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EFFECT OF SALT STRESS ON CHLOROPHYLL AND PROLINE ACCUMULATION IN LEAVES OF *Heritiera fomes* Buch.-Ham. AND *Xylocarpus moluccensis* (Lam.) M. Roem. SEEDLINGS REGULATING ITS SURVIVAL AND GROWTH

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Abstract: *Heritiera fomes* Buch.-Ham. and *Xylocarpus moluccensis* (Lam.) M. Roem. are the two most important trees of the *Sundarbans*. Survival and biomass increment, chlorophyll and proline concentration in leaves were investigated in seedlings of the two species grown at different levels of salinity in hydroponic culture. Seedlings were grown at 0, 5, 10, 15, 20, 25, 30 and 35 ppt salinities for 90 days. Significant reduction of chlorophyll concentration was found with increasing salinity for both the species. Total chlorophyll concentration halved for both the species as the salinity was increased from 0 to 35 ppt (0.34 to 0.17 mg/g for *H. fomes* and 0.07 to 0.03 mg/g for *X. moluccensis*). In addition, the *H. fomes* leaves have five times more chlorophyll concentration than *X. moluccensis*. Proline accumulation in leaves of *X. moluccensis* was found considerably high compared to *H. fomes* and increase with salinity for both the species. It was quadrapoled (254 to 1462 μmoles/g) in *X. moluccensis* and doubled (8 to 16 μmoles/g) in *H. fomes*. Seedling survival was found to vary with species and salinity levels. Survival of *H. fomes* seedlings decreased from 83% to 58% at 15 to 35 ppt salinity and no mortality was observed at lower salinities of 0 to 10 ppt. In contrast no mortality was observed from 0 to 25 ppt salinity in *X. moluccensis* seedlings and 92% seedling survived at 30 and 35 ppt salinities. Both the species showed lower biomass accumulation at higher salinities. Higher biomass increment (38 to 52%) was observed for *X. moluccensis* than that of *H. fomes*(33%) at lower salinities (0 to 5 ppt) and reduced gradually at higher salinities. This study revealed that *X. moluccensis* is more salt tolerant than *H. fomes* considering higher survival as a result of high proline accumulation in leaves that helps to overcome salt induced negative osmotic pressure. But growth is reduced due to lowering of chlorophyll accumulation at higher salinities for both the species.

Keywords: Chlorophyll, proline, salinity, *Heritiera fomes*, *Xylocarpus moluccensis*

Introduction

Mangrove ecosystems along the sheltered coast of tropical and subtropical regions are characterized by saline environment (Saenger, 2002). The *Sundarbans* located in south western region of Bangladesh on the interface between the Ganges-Brahmaputra river system and the Bay of Bengal covers the largest single tract mangrove forest in the world (Hussain & Karim, 1994). It is one of the biologically diverse mangrove forests with 182 plant species of which 52 are trees (Islam et al., 2016). The spatial

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distribution of the tree species is mostly regulated by heterogeneous physical environment of the ecosystem and salinity is one of the most important parameters of this kind (Islam et al., 2016; Iftekhar & Saenger, 2008). Based on the level of salinity, *Sundarbans* has been divided into less saline (LS), moderately saline (MS) and strongly saline (SS) zones having salinities ranging from 0.5-5 ppt, 5-18 ppt and 18-30 ppt, respectively (Siddiqi, 2001). The vegetation composition varies in different zones due to the varied salt tolerance of different species (Iftekhar & Saenger, 2008; Siddiqi, 2001).

Xylocarpus moluccensis (Lam.) M. Roem. and*Heritiera fomes* Buch.-Ham. are the two most important timber species of the *Sundarbans*. *H. fomes* is found in the less to moderately saline zones, while *X. moluccensis* is mostly found in the moderately to strongly saline zones of the *Sundarbans* (Islam et al., 2016; Sarker et al., 2016). Though species occurrence and growth are regulated by several biotic and abiotic factors, salinity is one of the blazing factors as it has been increasing in the *Sundarbans*.

