https://doi.org/10.26525/jtfs32.2.179

ADAPTIVE RESPONSES TO SALINITY: SEED GERMINATION TRAITS OF *SONNERATIA APETALA* ALONG SALINITY GRADIENT IN SUNDARBANS, BANGLADESH

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Submitted May 2019, accepted August 2019

Water and soil salinity of the Sundarbans are increasing due to continuous reduction in fresh water flow from upstream and noticeable change in rainfall pattern. *Sonneratia apetala* is an important tree species for coastal afforestation in Bangladesh. It was hypothesised that *S. apetala* may show adaptive responses to salinity gradient in terms of morphological and physiological traits. This study examined the adaptive responses of *S. apetala*, growing in three saline zones (less, moderate and high) of the Sundarbans, in terms of fruit weight and seed germination traits. The fruits of less saline zone was comparatively heavier (9.09 ± 0.08 g) than moderate and high saline zone. The final germination percentage (FGP), germination initiation time (GIT) and germination index (GI) of *S. apetala* seeds varied significantly (ANOVA, $p < 0.05$) among the saline zones and saline treatments. The FGP and GI showed a significant ($p < 0.05$) negative correlation with salinity levels. While, GIT showed a significant ($p < 0.05$) positive correlation with salinity treatments. Seeds of moderate and high saline zones germinated faster and vigorously than the seeds of low saline zone at higher salinity levels.

Keywords: Adaptation, germination traits, *Sonneratia apetala*, Sundarbans, salinity

INTRODUCTION

Mangroves is one of the productive ecosystems in the tropical and sub-tropical coastal zones of the world with fluctuating tidal and saline regimes (Mattes & Kapetsky 1988). Mangroves play a vital role in supporting food webs and nutrient cycles in the adjacent coastal ecosystems (Alongi et al. 2000, Machiwa & Hallberg 2002, Mumby et al. 2004, Mahmood et al. 2008). About 75% of the world's tropical coastline was dominated by mangroves during the 1900's, which has been significantly reduced due to natural and human-induced stresses (López-Portillo et al. 2017). Salinity is an important factor that controls seed germination and survival of seedling, its growth and development, and distribution of mangrove plant species (Waisel 1972, Mahmood et al. 2014). Salinity can influence the morphology and physiology of mangrove plants (Ball & Pidsely 1995). High salinity induces high osmotic potential and ion toxicity, restricting water availability to seeds, which in turn affects imbibition, enzyme activity and cell division, thereby delaying growth initiation (Waisel 1972, Atak et al. 2006, Kim et al. 2013). However, some mangrove species showed adaptive responses in seed germination with increased salinity (Bhosale & Sinde 1983, Alam et al. 2018).

Increasing salinity in the coastal areas and the Sundarbans of Bangladesh is a threat to floral diversity (Siddiqi 2001, Kathiresan & Arif 2006). Reduced flow of fresh water from upstream, change in rainfall pattern and sea level rise are the major causes of salinity increase in coastal areas and the Sundarbans of Bangladesh (Gopal & Chauhan 2006). Three major distributaries (Gouri, Kapatakkah and Baleshwari rivers) of the Ganges are responsible for fresh water flow to the Sundarbans. Presently, the Baleshwari River is the only source of fresh water flow to the Sundarbans, and the amount has dropped from 3700 $m^3 S^{-1}$ to 364 $m^3 S^{-1}$ after the construction of Farakka dam in India during 1974 (Wahid et al. 2007). In addition, the rate of sea level rise in the coast of Bangladesh is about 6–20 mm yr^{-1}, which has accelerated saline intrusion in the coastal areas of Bangladesh

(Karim & Mimura 2008). Therefore, increasing salinity in the coastal zone may create a challenge to select suitable mangrove species for future plantation in the newly accreted lands and offshore island in the Bay of Bengal. *Avicennia officinalis*, *Excoecaria agallocha*, *Sonneratia apetala* and *S. caseolaris* were planted for coastal afforestation in Bangladesh. *Sonneratia apetala* grows along a salinity gradient (less, moderate and high) in the Sundarbans of Bangladesh and this species has appeared as a principal species in coastal afforestation programme due to its higher survival and growth performance (Mahmood 2015, Islam et al. 2016). It can be hypothesised that *S. apetala* may show adaptive responses to salinity gradient in terms of morphological and physiological traits. Therefore, the objectives of the present study were i) to evaluate the adaptive response to salinity in terms of fruit weight of *S. apetala* that grows in less, moderate and high saline zones of the Sundarbans, and ii) to examine the adaptive response to salinity in terms of seed germination traits of *S. apetala* that grows in different saline zones of the Sundarbans under varying saline treatments. The outcome of this study will be helpful in understanding the fate of this species by the changing salinity, as well as identifying and selecting salt-tolerant population(s) in the Sundarbans of Bangladesh.

