



**LEAF PIGMENT AND TOTAL CARBOHYDRATE CONTENT AT EARLY STAGES OF
HERITIERA FOMES BUCH. SEEDLINGS GROWN AT THREE SALINE ZONES OF THE
SUNDARBANS, BANGLADESH**

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Abstract

The experiment was conducted to find the leaf pigments and carbohydrate content in *Heritiera fomes* at different growth stages exposed to different salinity levels in the Sundarbans areas, Bangladesh. Three leaf pigments, viz., chlorophyll a, chlorophyll b, and carotenoid, total chlorophyll, and total carbohydrate content of *Heritiera fomes* Buch. Ham seedlings were determined in the leaves grown in the oligohaline, mesohaline, and polyhalite zones at early (6 and 9 months) and survival ages (30 months) following standard methods. Leaves from seedlings at the age of 30 months (survival aged) show relatively high leaf pigments than that of the 6 and 9-month-old seedlings grown in the three saline zones. Total chlorophyll content in leaves was found low in the oligohaline zone at the early and survival ages, but higher in the polyhalite zone at the early ages, and higher in the mesohaline zone at the survival age. Chlorophyll b was relatively higher than chlorophyll a. Leaves of 30 months seedlings showed relatively higher chlorophyll a than chlorophyll b at the polyhalite zone, but higher chlorophyll b was noted at the mesohaline zone. The highest content of total carbohydrates was observed in the shoots of 6, 9, and 30 months ages *H. fomes* seedlings in the polyhalite zone, while the lowest was in the mesohaline zone. At the same age periods in the mesohaline zone, the roots of the *H. fomes* seedlings showed relatively higher carbohydrate content and lower at the 30 months seedlings in the all saline zones. Chlorophyll a was found to be more sensitive than chlorophyll b and carotenoid at different salinity levels tested. Higher carbohydrate content was discovered as an additional mechanism to prevent salt toxicity at early ages of high saline conditions (polyline zone).

Keywords: Leaf pigment, chlorophyll, carbohydrate, oligohaline, mesohaline, polyline salinity zones

Introduction

The Sundarbans are the largest and most unique natural mangrove forests in the world and have been shown as the outer deltas of the Ganges, Brahmaputra, and Meghna rivers. About 10,000 km² of total forest area is located in the southwestern parts of Bangladesh and West Bengal, India. Among them, 62%, or 6017 km², of the Sundarban forest in the southwestern part of Bangladesh between latitudes 21°31' N and 22°30' N and between longitudes 89°18' E and 90°18' E lies in the Khulna region (Katebi, 2001). In this area, about 4016 km² is occupied as forestland, and the remaining 1874 km² is made up of rivers, creeks, and canals (Wahid, 1995). The natural vegetation of the Sundarbans is composed of sixty-six halophytic species, mainly herbs, shrubs, climbers, and tree species (Chaffey et al., 1985). However, the trees and shrubs are dominant in the ecosystem. Among them, tree species are belonging to 30 genera under 22 families. Generally, members of Rhizophoraceae, Avicenniaceae, and Sonneratiaceae family are dominating among the most mangrove forests.

The Sundarbans is a region of transition between the freshwater of the rivers originating from the Ganges and the saline water of the Bay of Bengal (Wahid et al., 2007). So, the growth and distribution of the mangroves depend on different levels of salinity (Chanda & Dutta, 1986). Though mangroves are remarkably adapted to tidal and coastal land

through their ability to live in poorly oxygenated sediment and can tolerate inundation by salt water through physiological and chemical mechanisms (Morley, 2000). In general, wetland ecosystems are physical and dynamic links between terrestrial and aquatic resources developed by integrated relationships (Erickson, 1994; Curry & Meguire, 2002). In this development, they show a combined relationship between the atmosphere, soil, and water by developing mineral cycling in wetland ecosystems (Dykyjova & Ulehlova, 1998; Mitsch & Gosselink, 2000). However, increasing sediment salinity generally affects the growth of mangroves, but extremely high salinity has been found to negatively influence the growth of mangrove seedlings (Clough et al., 1982). The osmotic adjustment of water uptake of mangrove seedlings applied distinct strategies or physiological adaptation among the different stages of seedling's growth with their differential ability of salt tolerance (Shan et al., 2008). The condition of the juvenile or early stage of the tree is quite different from the adult stage (Khan et al., 2006). At the adult stage survivability of mangroves occurred due to the presence of sequester and compartmentalization in the presence of a high concentration of ionic compounds (salt, including metals) in the soil. However, at the early or juvenile stage, they are affected by high ionic content. Biochemical mechanisms as well as various metabolites of the species counter the high osmoregulatory of salts that accumulate from compatible solutes (Taskemura et al., 2000). zone. It was reported that at the high saline condition, higher carbohydrate content was regarded as an additional mechanism to prevent salt toxicity (Rathert, 1982). However, leaf pigment content was found to decrease with the increase in salinity (Mitra & Banerjee, 2010; Shinde & Bhosale, 1985; Alamgir et al., 1992). So, in unfavorable environmental conditions, mangroves accumulate different types of ions or osmolytes like proline or carbohydrate, which allow them to grow and survive by preventing water loss and ionic toxicity of the plant cells (Rajaravindran & Natarajan, 2012).

