



Effects of Salinity Level on Biochemical Constituents of *Heritiera fomes* Buch-Ham. and *Excoecaria agallocha* L. at the Three Saline Zones of the Sundarban Mangrove Forest

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Abstract

Biochemical constituents *viz.* protein and proline (Amino acid) content of early (6, 9 months) and grown-up ages (30 months) of *Heritiera fomes* Buch-Ham and *Excoecaria agallocha* L. seedlings were investigated at three saline zones *viz.* Chandpai (Oligohaline), Jungra (Mesohaline) and Munshiganj (Polihaline) of the Sundarban mangrove forest. In the leaf of early ages *H. fomes* showed comparatively higher protein content when grown in oligohaline zone but *E. agallocha* showed highest protein content when grown in mesohaline zone. But at the grown-up age, both species showed lowest content of protein. On the other hand, proline content showed highest in the leaf of early stage of *H. fomes* seedlings at the polihaline zone. But in the stem and the root of *H. fomes* seedlings found relatively higher proline content when grown in oligohaline zone. *E. agallocha* showed relatively highest proline content in the early age stems at oligohaline zone. In the grown-up age comparatively highest proline content was found in all parts of both species at polihaline zone. At this unfavorable saline environment, the species survive by accumulation of osmolytes like proline which prevents water loss and ion toxicity. So, their adaptation to high salinity is closely associated with proline accumulation.

Subject Areas

Edaphology, Environmental Sciences

Keywords

Biochemical, Proline, Protein, Oligohaline, Mesohaline, Polyhaline

1. Introduction

The Sundarban is one of the largest natural mangrove forests of the world. It lies across the outer deltas of the Ganges, Brahmaputra and Meghna rivers and covers an area of 10,000 km² in the south western part of Bangladesh and West Bengal of India. It is situated in the southern part of Bangladesh between latitudes 21°31'N - 22°30'N and between longitudes 89°18'E - 90°18'E [1]. The Bangladesh portion of the Sundarban covers an area of 6017 km² [1] of which 1874 km² are made up by rivers, creeks and canals and remaining 4016 km² areas are occupied as forestland [2]. The Sundarban is crisscrossed by a network of complex estuarine system created by the different river systems. They carry a large amount of nutrient rich alluvial soil and sediments and enrich the productivity, expansion and dynamics of the forest area of this delta [3] [4]. These influence the growth of tree composition and quality of the Sundarban forest.

The natural vegetation of the forest is mainly composed of halophytic herbs, shrubs, climbers and tree species. The floristic compositions of the Sundarban are very rich compared to many other mangroves of the world. Among them the Sundarban mangrove forest is dominated by *Heritiera fomes* (Sundari) of Sterculiaceae and *Excoecaria agallocha* (Gewa) of Euphorbiaceae family. Besides these, they grow exclusively in these tidal areas in large stands or grove which reflect their own ecological community with halophytic or mangrove associations [5].

Salinity is a key factor for regulating growth and distribution of the mangroves. In the Sundarban mangrove forest, salinity content of rivers shows a special variability. Because it is a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal [2]. So, different saline concentration prevails between the western and eastern zones of the Sundarban forest as the sweet water influx in the west is much less, thus become more saline than that of east [6]. Based on the level of soil salinity [7], the Sundarban has been divided into three ecological zones, such as—Oligohaline or less saline zone (north and eastern part), soil salinity 4 - 8 dS⁻¹.m, Mesohaline or moderate saline zone (middle portion), soil salinity 8 - 15 dS⁻¹.m and Polyhaline or strong saline zone (south and western part), soil salinity > 15 dS⁻¹.m.

The mangroves inhabit in a sensitive ecosystem that link terrestrial and marine environments [8]. Mangroves are adapted to a harsh ecological condition with waterlogged soils, highly variable salinity and nutrients, loose substrate and tropical storm recurrence [9] [10]. Among them salinity is a major environmental stress that reduces plant growth through both ionic toxicity and osmotic stress. Salt induces osmotic stress limit absorption of water from soil and made an ionic stress, therefore, high concentration of potentially toxic salt ions enters within plant cells. They are adapted to such environment by developing various physiological adaptations due to saltwater and fluctuation of tide level. Therefore, they applied distinct strategies for growing and reproducing in saline conditions with their differential ability of salt tolerance for their continued survival