Salinity afflicts the survival and growth of mangrove plants (Nasrin et al., 2016; Munns & Tester, 2008; Parida & Das, 2005). It creates high negative osmotic potential in the plant root system that makes uptake of water and nutrients difficult for optimum physiological response (Hogarth, 2015; Flowers & Colmer, 2008; Munns, 1993). To cope with this difficulty, some mangrove species accumulate low molecular compatible solutes in cytoplasm (Hogarth, 2015; Parida et al., 2004; Takemura et al., 2000). Of the compatible solutes proline is reported to be frequently occurring in high salt tolerant species (Popp et al., 1985). Again, salinity induced stress affect photosynthesis through the reduction of chlorophyll production. It was reported that the thylakoid membranes of chloroplast are damaged due to high salt concentrations that causes growth reduction (Omoto et al., 2010; Wu &Zou, 2009).

This study aims to assess the response of *H. fomes* and *X. moluccensis* seedlings under salinity stress by means of survival, growth, chlorophyll synthesis and proline accumulation in control condition. This information may indicate the underlying causes of species distribution in varied salinities in the *Sundarbans*.

Materials and methods

Experiment setup: Seeds of *H. fomes* and *X. moluccensis* were collected from the *Sundarbans* and seedlings were raised on nursery bed of coarse sand. Six months old seedlings were taken from the nursery bed by careful washing of seedling root with minimal damage. The initial fresh weight of seedlings was taken immediately. The experiment was carried out in pot culture with full strength modified Hoagland's solution according to Hoque et al. (2006). Each seedling was placed in a perforated plastic pot containing coarse sand. Thus, twelve pots with seedling were placed in a plastic box containing 8 litres of nutrient solution. A total of 24 boxes were prepared for each species. Salinities were maintained in every box by adding salt water. Eight salinity levels i.e., 0, 5, 10, 15, 20, 25,30 and 35 ppt were adopted gradually with three replications in a completely randomized design (Fig. 1). This experiment was carried out for three months and Hoagland's solution and salinity levels were renewed every week.

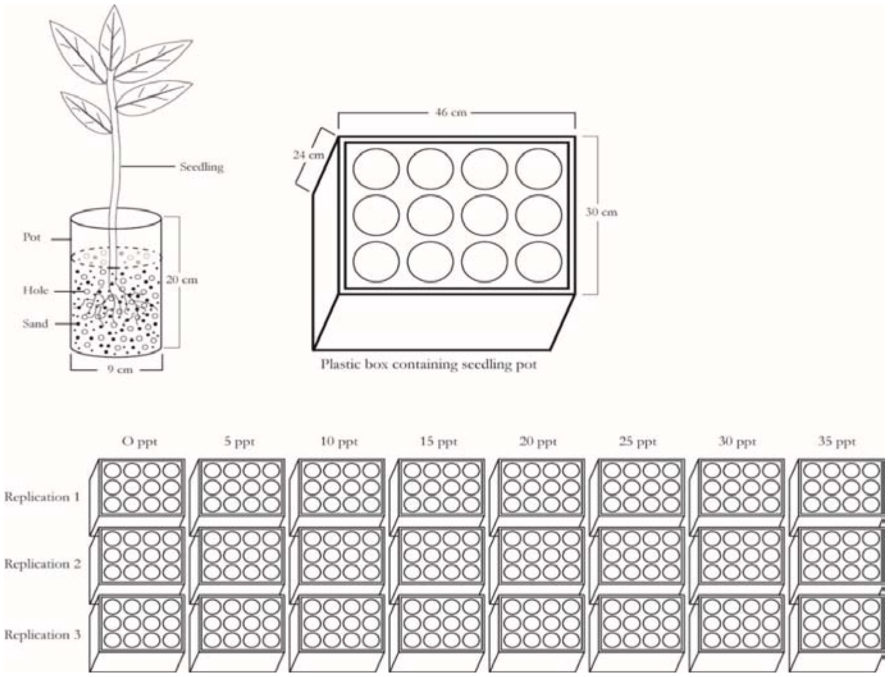

Fig. 1: Experimental design of salinity treatments (0 to 35 ppt) with replications

Survival and growth measurement: At the end of the experiment, surviving seedlings in each salinity treatment were counted and survival percentage was estimated. All the seedlings were harvested and their fresh weights were measured and recorded against treatments. The growth increment (in %) was estimated from the initial and final biomass of the seedlings.