Several factors contribute to the productivity of a forest for future stocking and its continuity. Although propagules of mangroves can exploit their habitat (the intertidal zones) by developing physiological, anatomical, and morphological adaptations to waterlogged or saline environments (Saenger, 2002), they are negatively influenced by excessive salinity (Wang et al., 2007). Due to changing environmental conditions, they face obstacles to their physiological activities and the early natural growth of the species (Ungar, 1995; El-Keblawy & Al-Rawai, 2005). Under these circumstances, the present paper tried to determine leaf chlorophyll and carbohydrate content at the early and mature growth stages of *Heritiera fomes* seedlings as well as the best salinity level for the establishment of such mangroves.

Materials and Methods

Heritiera fomes Buch-Ham species were selected for the present investigation. Three different areas of the Sundarbans mangrove forest were chosen as an experiment sites viz. Chandpai, Jungra, and Munshiganj as oligohaline (4-8 dS-1m), mesohaline (8-15 dS-1m), and polyline (> 15 dS-1m) zones (SRDI, 2010). The Chandpai and Jungra zones are in the Chandpai range and the Munshiganj zone is in the Satkhira range of the Sundarbans forest. *Heritiera fomes* seedlings were collected from naturally regenerated sample plots randomly at the ages of 6 months, 9 months, and 30 months old as an early stage and a survival stage was collected from the selected field of three saline zones of the Sundarbans mangrove forest.

Determination of leaf pigment

Leaf pigment (chlorophyll a, chlorophyll b, and carotenoid) was determined using leaves from 6, 9, and 30 months old seedlings collected from three saline zones of the Sundarbans. After collection, 0.5g of leaves were chopped into small pieces and homogenized in a mortar with 10 mL of 100% acetone, and the supernatant was decanted into a centrifuge tube. To the residue, 10 ml of acetone was added for further extraction, and the process was repeated for total extraction of the pigments. The combined extract was balanced and centrifuged in the cold (0-4°C) at 1000g for 10 minutes, and the clear supernatant was taken and volume was made up to 50 ml in a volumetric flask by adding more 100% acetone. The concentration of chlorophyll a, b, and carotenoids were determined following Wettstein (1957) by taking absorbance in a spectrophotometer at 662 nm, 644 nm, and 440.5 nm for chl a, b, and carotenoids, respectively, after proper dilution against 100% acetone.

The formula used for calculation was:

Concentration of chlorophyll-a, ca = $9.784 \times E662 - 0.990 \times E644$ mg/L

Concentration of chlorophyll-b, cb = $21.426 \times E644 - 4.650 \times E662$ mg/L

Concentration of carotenoids-car, c car = $4.695 \times E440.5 - 0.268$ (chl.a+chl.b) mg/L

$$A = \frac{C \times U \times V}{1000 \times Wt}$$

Where,

- A = Pigment content mg/g fresh weight
- C = Concentration of the corresponding pigment in mg/L
- V = Volume of the pigment
- U = Dilution factor
- Wt = Fresh weight of the leaf, g.

Determination of carbohydrates in the seed and the plant materials

Total carbohydrates content was determined at the root and shoot of the 6 months, 9 months as early stage, and 30 months as survival stage of the *Heritiera fomes* seedlings.

The anthrone method (Hedge and Hofreiter 1962) was used for the determination of the total carbohydrate content of *H. fomes* seedlings. This compound forms a green-colored product with an absorption maximum of 639 nm.

Analysis the samples

By adding extra distilled water, all of the sample and standard solution tubes were filled to 1 ml. Then they added 4 ml of anthrone reagent and heated them for eight minutes in a boiling water bath. Then, after cooling, read the OD at 630nm. A standard curve was drawn by plotting the concentration of the standard on the x-axis versus absorbance on the y-axis.