MATERIALS AND METHODS

Study site

The Sundarbans is the world largest single tract mangrove forest, situated at the southwest corner of Bangladesh in between 21°30' to 22°30' N and 88°10' to 89°51' E (Figure 1) (Chaffey et al. 1985). The Sundarbans is a unique habitat for diversified flora and fauna and it contains almost 50% of mangrove flora of the world. There are 334 species of plants, 49 species of mammals, 315 species of birds, 400 species of fish and 53 species of reptiles in the Sundarbans (Karim 1995). It is a natural home for *Panthera tigris* and *Axis axis* (Moss 1993). However, the major tree species

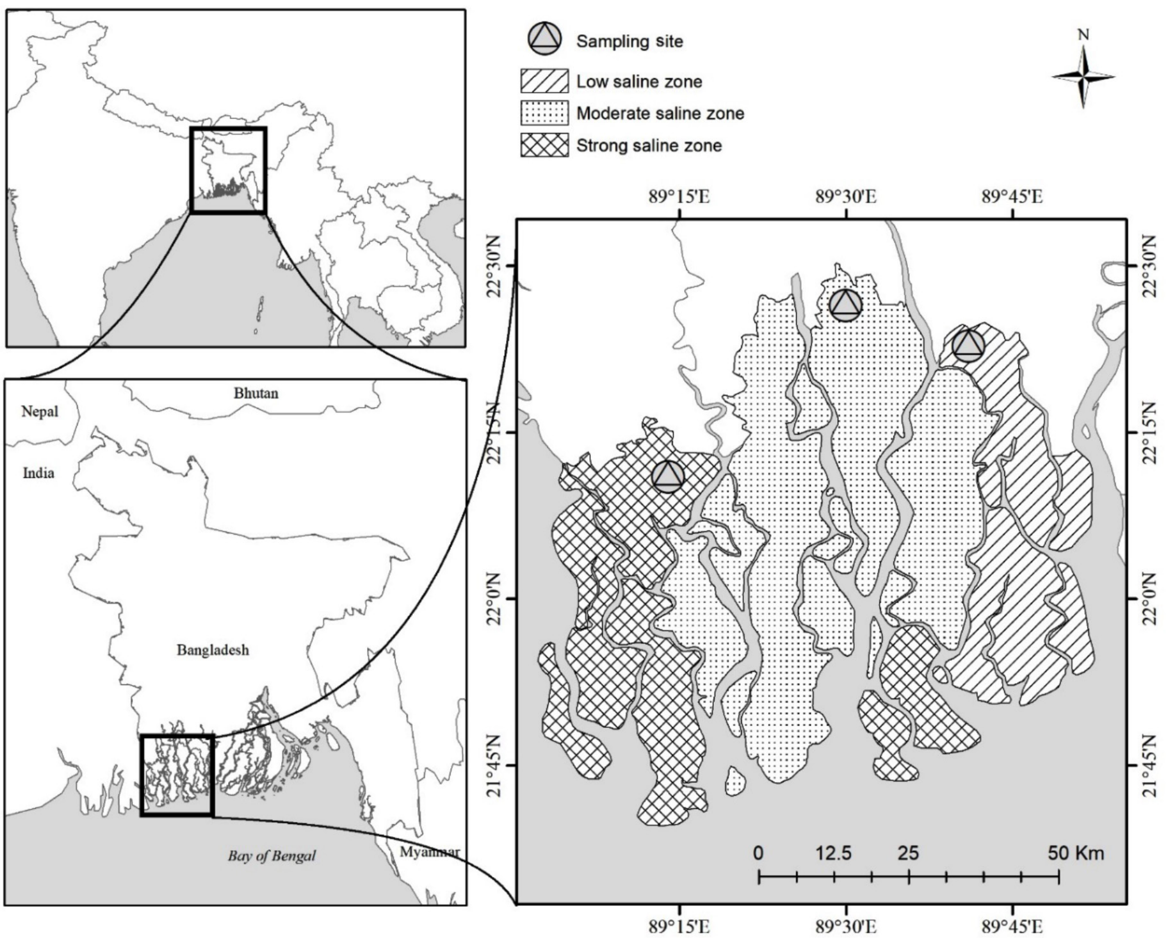

Figure 1 Map showing study area in the Sundarbans mangrove forest of Bangladesh

of the Sundarbans are *Heritiera fomes*, *Excoecaria agallocha*, *Ceriops decandra*, *Avicennia officinalis*, *Sonneratia apelala*, *S. caseolaris*, *Bruguiera sexangula*, *Xylocarpus molucensis*, *X. gratatum*, *Rhizophora apicilata* and *R. mucronata*. Besides these, there are different species of phytoplankton, bacteria, fungi and many other microorganisms (Mahmood 2015). Based on salinity (practical salinity unit, psu), this forest is divided into low (0.5 to 5 psu), moderate (5 to 18 psu) and high or strong (18 to 30 psu) saline zones (Siddiqi 2001). The climate is strongly seasonal with 87% of the mean annual precipitation (1,500 mm) occurring between May and October, and the remaining months are considered as dry season with no or less precipitation. The range of maximum and minimum temperatures are 18 to 35 °C in the summer and 12 to 29 °C during the winter. Soil is silty clay, *in situ* pH is neutral to alkaline and range of organic carbon is 1.5 to 3% (Mahmood 2015).

Fruit collection

The bulk of *S. apetala* mature fruits were collected from the pure patch of the species, located in the three saline zones of the Sundarbans. The locations of the pure patch were identified using the vegetation map derived by Chaffey et al. (1985), which was further confirmed by field visits. Fruits were collected from 15 selected mother trees (DBH > 40 cm, height > 10 m, straight bole without broken top and unaffected by disease) for each saline zone: less (89°41' E and 22°22' N), moderate (89°29' E and 22°26' N) and high (89°14' E and 22°10' N) (Figure 1).

Phenology and fruit weight

Phenology of *S. apetala* varies with site conditions (Islam & Siddiqi 1987). The flowering and fruiting seasons of this species are April to June and June to September, respectively (Das & Siddiqi 1985, Mahmood 2015). The collected fruits from low (0.5 to 5 psu), moderate (5 to 18 psu) and high (18 to 30 psu) saline zones were sorted manually to discard the defective ones. One hundred randomly selected, defect free fruits constituted a sample. Thus, three samples of fruits (100*3 = 300 fruits) were collected from each saline zone to obtain zone wise fruit weight. The fruit of *S. apetala* is round in shape, hence, length and width were not measured.