Chlorophyll and proline concentration in leaves: Dimethyl sulphoxide (DMSO) extraction method of Hiscox and Israelstam (1979) was followed for chlorophyll extraction. Chlorophyll was estimated by using Arnon's (1949) equations as follows

Total Chlorophyll (g/l) = 0.0202 A_{663} + 0.00802 A_{645}

where, A_{663} and A_{645} denote the absorbance of extract at 663nm and 645nm wavelengths respectively.

The proline was estimated according to Bates et al. (1973) and proline concentration was determined from a standard curve and calculated on fresh weight basis as follows:

$$Mole\ proline.g^{-1} fresh\ weight = \frac{g\ proline.ml^{-1} \times \frac{ml\ of\ toluene}{115.5}}{\frac{g\ of\ sample}{5}}$$

***Statistical analysis*:** Survival, biomass increment, chlorophyll and proline concentration were compared among different level of salinity treatments using one-way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). Correlation was analysed for survival, growth, chlorophyll and proline content in relation to salinity. All the analyses were performed in Statistical Analysis Software (SAS 6.12).

Results

Survival and growth: For *H. fomes* within the salinity range 0 to 10 ppt there was no seedling mortality. However, the seedling survival was gradually decreased from 83% to 58% at salinities from 15 to 35 ppt. On the other hand, no mortality was observed within the salinity range 0 to 25 ppt for *X. moluccensis* and 8% seedling mortality was found at 30 and 35 ppt salinities. Significant ($p<0.05$) negative correlation between survival percentage and salinities was observed for *H. fomes* ($r = -0.94$) and *X. moluccensis* ($r = -0.76$). Decreasing survival of *H. fomes* seedlings at salinity more than 10 ppt may restrict its occurrence at higher salinity but seedlings of *X. moluccensis* can withstand at wider range salinity (Fig. 2).

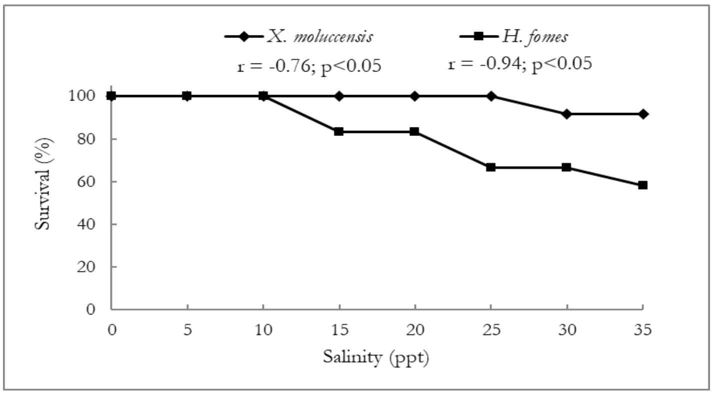

Fig. 2: Survival (%) of *X. moluccensis* and *H. fomes* seedlings at different salinity

At non-saline condition (i.e., 0 ppt), biomass increment of seedlings was 38% and 33% for *X. moluccensis* and *H. fomes* respectively. At 5 ppt salinity, about 52% biomass increment was observed for *X. moluccensis* indicating its preference for some salt for better growth. In contrast the growth of *H. fomes* remained same at 0 and 5 ppt salinities. At higher salinities (i.e., 10 to 35 ppt) the growth of both the species was found to decrease compared to non-saline condition (Fig. 3).

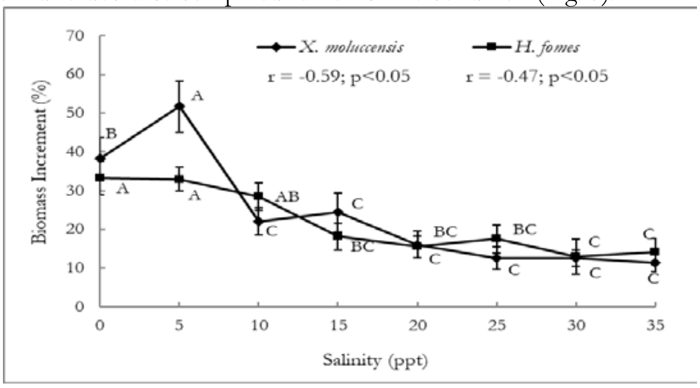

Fig. 3: Biomass increment (%) of *X. moluccensis* and *H. fomes* seedlings at different salinity. Lines represent means ± standard errors. Same letter for individual species represents no significant ($p<0.05$) difference among the treatments (DMRT).