Co-efficient was calculated following the formula.

$$\text{Co- eff.} = \frac{\text{Concentration of the standard solution}}{\text{OD for each standard solution}}$$

Results and Discussion

a. Leaf Pigment (Chlorophyll a, Chlorophyll b, and Carotenoid) content at the early (6 and 9 months) and survival age (30 months) of *Heritiera fomes* seedlings grown at the three saline zones of the Sundarbans

Three leaf pigments like chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b content were determined in the leaves of *Heritiera fomes* seedlings at the ages of 6, 9, and 30 months in three saline zones viz., oligohaline, mesohaline, and polyhaline of the Sundarbans. At the three saline zones, 30-month-old *Heritiera fomes* seedlings had a higher content of chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b. But at the ages of 6 and 9 months, the leaves of the species contain relatively higher chlorophyll a, chlorophyll b, carotenoid, and chlorophyll a+b at the polyhaline zone (Munshiganj) but lower at the oligohaline zone (Chandpai). In 30 months, *Heritiera fomes* had relatively higher chlorophyll a and carotenoid content in the Munshiganj (strong saline) zones, but the Jungra (moderate saline) zone had relatively higher chlorophyll b and chlorophyll a+b content. As observed by many authors (Mitra & Banerjee, 2010; Shinde & Bhosale, 1985; Alamgir et al., 1992), chlorophyll content in the leaf was less affected in less saline conditions than that in the leaf under high salinity. Although leaf pigment content decreased as salinity increased, similar results to the present findings are also observed in other mangroves by many authors (Rajesh et al., 1998; James et al., 1999). In the current study, chlorophyll b content was found to be relatively higher than chlorophyll a. in the leaves of *H. fomes* grown in the three saline zones. Higher chlorophyll b content is also noticed by previous findings (Rajaravindran & Natarajan, 2012; Ramani et al., 2006) and also remarked by other findings that Chlo. b found comparatively higher saline tolerance than Chlo.a (Singh & Dubey, 1995)

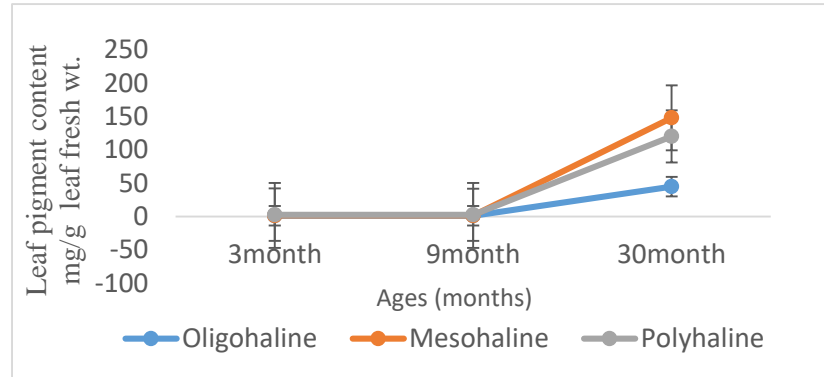


Figure 1. Total Chlorophyll content in the leaves of *Heritiera fomes* seedlings at the three saline zones of the Sundarbans

Table 1. Chlorophyll a, Chlorophyll b, and Carotenoid content in the leaf of *Heritiera fomes* Buch, Ham seedlings at the early (6 months) and the survival (30 months) ages at three saline zones of the Sundarbans

Ages	Saline zones	Leaf pigment content mg/g leaf fresh wt.		
		Chlorophyll a	Chlorophyll b	Carotenoid
6 month	Oligohaline	0.50 ±0.01	0.98 ±0.01	0.52 ±0.02
	Mesohaline	0.52 ±0.02	1.30 ±0.01	1.07 ±0.01
	Polyhaline	0.59 ±0.01	2.44 ±0.01	1.28 ±0.01
9 month	Oligohaline	0.48 ±0.02	0.94 ±0.01	0.44 ±0.01
	Mesohaline	0.51 ±0.01	1.24 ±0.01	0.90 ±0.05
	Polyhaline	0.61 ±0.01	2.33 ±0.01	1.26 ±0.12
30 month	Oligohaline	44.47 ±0.87	67.32 ±1.51	12.48 ±0.08
	Mesohaline	60.50 ±0.33	87.62 ±0.33	15.66 ±0.34
	Polyhaline	77.84 ±1.03	42.43 ±0.40	26.56 ±0.63

b. Carbohydrate content in the seedlings of *Heritiera fomes* at the early (6 and 9 months) and survival ages (30 months) grown at three saline zones of the Sundarbans

Total carbohydrate content was determined in the root and shoot of early (6 and 9 months) and survival age (30 months) seedlings of *Heritiera fomes* Buch. Ham is grown at three saline zones viz. oligohaline, mesohaline, and polyhaline zones of the Sundarbans.