in this habitat which does not preclude other plants [11] [12] [13] [14]. On the other hand, the environment for tree seedlings of the young stage is quite different from the environment experience of the adult, as these stage mangroves have ability on osmotic adjustment by allowing water uptake into the plant despite the salt content [12]. They show sequester and compartmentalize ionic compounds (salt, including metals) even when these ionic compounds are in high concentration in the soil. But at the early stage due to the lack of sequester and compartmentalization of ionic compounds, they affected by high ionic contents. Moreover, plants have evolved a variety of protective mechanisms *viz.* accumulation of different ions and osmolytes like proline, protein, sugar etc. which allowed them for survival and growth at the unfavorable environmental condition. Therefore, accumulation of these compounds prevents water loss and ionic toxicity of the plant cells [15]. Therefore, biochemical mechanisms of mangroves counter the high osmolarity of salts by accumulation of compatible solutes [16] which do not interfere with the plant metabolism but contribute to turgor maintenance and osmoprotectance in plant [17]. However, they vary considerably in their ability to tolerate salt. So, the paper presents the effects of salinity levels on proline and protein content in *Heritiera fomes* and *Excoecaria agallocha* grown at the three saline zones of the Sundarban mangrove forest an aim to obtain insights into the changes in osmotic composition associated with salt accumulation.

2. Materials Methods

2.1. Materials

Two mangrove tree species of the Sundarban forest *viz.* *Heritiera fomes* Buch-Ham and *Excoecaria agallocha* L. were selected for the present investigation. Three different areas *viz.* Chandpai, Jungra and Munshiganj as Oligohaline (4 - 8 dS⁻¹.m), Mesohaline (8 - 15 dS⁻¹.m) and Polihaline (>15 dS⁻¹.m) zones respectively were selected as an experiment site. The Chandpai and Jungra zones are in Chandpai range and Munshiganj zone in Satkhira range of the Sundarban forest.

2.2. Methods

The experiments were conducted at the field condition by plot preparation at three different saline zones *viz.* Chandpai, Jungra and Munshiganj as Oligohaline, Mesohaline and Polihaline zone. Analytical works were done at Bangladesh Forest Research Institute (BFRI) and Department of Botany Chittagong University. Seedling samples were collected from each plot after the age of Six (06), nine (9) and thirty (30) months of each species (*Heritiera fomes* and *Excoecaria agallocha*). Collected fresh roots of the seedlings were washed with the water of the similar saline zone and then they were taken in plastic bags. However, for proline determination roots of the plant samples were collected with soil roofed.

2.2.1. Determination of Proline in the Plant Sample

Extraction of proline was determined by following the methods of Troll and

Lindsley (1954). Proline was extracted from 1 g fresh plant sample in 5 ml of 90% alcohol. Plant material was pasted in mortar by 90% alcohol (1:5). Extract was poured into a centrifuge tube and to residue more alcohol was added. The process was repeated 2 - 3 times. The added extract was then centrifuged at 1000 g for 10 minutes and supernatant was collected in separate test tube. Volume of the extract was adjusted to 25 ml by adding more alcohol. From this 2 ml extract was taken in a poroceline cup and was evaporated to dryness under strong ventilation at room temperature (25°C) and then it was diluted to 2 ml with distilled water. 2 ml of sample extract was taken in a 20 ml test tube. Added 2 ml glacial acetic acid and mixed them. Then added 2 ml ninhydrine reagents, after that the mixture was heated for 1 hour in the boiling water bath. After boiling, the solution was cooled to the room temperature and then 5 ml of benzene was added. Around 2 minute's air bubbles were passed through the solution. Then two layers were distinct into the solution. The upper characteristic light red or pinkish colour of layer was taken with the pipette. Optical density (OD) of this layer was measured in a Spectrophotometer by absorbance of 515 nm. The amount of proline was calculated with the help of a mean co-efficient derived from the standard curve for proline.

2.2.2. Determination of Protein in the Plant Sample

Protein in the plant sample was estimated by standard Kjeldahl method in the trichloro acetic acid precipitate following Pleshkov (1976). 0.4 gm dried and grinded powdered sample taken in a test tube and then add 15 ml 20% TCA and stirred than filtered and the residue with filter paper was transferred in the kjeldahl flask. After that 5 ml H₂SO₄ and 2 ml H₂O₂ were added and then heated in electric heater and continued till the residue dissolved and transparent liquid appeared. Then cooled and filtered the solution into a 25 ml volumetric flask by repeating washed with distill water. Distillation and titration with standardized 0.1N Na₂CO₃ for the estimation of nitrogen in the digest was done. Protein value in the sample was calculated by multiplying protenaeous nitrogen in the TCA precipitated digest by protein conversion co-efficient 6.25.