Germination experiment

The sorted fruits of each saline zone were stacked separately and covered superlatively with straw for 4–5 days, and watered daily to partially decompose. The partial decomposed fruits were then washed to get the seeds. Seed germination experiment was conducted according to Alam et al. (2018). In this experiment, seeds were laid in a randomised block design with eight treatments of salinity (0, 5, 10, 15, 20, 25, 30 and 35 psu) and three replications for each saline zone. Twenty-four (8 treatments × 3 replications) germination trays (75 × 75 × 6 cm) were prepared for each saline zone. Trays were filled with 3 cm thick layer of coarse sand, and one hundred seeds were sown in each tray. A total of 2400 seeds (8 treatments × 3 replications × 100 seeds) were used for the germination experiment for each saline zone. The experiment was conducted in a forest nursery glass house of Khulna University.

Crude sea salt (unrefined sea salt containing all chemical constituents) was used for preparing salinity treatments of 5, 10, 15, 20, 25, 30 and 35 psu. Distilled water was used for 0 psu treatment. Water solution of appropriate salinity treatment was added to the respective germination trays until the sand became saturated and a thin film of water was conspicuous. Water and salinity levels of each tray were checked daily and adjusted as required. The range of daily maximum temperature and relative humidity during the experimental period were recorded as 31 to 35 °C and 64 to 75%, respectively. Germination was defined as the initiation of the first root, and the seedlings were removed from the trays immediately after germination. The number of germinated seeds were counted and recorded at 24-hour intervals for four weeks.

Germination traits

Final germination percentage (FGP) is the percentage of sown seeds that germinated at the end of the experiment:

FGP = (Number of germinated seeds/total number of sown seeds) × 100

Germination initiation time (GIT) is the time required to initiate germination of seeds:

GIT = Day of first germination - day of seed soing (Ellis & Roberts 1981)

Germination index (GI) is calculated to find out vigorous seed. It is a good indicator of dormancy decay; higher value of GI indicates good quality of seeds.

$$GI = \sum n/d,$$

where n = number of seedling emerging in a day and d = the day after seed sowing (Karaguzel et al. 2004).