At the early (6 and 9 months) and survival (30 months) both ages, roots of the *H. fomes* seedlings showed relatively higher carbohydrate content grown in the mesohaline zone. However, among all ages (early and survival ages) relatively lower carbohydrates were found in the roots of 30 months old seedlings grown at the all saline zone. The highest content of total carbohydrates was found in the shoots of early (6 and 9 months) and survival (30 months) both ages *H. fomes* seedlings grown in the polyline zone, while the lowest and almost similar carbohydrate content was found in the mesohaline zone. At the high saline condition as a non-tolerant of high salinity *H. fomes* seedlings faced many obstacles like disturbances in metabolism. Previous findings show that in that situation plant tissues accumulate more carbohydrates as a dominant osmoregulation compound to prevent salt toxicity by acting as an additional mechanism. (Popp et al., 1985; Rathert, 1982).

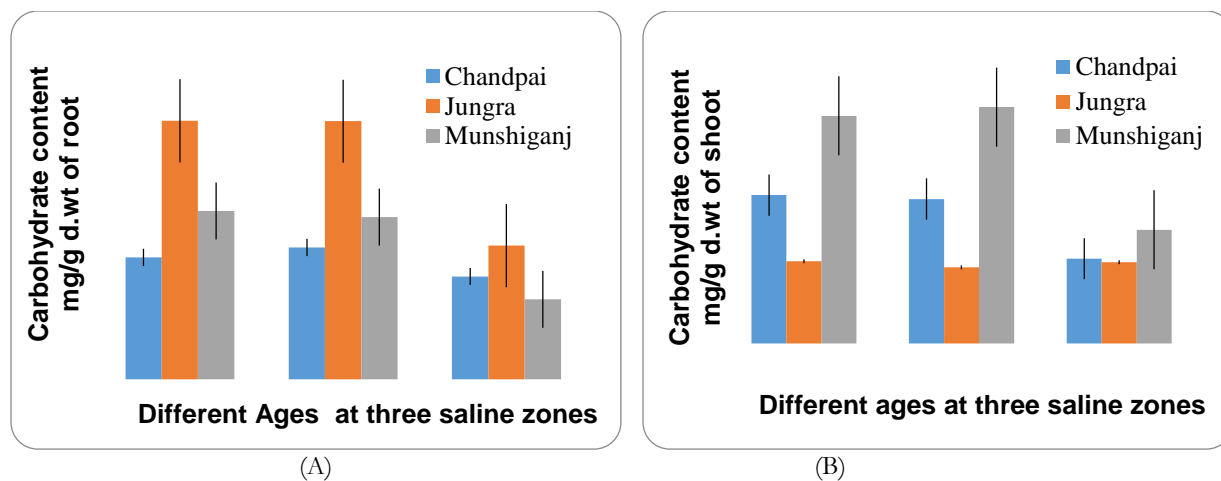


Figure 2. Carbohydrate content in the root (A) and the shoot (B) of *Heritiera fomes* seedlings at early (6 and 9 months) and survival (30 months) ages in three saline zones of the Sundarbans

Conclusion

Heritiera fomes Buch-Ham, as a moderately salinity-tolerant species, influenced the leaf pigments, viz., chlorophyll a, chlorophyll b, carotenoid, total chlorophyll, and carbohydrate content at the early and survival ages with three different salinity levels in the Sundarbans mangrove forest. The survival age (30 months) showed a relatively higher content of leaf pigments (chlorophyll a, chlorophyll b, and carotenoid) in the three saline zones. However, at the same ages, total chlorophyll (leaf pigments) content in leaves and carbohydrate content in roots of *H. fomes* species were comparatively higher in the mesohaline zone. Furthermore, in the shoots of early and survival, both ages of *H. fomes* species remarked the highest content of total carbohydrate grown in the polyline zone as a dominant osmoregulation compound to prevent salt toxicity by acting as an additional mechanism for survival.

Conflict of Interest

The authors declares no conflict of interest.

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