3. Results and Discussion

Protein and Proline content were observed in leaf, stem and root of 6, 9 and 30 months seedlings of *Heritiera fomes* and *Excoecaria agallocha* at the ages of grown at three saline zones of the Sundarban forest.

3.1. Protein Content in the Leaf, Stem and Root of *Heritiera fomes* and *Excoecaria agallocha* Seedlings

Protein content was found comparatively higher in the ages of 6 and 9 months in the leaf of *H. fomes* (58.77 mg/g and 68.9 mg/g) and *E. agallocha* (121.0 mg/g 137.88 mg/g) both but lower in 30 months (41.03 mg/g and 50.97 mg/g) grown at the Chandpai zone (Oligohaline) shown in **Figure 1(a)** & **Figure 2(a)**. On the other hand, **Figure 1(b)** shown in the stem of the *H. fomes* seedlings at the

Munshiganj (polihaline) zone found relatively higher protein content in the 6, 9 & 30 month ages were 43.03 mg/g 55.03 mg/g and 12.17 mg/g respectively. However, **Figure 2(c)** shown that in the root of *E. agallocha* seedlings have relatively higher protein content at the Munshiganj (strong saline) zone in the ages of 6, 9 and 30 months (35.6 mg/g, 41.03 mg/g and 20.97 mg/g). At the high salinity increasing trend of protein content also noticed by other observations in [18] and [19]. In general, the protein content increased with increasing concentration up to an optimal level, beyond the optimal level the protein content decreased due to proteolysis and decreased protein synthesis showed in [20] [21] and [22]. However, both species showed comparatively lower protein content in the leaf, stem and root (12.7 mg/g, 10.02 mg/g and 12.17 mg/g & 10 mg/g, 3.53 mg/g and 20.87 mg/g) of the seedlings in the age of 30 months but higher in 6 and 9 months ages. Previous findings on mangroves also showed relatively higher protein content in the early stages as a protective phenomenon for the early development showed in [15].

3.2. Proline Content in the Leaf, Stem and Root of *Heritiera fomes* and *Excoecaria agallocha* Seedlings

Proline content was determined in leaf, stem and root of *Heritiera fomes* and *Excoecaria agallocha* seedlings in the ages of 6, 9 and 30 months. *H. fomes* showed relatively higher proline content in the stem and the root of 6 and 9 months seedlings grown at the Chandpai (Oligohaline) zone in **Figure 3(b)** & **Figure 3(c)**. But in the leaf of the seedlings were showed higher proline content when grown at the Jungra (mesohaline) and the Munshiganj (polihaline) zone in the ages of 6 and 9 months as shown in **Figure 3(a)**. On the other hand 6 and 9 months ages stem of *E. agallocha* seedlings showed relatively higher proline content at the Chandpai (oligohaline) zone as shown in **Figure 4(b)**. Whereas in the 30 months age leaf, stem, and root of both species *viz.* *H fomes* (2.11 µg/g, 2.2 µg/g and 3.1 µg/g) and *E. agallocha* (2.1 µg/g, 2.33 µg/g and 2.4 µg/g) seedlings showed comparatively higher proline content grown at the Munshiganj (Polyhaline) zone as shown in **Figure 3** & **Figure 4**. The earlier findings also noticed in [17] & [23] showed relatively higher leaf proline content in higher saline condition and they also observed the gradual increasing trend of proline with the increasing level of salinity. At the saline environment the mangroves have evolved a variety of protective mechanisms to survive these unfavorable environmental conditions by accumulation of some ions and osmolytes like proline which prevents water loss and ion toxicity. Many researchers several times described about the cellular salt tolerance mechanism of mangroves [15] [23] [24] & [25]. With this mechanism, the ability of plant cells tried to adjust osmotically and to accumulate organic solutes like proline, protein, sugar etc. The accumulation of these compounds is not only important for osmoregulation but also protection of sub cellular structure. So, adaptation to high salinity is closely associated with proline accumulation [5] [6] [26].