FGP, GI and GIT were calculated for seeds of all saline zones and salinity treatments.

Statistical analysis

One-way analysis of variance (ANOVA) was performed to compare the fruit weights of different saline zones. Two way ANOVA was also conducted for FGP, GIT and GI values of different salinity treatments and zones to understand the influence of salinity on germination traits. The SAS statistical software (ver. 6.12) was used for ANOVA analysis. Pearson correlation analysis was conducted among germination parameters (FGP, GI and GIT), and salinity treatments using IBM SPSS statistical software (ver. 20) to evaluate the relationship among salinity treatments and seed germination parameters. Bonferroni adjustment analysis was also performed as post-ANOVA tests for pairwise comparisons among the germination parameters (FGP, GIT and GI) for all zones and salinity treatments using IBM SPSS (ver. 20) statistical software.

RESULTS

Fruit weight

Comparatively ($p < 0.05$) higher weight (9.09 ± 0.08 g) was observed in the less saline zone and lowest weight (6.10 ± 0.18 g) in the high saline zone (Figure 2). Moreover, fruit weights significantly ($p < 0.05$) varied between less saline zone (LSZ) and medium saline zone (MSZ) as well as between LSZ and high saline zone (HSZ), while fruit weight did not significantly ($p > 0.05$) vary between MSZ and HSZ.

Germination traits

The FGP of seeds varied significantly ($p < 0.05$) among the salinity treatments and among the saline zones. The FGP showed significant negative correlation ($p < 0.05$, $r = -0.81$) with the salinity treatments (Table 1). The FGP of seeds for LSZ was lowest (28.33%) compared with seeds of MSZ (49.67%) and HSZ (59.67%), at the higher salinity treatments of 35 psu (Figure 3a). Bonferroni adjustment analysis showed no significant ($p > 0.05$) differences in FGP among saline zones at 0 to 15 psu salinity treatments. However, at 20 to 35 psu salinity treatments, the differences in FGP between LSZ and MSZ, and between LSZ and HSZ were significant ($p < 0.05$), while the differences in FGP between MSZ and HSZ was not significant ($p > 0.05$) (Table 2).

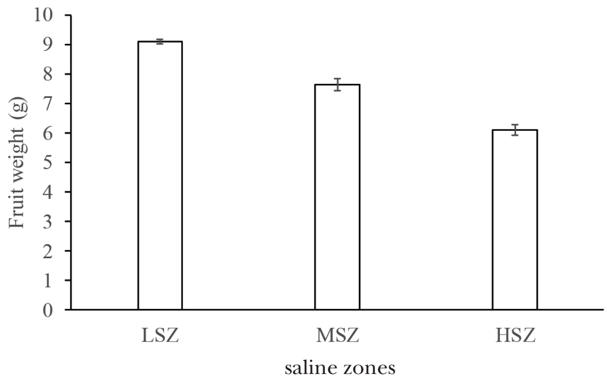

Figure 2 Fruit morphology (fruit weight) from three different saline zones; LSZ = less saline zone, MSZ = medium saline zone, HSZ = high saline zone

The GIT was found to vary significantly ($p < 0.05$) among salinity treatments and zones. The GIT showed a significant positive correlation ($p < 0.05$, $r = 0.87$) with salinity treatments (Table 1). The lowest GIT (2 days) was observed at 0 psu for seeds of all saline zones. But, highest GIT (8 days) was observed at 35 psu salinity treatment for LSZ followed by MSZ and HSZ (Figure 3c). Bonferroni adjustment analysis showed no significant ($p > 0.05$) differences in GIT among the saline zones at 0 to15 psu salinity treatments. However, at 20 to 35 psu salinity treatments, GIT was found to vary significantly ($p < 0.05$) between LSZ and MSZ, and between LSZ and HSZ, while GIT between MSZ and HSZ did not vary significantly ($p > 0.05$) (Table 2).

