Research Article

How Bruguiera gymnorhizza seedlings respond to climate change induced salinity rise?

Prosenjit Pramanick¹, Subrata Trivedi², Sufia Zaman¹ and Abhijit Mitra³*

¹Department of Oceanography, Techno India University West Bengal, Salt Lake Campus, Kolkata 700091, India
²Department of Biology, Faculty of Science, University of Tabuk, Ministry of Higher Education, Kingdom of Saudi Arabia
³Department of Marine Science, University of Calcutta, 35 B.C. Road, Kolkata 700019, India

Abstract

A study was undertaken during August 2017 to evaluate the effect of salinity on chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid and proline contents of hydroponically grown seedlings of Bruguiera gymnorhizza. The primary aim was 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 2.39 to 3.71 throughout 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 Bruguiera gymnorhizza 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

Bruguiera gymnorhizza belongs to the "true mangroves" and is very common in the Orient. The name gymnorhizza comes from two Greek words “gymno” naked and “rhizza” root, naked root which refers to the exposed knee roots of Bruguiera gymnorhizza emerging from the ground. The main difference from other Bruguiera species is that Bruguiera gymnorhizza has the largest leaves, flowers, propagules and lenticels of all Bruguiera species. The name Large-Leafed Orange Mangrove of Bruguiera gymnorhizza comes from the orange flowers and the large leaves that can reach up to 25 cm in length.

Salinity plays a crucial role in the growth and survival of mangroves. However saline condition is not a pre-requisite for their development, rather mangroves choose saline condition to avoid the competition with the more vigorous terrestrial plants. Based on the physiological studie [1,2] 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 [3]. However no study has yet been carried out on the effect of salinity fluctuation on the biochemical components of mangroves under culture conditions.
How Bruguiera gymnorhizza seedlings respond to climate change induced salinity rise?

from this part of the Indian subcontinent. The effects of salinity on mangroves have been studied in relation to antioxidative enzymes [4,5], leaf structure, rates of transpiration, stomatal conductance and rates of photosynthesis [6,7] and changes in chloroplast structure and function [8,9] 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 [10]. Further, one of the biochemical mechanisms by which mangroves counter the high osmolarity of salt was accumulation of compatible solutes [4]. 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 this paper, we present the effect of increasing salinity on pigments and proline content of hydroponically grown seedlings of Bruguiera gymnorhizza with an aim to obtain insights into the changes in these biochemical components with salt acclimation.

Materials and Methods

Plant materials and culture conditions

Seedlings of Bruguiera gymnorhizza were collected from Sundarbans mangrove ecosystem of India (22°16′40.6″ N latitude and 88°38′18.4″ E longitude) during August, 2017. They 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⁻²s⁻¹. The preliminary experiments were carried out in the selected species at five different salinities (2psu, 5psu, 10psu, 15psu and 20psu 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 14h photoperiod at PAR of 300 μmol m⁻²s⁻¹, 26±3°C and 80% RH. The culture medium was changed every 7days. Leaves were harvested at 7, 14, 21 and 30 days intervals to measure the pigment and proline concentrations.

Extraction and estimation of pigments

Leaves (0.5g) were homogenized in chilled N, N-dimethylformamide (DMF) in a mortar and pestle in dark at 4°C and the homogenates were centrifuged at 8800×g for 10 min. The supernatants were collected and absorption spectra at 663.8 and 646.8nm were recorded using Jasco V-530 UV–vis spectrophotometer for estimation of chlorophyll a, chlorophyll b and total chlorophyll following the procedure of [11]. For estimation of total carotenoids, leaf tissues (0.5g) were homogenized in chilled 80% (v/v) acetone and the homogenates were centrifuged at 8800×g for 10 min at 4°C in the dark. The absorbance of the acetone extracts was measured at 663, 645 and 470 nm. Total carotenoids were calculated according to [12].

Estimation of free proline

Free proline content was measured from leaf using 3% sulphosalicylic acid following the method of [13], 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 [14].
How Bruguiera gymnorhizza seedlings respond to climate change induced salinity rise?

Results

All the collected seedlings of *Bruguiera gymnorhizza* could tolerate salinity up to 20psu 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 considerably (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 2.39 to 3.71 (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 [15].