Chlorophyll and proline concentration in leaves: Comparatively higher concentration of chlorophyll was observed in the leaves of *H. fomes* seedlings than that in *X. moluccensis* at all salinity levels (0 to 35 ppt). Chlorophyll concentration in the leaves of *H. fomes* seedlings decreased significantly from 0.34 to 0.17 mg/g as the salinity was increased from 0 to 35 ppt (Fig. 4). In slightly saline condition (0 to 5 ppt), chlorophyll concentration in *H. fomes* did not vary significantly ($p>0.05$), but it was found to decrease sharply at higher salinities and showed strong negative correlation ($r=-0.89$; $p<0.05$). Conversely, chlorophyll concentration was found to increase from 0.07 mg/g at 0 ppt salinity to 0.21 mg/g at 5 ppt salinity in the leaves of *X. moluccensis* and sharply decreased to less than 0.05 mg/g at 10 ppt salinity and then maintained the level at higher salinity (Fig. 4).

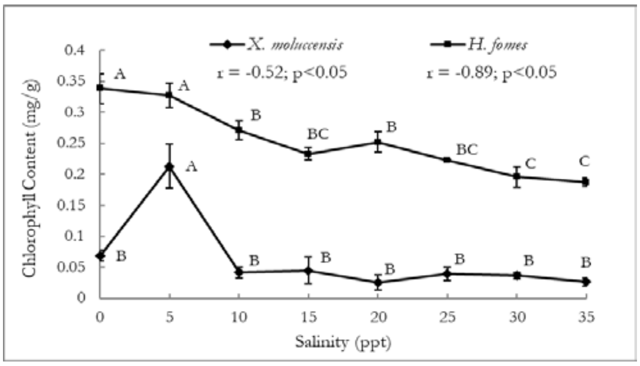

Fig. 4: Chlorophyll (mg/g) concentration in leaves of *X. moluccensis* and *H. fomes* seedlings at different salinity. Lines represent means ± standard errors. Same letter indicates no significant difference for individual species (DMRT).

Proline concentration was much higher in the leaves of *X. moluccensis*as compared to that in *H. fomes*. The proline concentrations were almost same (254 to 296 μmoles/g) in leaves of *X. moluccensis* at 0 to 10 ppt salinities. However, it was found to increase sharply to 1389 μmoles/g at 15 ppt and remained almost similar up to 35 ppt salinity (Fig. 5a). Conversely, very low concentration (8 to 16 μmoles/g) of proline was detected in the leaves of *H. fomes* at different salinities. A slight increase of proline concentration was observed in *H. fomes* leaves at 10 to 20 ppt from that at 0 to 5 ppt salinities and decreased again at salinities ≥20 ppt (Fig. 5b).

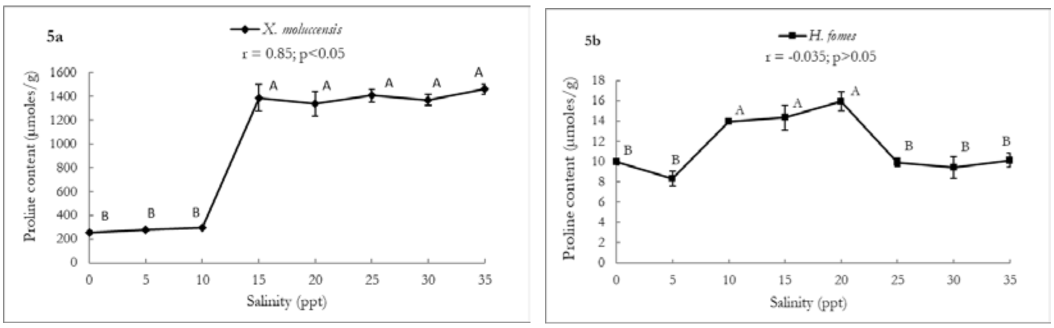

Fig. 5: Proline concentration (µmoles/g) in seedling leaves of (a) *X. moluccensis* and (b) *H. fomes* seedlings at different salinity. Lines represent means ± standard errors. Same letter indicates no significant difference for individual species (DMRT).