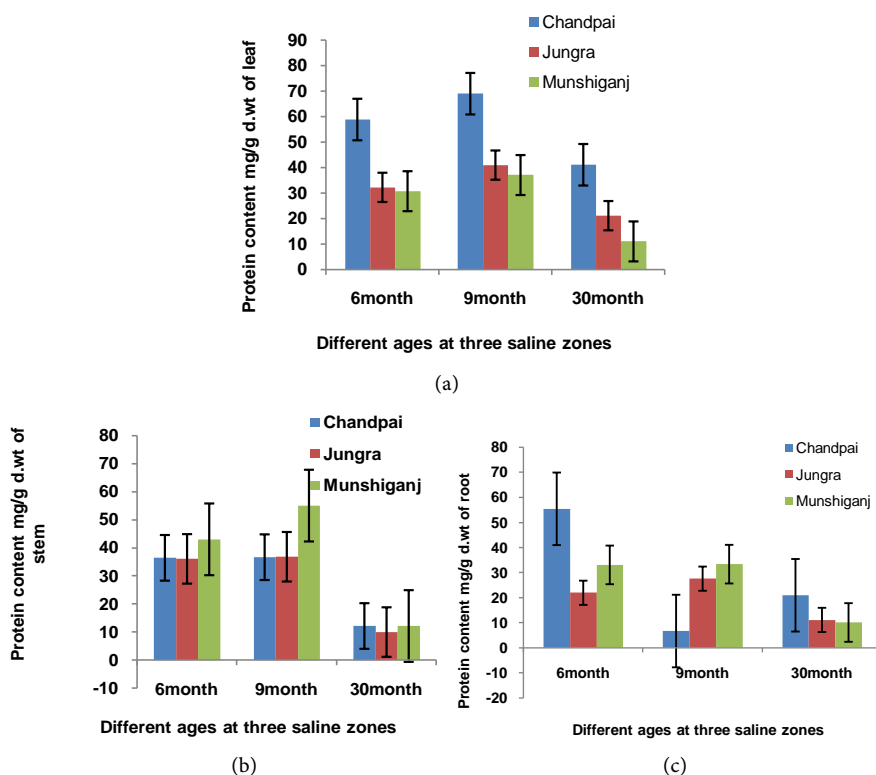


Figure 1. Protein content in different ages (6, 9 and 30 months) seedlings of *Heritiera fomes* leaf (a), stem (b) and root (c) grown in three saline zones of the Sundarban forest.

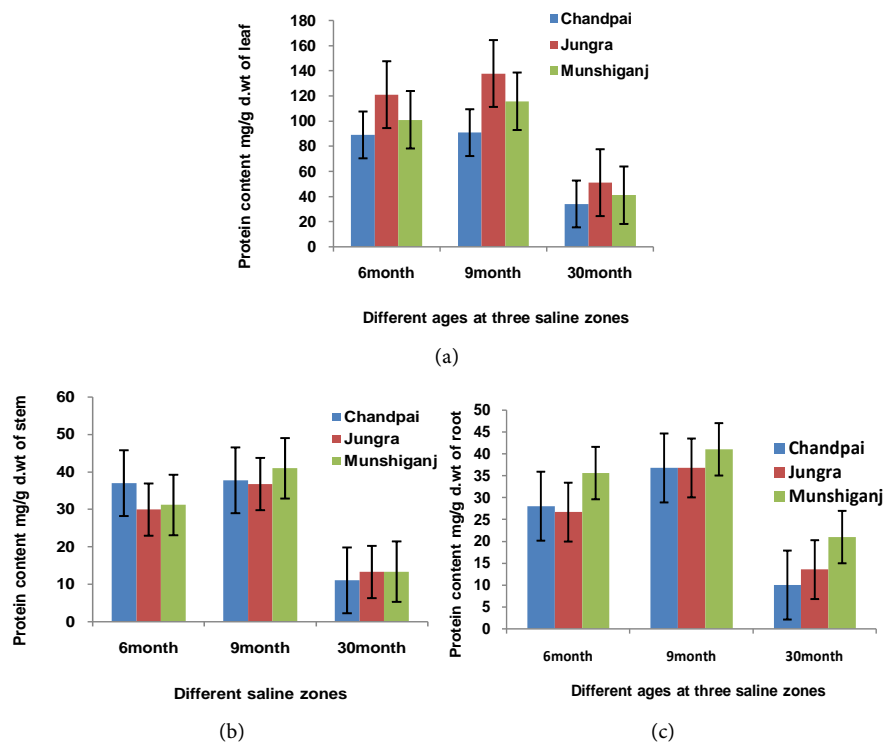


Figure 2. Protein content in different ages (6, 9 and 30 months) seedlings of *Excoecaria agallocha* leaf (a), stem (b) and root (c) grown in three saline zones of the Sundarban forest.