Till date there have been few studies on the effect of salinity on photosynthetic gas exchange in mangroves. [16] 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

### Table 1: Effects of different salinities on pigment level and proline concentration in *Bruguiera gymnorhizza*.

| Duration of treatment (d) | Salinity (psu) | Chl a | Chl b | Total Chlorophyll | Chl a:b | Carotenoid | Proline |
|--------------------------|---------------|-------|-------|------------------|---------|------------|---------|
| 7                        | 2             | 0.67±0.03ab | 0.28±0.007ab | 0.95±0.03ab | 2.39     | 0.23±0.03a | 1.20±0.3a |
|                          | 5             | 0.64±0.01a  | 0.24±0.004a  | 0.88±0.03a  | 2.67     | 0.19±0.03a | 1.30±0.3a |
|                          | 10            | 0.73±0.01ab | 0.26±0.006ab | 0.99±0.03ab | 2.81     | 0.24±0.03a | 1.40±0.3a |
|                          | 15            | 0.74±0.02ab | 0.27±0.008bc | 1.01±0.03ab | 2.74     | 0.21±0.03a | 1.70±0.3a |
|                          | 20            | 0.78±0.03b  | 0.21±0.006c  | 0.99±0.03b  | 3.71     | 0.22±0.03a | 1.50±0.3a |
| 14                       | 2             | 0.69±0.02ab | 0.26±0.005ab | 0.95±0.03ab | 2.65     | 0.24±0.03a | 1.40±0.3a |
|                          | 5             | 0.64±0.04a  | 0.21±0.005a  | 0.85±0.03a  | 3.05     | 0.19±0.03a | 1.20±0.3a |
|                          | 10            | 0.66±0.02ab | 0.20±0.004ab | 0.86±0.03ab | 3.30     | 0.16±0.03a | 1.50±0.3a |
|                          | 15            | 0.68±0.03ab | 0.27±0.007bc | 0.95±0.03ab | 2.52     | 0.22±0.03a | 0.70±0.3a |
|                          | 20            | 0.70±0.04b  | 0.29±0.009c  | 0.99±0.03b  | 2.41     | 0.21±0.03a | 1.30±0.3a |
| 21                       | 2             | 0.69±0.01ab | 0.28±0.003ab | 0.97±0.03ab | 2.46     | 0.20±0.03a | 1.60±0.3a |
|                          | 5             | 0.65±0.01a  | 0.23±0.004a  | 0.87±0.03a  | 2.83     | 0.18±0.03a | 0.60±0.3a |
|                          | 10            | 0.67±0.02ab | 0.20±0.003ab | 0.87±0.03a  | 3.35     | 0.17±0.03a | 1.90±0.3a |
|                          | 15            | 0.71±0.02ab | 0.25±0.005bc | 0.96±0.03ab | 2.84     | 0.13±0.03a | 1.50±0.3a |
|                          | 20            | 0.68±0.04b  | 0.27±0.005c  | 0.95±0.03b  | 2.52     | 0.15±0.03a | 1.30±0.3a |
| 30                       | 2             | 0.74±0.04ab | 0.27±0.002ab | 0.93±0.03ab | 2.74     | 0.24±0.03a | 1.40±0.3a |
|                          | 5             | 0.65±0.01a  | 0.23±0.002a  | 0.85±0.03a  | 2.83     | 0.19±0.03a | 1.70±0.3a |
|                          | 10            | 0.68±0.02ab | 0.22±0.004ab | 0.79±0.03ab | 3.09     | 0.21±0.03a | 1.50±0.3a |
|                          | 15            | 0.70±0.04ab | 0.29±0.007bc | 0.91±0.03ab | 2.41     | 0.17±0.03a | 1.80±0.3a |
|                          | 20            | 0.73±0.04b  | 0.25±0.009c  | 0.90±0.03b  | 2.92     | 0.22±0.03a | 1.20±0.3a |

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 \(p \leq 0.01\).

### Table 2: Inter-relationships between salinity and selected pigments in *Bruguiera gymnorhizza*.

| Combination               | \(r\) value | \(p\) value |
|---------------------------|-------------|-------------|
| Salinity\times Chl a      | 0.6943      | <0.01       |
| Salinity\times Chl b      | 0.8568      | <0.01       |
| Salinity\times Total Chl  | 0.7034      | <0.01       |
| Salinity\times Carotenoid | 0.07650     | IS          |
| Salinity\times Proline    | -0.1655     | IS          |

IS means insignificant?
How Bruguiera gymnorrhiza seedlings respond to climate change induced salinity rise?