Discussion

Higher percentage of seedling survival of *X. moluccensis* indicates that the species is more salt tolerant. However, the negative relation among salinity and seedling survival supports the similar observations as reported for *Avicenia germinans* (Lopez-Hoffman et al., 2007), *Ceriops australis* and *C. decandra* (Ball, 2002). Decrease in biomass increment with increasing salinity for the studied species is a phenomenon common for mangroves (Tomlinson, 1986). But higher biomass increment for *X. moluccensis* at 5 ppt salinity has similarity with that of *Rhizophora mangle* (Cardona-Olarte et al., 2006; Lopez-Hoffman et al., 2006), *Avicennia germinans* (Lopez-Hoffman et al., 2007), *Laguncularia racemosa* (Cardona-Olarte et al., 2006).

The result indicates non-saline or slightly saline condition≤5 ppt) are favourable for chlorophyll accumulation in the leaves of *H. fomes* seedlings. Similarly, slightly saline condition (5 ppt) promotes chlorophyll synthesis in the leaves of *X. moluccensis*. Some studies showed no significant change in chlorophyll concentration in leaves of different mangroves such as *Rhizophora mangle, Laguncularia racimosa* (Falqueto et al., 2008), *Avicennia officinalis, H. fomes, Excoecaria agallocha* (Panda et al., 2006). However, most of the studies found a decreasing trend of chlorophyll with increasing salinity in mangroves leaves such as *H. fomes* (Mitra & Banerjee, 2010), *Bruguiera gymnorrhiza, E. agallocha* (Nandy (Datta) et al., 2009), *Aegiceras corniculatum* (Parida et al., 2004), *Bruguieraparviflora* (Parida et al., 2002). On the contrary, chlorophyll concentration in leaves of *Phonixpaludosa, X. granatum* (Nandy (Datta) et al., 2009) and *Kandelia candel* (Qiu et al., 2007) was found to increase with increasing salinity. This comparison indicates that chlorophyll accumulation in leaves is species specific and most mangrove plants do not prefer high salt as their chlorophyll and biomass reduced significantly at salt stress (Nandy (Datta) et al., 2009; Parida & Das, 2005; Saenger, 2002).

Low concentration of proline in leaves of *H. fomes* and no correlation between proline concentration and salinity may cause the lower survival of seedlings at high saline condition. In contrast, high proline accumulation in leaves of *X. moluccensis* and significant positive correlation with salinity may lead less mortality at high saline condition for the species. Increased accumulation of proline at high saline condition may influence the higher survival of *X. moluccensis* seedlings. It indicates that proline accumulation may be an adaptive mechanism to cope with high salt stress for this species. Some studies showed the positive relation among proline concentration and salinity level especially for *B. parviflora* (Parida et al., 2002), *Ceriop stagal* (Aziz & Khan, 2001), *Ceriops roxburghiana* (Rajesh et al, 1999), *Aegialitis annulata*, *X. granatum* (Popp & Albert, 1995). Conversely, Parida et al. (2004) found decreasing concentration of proline with the increase of salinity for leaves of *A. corniculatum*. Moreover, many of the mangroves do not produce proline in their leaves tissue but they can able to survive in strong saline condition. They may produce other osmolytes such as glycinebetaine, pinitol and mannitol to regulate osmotic balance for coping with high salt (Hogarth, 2015; Parida & Das, 2005). The osmolytes may regulate survival of mangrove species at different salinity gradients.

Conclusion

Salinity is a major abiotic stress that affects the survival and growth of mangroves. High salt stress often hinders the production of photosynthetic pigments in the leaves and hence reduces the growth of plants. Moreover, to cope with high salt concentration mangroves often produce osmolytes in their cytoplasm to avoid osmotic imbalance. The response to salt stress is not same for all the species. Species like *X.moluccensis* may survive at high salinity as they produce proline in their leaves but growth can be reduced because of decreasing chlorophyll content. Conversely, high salinity may restrict *H. fomes* to survive due to low production of proline in leaves and severely reduce their growth by low level of chlorophyll synthesis.

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