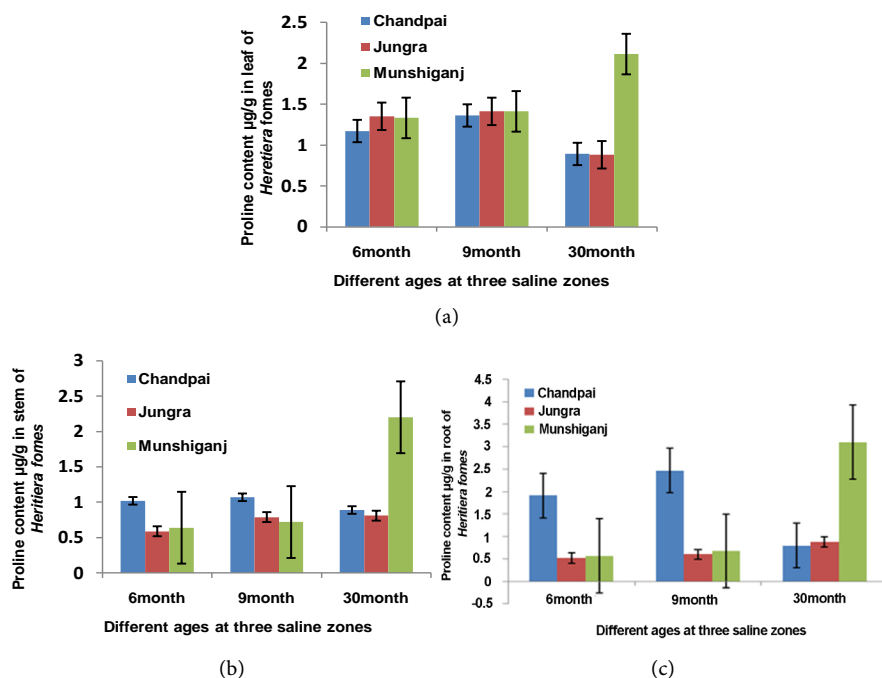


Figure 3. Proline content in different ages (6, 9 and 30 months) seedlings of *Heritiera fomes* leaf (a), stem (b) and root (c) grown in three saline zones of the Sundarban forest.

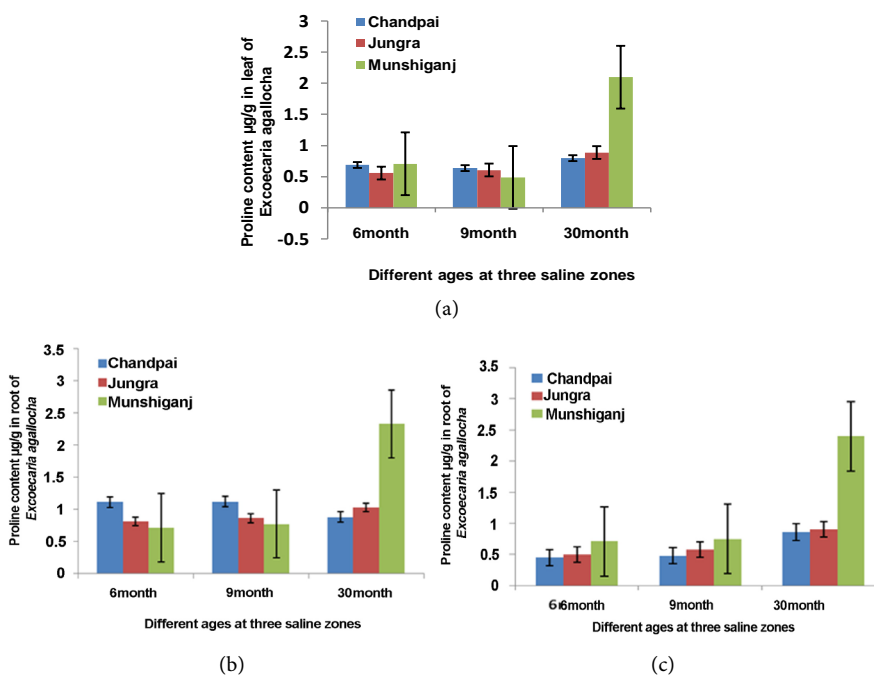


Figure 4. Proline content in different ages (6, 9 and 30 months) seedlings of *Excoecaria agallocha* leaf (a), stem (b) and root (c) grown in three saline zones of the Sundarban forest.

4. Conclusion

Heritiera fomes showed comparatively lower proline content in grown-up ages of 30 months grown at the Chandpai (less saline) and Jungra (moderate saline)

zone but relatively higher in Munshiganj (strong saline) due to surviving in the harsh environment. They could not tolerate high salinity. On the other hand, *Excoecaria agallocha* content is higher compared with proline content in the grown-up ages of 30 months in all the three saline zones and it tolerates high salinity and survives in strong saline zone (Munshiganj) better than *H. fomes*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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