Published: September 22, 2017

Salts of brackish water constant unlike several previous studies where the plants were exposed to different NaCl concentrations [17,18] that are not the real image of ambient seawater. Various studies have shown that a number of mangrove species grow best at salinities between 4psu and 15psu [19,14,20-22] and for Heritiera fomes, the preferred salinity range is much lower [23]. In this context, Bruguiera gymnorrhiza seems to be a befitting 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 [24,25].

Proline accumulation is a common phenomenon in halophytes. As Bruguiera gymnorrhiza 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 [26,27]. Thus, we monitored the proline levels in leaves of the species treated with 2,5,10,15,20psu 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 [26-28]. 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 [26]. It appears that the enzyme remain unaffected in Bruguiera gymnorrhiza even under high saline condition which is a unique adaptive mechanism.

Our results show that Bruguiera gymnorrhiza 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.

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 km² in the Indian part and 4500 km² 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 [15] due to complete obstruction of the freshwater supply of Ganga-Bhagirathi-Hooghly River as a result of heavy siltation since the 15th century [23] and rising sea level [29] 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 [30]. 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 [31-33].

In summary, the results of the present study show that the mangrove Bruguiera gymnorrhiza can easily be propagated under high salinity conditions and may be a better suited species for central sector of Indian Sundarbans where the environment is hypersaline in nature. The present study is relevant from the point of adaptation of the species to sea level rise and subsequent saline water intrusion into the islands of Indian Sundarbans. This deltaic system in the lower Gangetic region is vulnerable to climate change related effects owing to its location below the mean sea level and experiencing a sea level rise of 3.14 mm/yr.
How Bruguiera gymnorhiza seedlings respond to climate change induced salinity rise?

References

1. Bowman. Mangrove regeneration and management. In: AKF Hoque, 1995. Mimeograph. 1997.

2. Davis JH. The Ecology and Geological Role of Mangroves in Florida. Pap Tortugas Lab. 1940; 32: 303-412. Ref.: https://goo.gl/5aVa4k

3. Mitra A, Banerjee K, Bhattacharyya DP. The other face of mangroves. Department of Environment. 2004.

4. Takemura T, Hanagata N, Sugihara K, Baba S, Karube I, et al. Physiological and biochemical responses to salt stress in the mangrove, Bruguiera gymnorrhiza. Aquatic Botany. 2000; 68: 15-28. Ref.: https://goo.gl/YMaFBS

5. Parida AK, Das AB, Mohanty P. Defense potentials to NaCl in a mangrove, Bruguiera parviflora: differential changes of isoforms of some antioxidative enzymes. J Plant Physiol. 2004; 161: 531-542. Ref.: https://goo.gl/31anu2

6. Santiago LS, Lau TSP, Melcher J, Steele OC, Goldstein G. Morphological and physiological responses of Hawaiian Hibiscus tiliaceus population to light and salinity. International Journal of Plant Science. 2000; 161: 99-106. Ref.: https://goo.gl/1CiW75

7. Parida AK, Das AB, Mittra B. Effects of salt on growth, ion accumulation, photosynthesis and leaf anatomy of the mangrove Bruguiera parviflora. Trees-Structure and Function. 2004; 18: 167-174. Ref.: https://goo.gl/TTwKue

8. Parida AK, Das AB, Mittra B. Effects of NaCl stress on the structure, pigment complex composition and photosynthetic activity of mangrove Bruguiera parviflora chloroplasts. Photosynthetica. 2003; 41: 191-200. Ref.: https://goo.gl/zF3XRX

9. Tanaka Y, Fukuda A, Nakamura A, Yamada A, Saito T. Molecular cloning and characterization of mangrove Na+/H+ antiporter cDNA. Plant Cell Physiology. 2000; 41: 79.

10. Hotta M, Nemoto S, Mimura T. Re-evaluation of role of vacuole during salt adaptation in higher plant cells. Plant Cell Physiol. 2000; 41: 79.