The GI was found to vary significantly ($p < 0.05$) among saline treatments and zones. The GI showed a significant negative correlation ($p < 0.05$, $r = -0.91$) with salinity treatments (Table 1). Highest GI of seeds (18.87 ± 0.62 seed day^{-1}) was observed for HSZ at 0 psu treatment, however GI was found to decrease with increasing salinity

Table 1 Correlation matrix of all studied parameters of germination traits

	Salinity	FGP	GIT	GI
Salinity	1			
Final germination percentage (FGP)	-0.81*	1		
Germination initiation time (GIT)	0.87*	-0.76*	1	
Germination index (GI)	-0.91*	0.84*	-0.90*	1

* Significant at the 0.05 level

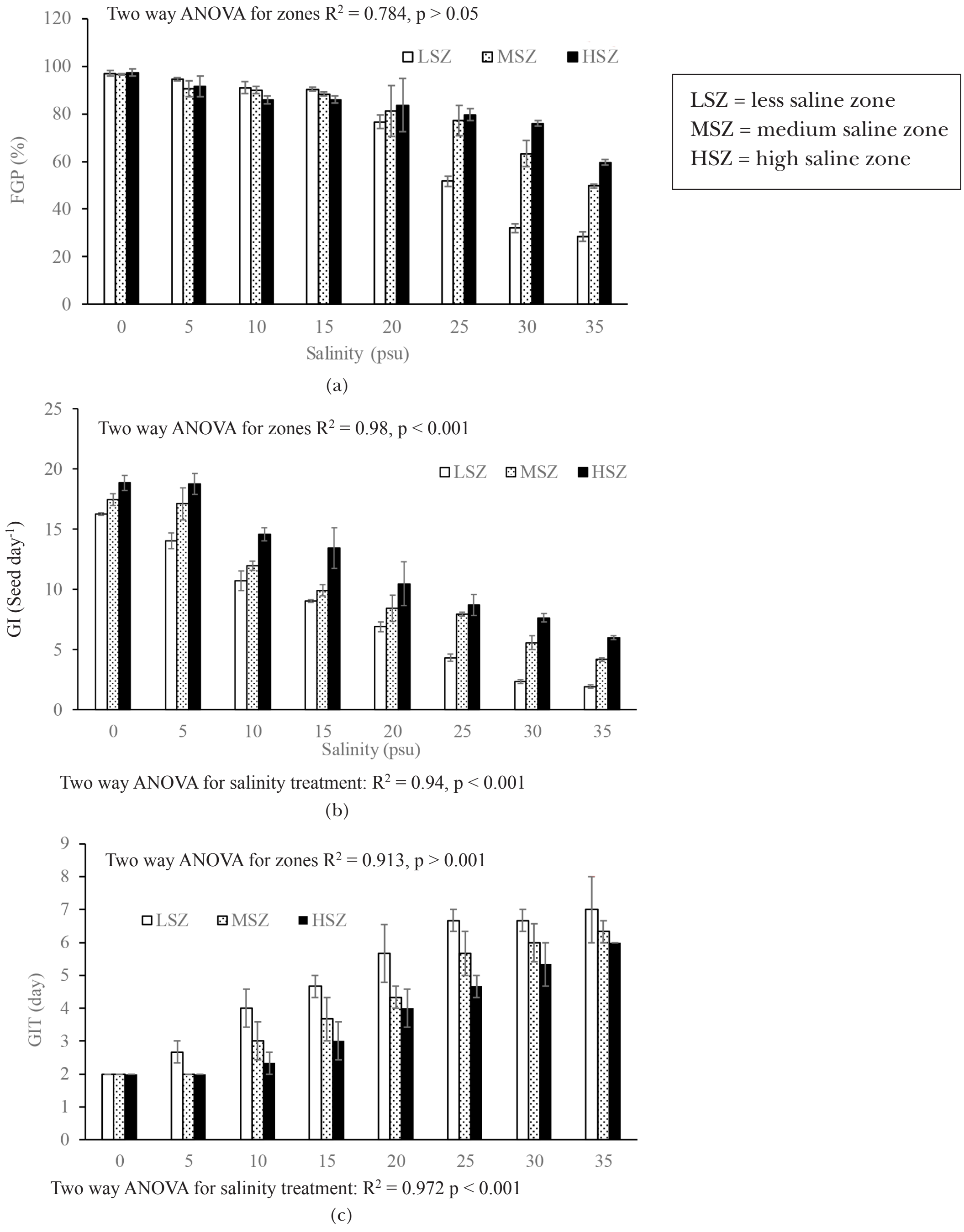

Figure 3 Germination traits of *Sonneratia apetala* (a) final germination percentage (FGP), (b) germination index time (GIT), (c) germination index (GI) at different salinity levels with three saline zones; means are significantly different as determined by two way ANOVA; vertical bars show standard errors of mean

