruguiera gymnorrhiza	2.2 (1.09)	16 (3.57)	–	0.75 (0.32)	1 (0.62)	–	2.11 (0.02)
B. sexangula	1.8 (0.06)	14 (1.58)	9.5 (2.12)	1.21 (0.31)	1.30 (1.03)	–	2 (0.68)
Ceriops decandra	17.3 (1.48)	8 (0.06)	–	1 (0.01)	1.88 (0.76)	–	1.41 (0.97)
Exoecaria agallocha	45.4 (2.49)	12 (3.06)	–	–	2 (0.20)	–	1.37 (1.04)
Heritiera fomes	23 (3.01)	13.5 (1.19)	–	2.50 (0.59)	–	–	1.92 (0.35)
Nypa fruticans	12 (1.44)	11.2 (0.08)	–	1.33 (0.43)	–	–	1.30 (0.62)
Phoenix paludosa	13.5 (4.07)	9.1 (2.68)	17.5 (3.85)	1.28 (0.64)	–	–	0.80 (0.03)
Rhizophora mucronata	6.7 (1.38)	6 (1.76)	–	–	–	–	1.59 (1.07)
Xylocarpus granatum	15 (1.42)	15 (3.43)	5 (2.08)	2.39 (1.07)	–	–	3.22 (1.29)
X. mekongensis	32 (3.21)	18.2 (2.65)	7 (0.11)	4 (1.62)	–	–	2.76 (1.38)

N.B. Values within parenthesis indicate +/– standard deviation.

detected in *Avicennia* spp, *B. sexangula, P. paludosa* and *Xylocarpus* spp. In *P. paludosa*, proline content was relatively high while alanine concentration is low, whereas a lower concentration of proline was estimated in *Avicennia* spp*., B. sexangula* and *Xylocarpus* spp., with a relatively high alanine content (Tab. 2). Where present together, these two amino acids showed a reverse trend while plotted against leaf OP (Fig. 3).

In seedling leaves, cysteine, aspartate, alanine and phenylalanine were obtained. Proline was detected only in *X. mekongensis*. As NaCl was enhanced in the soil, both cysteine and proline decreased gradually with an overproduction of alanine (Tab. 3). During photosynthesis in C$_3$ plants, as the O$_2$/CO$_2$ ratio increases inside the chloroplast,
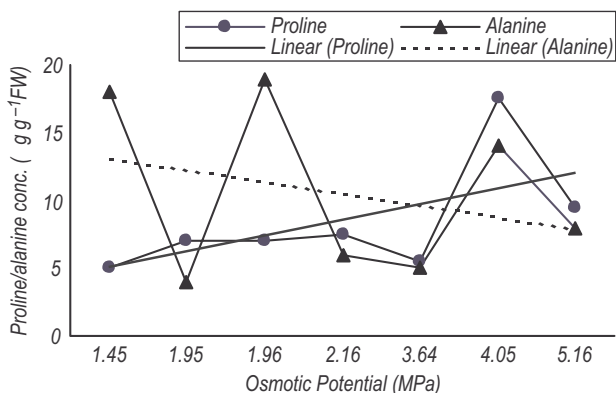
Fig. 3. Proline and alanine content in some mangroves. Data follow Tab. 2.

RUBISCO switches to oxygenase activity, leading to photorespiration. As a result, ribulose di-phosphate is converted to phosphoglycolate, which in turn produces serine, the precursor of cysteine. The gradual decrease in cysteine concentration with an increase in NaCl in the soil and the absence of free cysteine in mature plants probably indicates that photorespiration has either ceased or the rate is insignificant in mangroves under salinity stress. Mangroves restrict stomatal conductance in order to increase water-storage efficiency (NANDY DATTA and GHOSE 2001). At lower conductance, the intercellular CO_2 concentra-

Tab. 3. Free amino acids in seedling leaves (μg g^{-1} FW)

Species	Salinity (mol dm^{-3})	Cys	Asp	Ala	Pro	Phe
Bruguiera gymnorrhiza	0	0.7 (0.1)	1.5 (1.07)	9.5 (0.2)	–	1 (0.02)
	0.1	0.2 (0.01)	2.3 (0.4)	12 (1.4)	–	1.7 (0.4)
	0.3	0.02 (0.01)	2.5 (0.4)	14.3 (1.9)	–	2.3 (1.03)
Heritiera fomes	0	0.5 (0.03)	3 (1.1)	7 (0.03)	–	1.4 (0.6)
	0.1	0.3 (0.04)	5.1 (1.08)	9.5 (2.4)	–	1.8 (0.9)
	0.3	0.09 (0.08)	5.3 (0.6)	14.3 (1.6)	–	1.9 (1.08)
Xylocarpus mekongensis	0	1.3 (0.02)	3.4 (0.8)	13 (0.4)	1.5 (0.06)	0.2 (0.01)
	0.1	0.3 (0.1)	4.7 (2.6)	19.6 (3.1)	7.5 (1.4)	2.6 (1.06)
	0.3	0.12 (0.01)	6.9 (1.5)	22.7 (2.09)	5.5 (1.6)	2.9 (0.02)

N.B. Values within parenthesis indicate +/– standard deviation.

tion increases across stomata (Mulkey et al. 1996), so that the O_2: CO2 partial pressure in leaf cells probably never exceeds the threshold value beyond which photorespiration would take place.

The positive linear relation obtained between osmotic potential and proline concentration (Fig. 3) in *Avicennia* spp.*, B. sexangula, P. paludosa* and *Xylocarpus* spp. indicates that free proline acts as a major osmoprotectant at least in these mangrove taxa under moderate salinity stress. Heldt (1999) proposed that during water stress synthesis of pyrroline 5-carboxylate reductase induces the accumulation of proline in leaf cells. Thus, changes in cell turgor may activate and control the degree of proline synthesis to provide a water potential balance between cytoplasm and the surrounding environment of water stress. In addition to the reverse trend of proline and alanine (Fig. 3) and the elevated proline level estimated in species with sufficiently low alanine concentration and vice versa (Tab. 2), NaCl shock induced alanine in seedling leaves, while reducing proline content in *Xylocarpus mekongensis* (Tab. 3). This indicates that, free alanine may to some extent, substitute free proline under salt stress.

Das and Ghose (1996) reported that *Nypa fruticans* shows a thin cuticle, absence of water storing terminal tracheoids and loosely arranged mesophyll tissue in its leaves. These structural inadequacies, in addition to lack of proline content, considerably lower leaf OP (–1.89 MPa) than many of the other taxa studied and a rather low difference between leaf and root OP (–0.23 MPa) may indicate the probable reason for the gradual extinction of this species from the extremely saline soil of the Indian part of the Sundarbans.

Conclusion

The present investigation may throw some light on the adaptability of the studied taxa in conditions of salt stress. High negative leaf OP points to their efficiency in water uptake from an extremely saline substrate with a water potential as high as that of seawater. Osmotic potential is more negative mostly in leaves where the hyaline endodermal water storage tissue content is less. The positive linear relation between leaf OP and proline concentration establishes the fact that proline acts as an osmoticum in some of the studied taxa. Free alanine may substitute free proline to some extent under extreme salt stress. In seedling leaves, the gradual reduction in cysteine content due to NaCl shock indicates that photorespiration is reduced in conditions of enhanced salinity, another adaptation to salt stress.

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