11. Porra RJ, Thompson WA, Kriendemann PE. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophyll a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochimica Biophysica Acta. 1989; 975: 384-394. Ref.: https://goo.gl/x4GYSU

12. Arnon DI. Copper enzymes in isolated chloroplasts, polyphenol oxidase in Beta vulgaris. Plant Physiol. 1949; 24: 1-15. https://goo.gl/pksav9

13. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant Soil. 1973; 39: 205-207. Ref.: https://goo.gl/hxsXPe

14. Sokal RR, Rohlf FJ. The Principles and Practice of Statistics in Biological Research. Biometry. 1995; 887.

15. Mitra A, Gangopadhyay A, Dube A, Schmidt CK, Banerjee K. Observed changes in water mass properties in the Indian Sundarbans (Northwestern Bay of Bengal) during 1980 - 2007. Current Science. 2009; 97: 1445-1452. Ref.: https://goo.gl/NnTykA

16. Clough BF. Effect of nutrient supply on photosynthesis in mangroves. In: L.J. Bhosale (ed.) The Mangroves. Proc Natl Symp Biol Util Cons. 1985; 80-88.

17. Mishra S, Das AB. Effect of NaCl on leaf salt secretion and antioxidative enzyme level in roots of a mangrove, Aegiceras corniculatum. Indian Journal of Experimental Biology. 2003; 41: 160-166. Ref.: https://goo.gl/oQAjxE

18. Netondo GW, Onyango JC, Beck E. Sorghum and salinity: I. Response of growth, water relations and ion accumulation to NaCl salinity. Crop Science. 2004; 44: 797-805. Ref.: https://goo.gl/Ct4DvH

19. Connor DJ. Growth of grey mangrove (Avicennia marina) in nutrient culture. Biotropica. 1969; 1: 36-40. Ref.: https://goo.gl/JdmXX4

20. Downton WJS. Growth and osmotic relations of the mangrove Avicennia marina, as influenced by salinity. Australian J Plant Physiol. 1982; 9: 519-528. Ref.: https://goo.gl/3kTsQ0

21. Burchett MD, Field CD, Pulkownik A. Salinity, growth and root respiration in the grey mangrove Avicennia marina. Physiologia Plantarum. 1984; 60: 113-118. Ref.: https://goo.gl/trHSSZ

22. Clough BF. Growth and salt balance of the mangroves Avicennia marina (Forsk.) Vierh, and Rhizophora stylosa Griff. in relation to salinity. Australian J Plant Physiol. 1984; 11: 419-430. Ref.: https://goo.gl/cyw3UR
23. Chaudhuri AB, Choudhury A. Mangroves of the Sundarbans. 1994; 1: 284. Ref.: https://goo.gl/wf1tQA

24. Gadallah MAA. Effects of proline and glycinebetaine on Vicia faba in response to salt stress. Biologia Plantarum. 1999; 42: 249-257. Ref.: https://goo.gl/QbLXJG

25. Agastian P, Kingsley SJ, Vivekanandan M. 2000. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica. 2000; 38: 287-290. Ref.: https://goo.gl/cYqKxh

26. Lee TM, Liu CH. Correlation of decreases calcium contents with proline accumulation in the marine green macroalga Ulva fasciata exposed to elevated NaCl contents in seawater. Journal of Experimental Botany. 1999; 50: 1855-1862. Ref.: https://goo.gl/ZwEzca

27. Hernandez S, Deleu C, Larher F. Proline accumulation by leaf tissues of tomato plants in response to salinity. C R Acad Sci III. 2000; 323: 551-557. Ref.: https://goo.gl/pDxE7k

28. Parida A, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove Bruguiera parviflora in hydroponic cultures. J Plant Biology. 2000; 45: 28-36. Ref.: https://goo.gl/7RNhA1

29. Hazra S, Ghosh T, Dasgupta R, Sen G. Sea level and associated changes in Sundarbans. Science and Culture. 2002; 68: 309-321. Ref.: https://goo.gl/MQjaUe

30. Lal M, Aggarwal D. Climate change and its impacts in India, Asia-Pacific Jr. Environment & Development. 2000; 7: 1-41.

31. Ellison JC. How South Pacific mangroves may respond to predicted climate change and sea level rise. In Climate Change in the South Pacific: Impacts and Responses in Australia, New Zealand and Small Islands States. Kluwer Academic Publishers. 2000. Ref.: https://goo.gl/XUjMMY

32. Ellison JC. Vulnerability of Fiji’s mangroves and associated coral reefs to climate change. Review for the World Wildlife Fund. 2004.

33. Ellison JC, Stoddart DR. Mangrove ecosystem collapse during predicted sea-level rise: Holocene analogues and implications. J Coastal Research. 1991; 7: 151-165. Ref.: https://goo.gl/EcT6fv