Table 2 Pairwise comparisons (LSD) with Bonferroni adjustment results between different saline zones in respect of final germination percentage (FGP), germination initiation time (GIT) and germination index (GI)

Salinity	Between zones	FGP	GIT	GI
	LSZ and MSZ	0.98	1.00	1.00
0	LSZ and HSZ	0.99	1.00	1.00
	MSZ and HSZ	0.95	1.00	0.69
	LSZ and MSZ	0.98	0.15	0.21
5	LSZ and HSZ	1.00	0.15	0.04
	MSZ and HSZ	0.79	1.00	0.84
	LSZ and MSZ	1.00	0.64	0.56
10	LSZ and HSZ	0.57	0.18	0.05
	MSZ and HSZ	0.60	1.00	0.07
	LSZ and MSZ	0.78	0.73	0.06
15	LSZ and HSZ	0.10	0.22	1.00
	MSZ and HSZ	0.60	1.00	0.14
	LSZ and MSZ	0.43*	0.03*	0.01*
20	LSZ and HSZ	0.06*	0.01*	0.04*
	MSZ and HSZ	0.49	1.00	0.90
	LSZ and MSZ	0.01*	0.01*	0.01*
25	LSZ and HSZ	0.01*	0.01*	0.01*
	MSZ and HSZ	0.87	0.55	1.00
	LSZ and MSZ	0.00*	0.01*	0.01*
30	LSZ and HSZ	0.00*	0.01*	0.00*
	MSZ and HSZ	0.07	0.25	0.08
	LSZ and MSZ	0.00*	0.00*	0.00*
35	LSZ and HSZ	0.00*	0.01*	0.00*
	MSZ and HSZ	0.06	0.24	0.80

* Significant at 0.05 level; LSZ = less saline zone, MSZ = medium saline zone, HSZ = high saline zone

treatments for all zones. However, lowest (1.92 ± 0.11) GI was observed for LSZ at 35 psu salinity (Figure 3b). Bonferroni adjustment analysis showed no significant ($p > 0.05$) differences in GI among the saline zones at 0 to 15 psu salinity treatments. However, at 20 to 35 psu salinity levels, the differences in GI between LSZ and MSZ, and between LSZ and HSZ were significant ($p < 0.05$), while GI between MSZ and HSZ did not vary significantly ($p > 0.05$) (Table 2).

DISCUSSION

Sonneratia apetala fruits of HSZ of the Sundarbans were lighter than the fruits of LSZ and MSZ. Similar findings were also observed by Alam et al. (2018) while working with *A. officinalis* of the Sundarbans. Higher salt stress may reduce CO_2 assimilation, inhibiting photosynthesis which affects the energy source for growth and development (Naidoo 1987, Lin & Sternberg 1992, Siddiqi 2001). In other ways, most of the acquired energy was invested to fight against saline condition e.g. desalinisation and water uptake against an osmotic gradient (Waisel 1972). Salinity exerts complex effects on plants through ionic, osmotic and nutritional interactions (Zhu 2010, Liu et al. 2015). Salinity and other environmental factors also induce changes in the anatomy and morphology of mangrove plants, which may result in inter- and intra-specific variability among mangroves (Kathiresan &

Bingham 2001, Alam et al. 2019). These changes are considered as adaptive responses that may increase the survival chances of mangroves and endure salinity stress (Zhu 2001, Alam et al. 2018).

The FGP and GI (germination vigorousness) were lower at higher salinity levels. There was no significant differences in final germination percentage up to 15 psu among the three saline zones. The highest germination at 0 psu, irrespective of saline zones, indicated the facultative nature of *S. apetala*. At and above 20 psu salinity, the FGP were higher for MSZ and HSZ, showing adaptive responses to higher saline environments as stated by Waisel (1972) and Alam et al. (2018). This phenomenon explains the potentiality of a population to adopt with environmental conditions, especially with high salinity level (Nasrin et al. 2019). Increased salinity creates high osmotic potential in the germination medium, affecting imbibition and potentially inducing Na ion toxicity in seeds, and influencing enzyme activity and cell division (Waisel 1972, Clough 1984, Atak et al. 2006, Kim et al. 2013). These inhibitory effects may be responsible for the increased GIT, and decreased FGP and GI of *S. apetala* seeds. Similar observation was reported by Mahmood et al. (2014) with different mangrove species (*H. fomes, X. mekongensis, X. granatuma* and *Aglaia cucullatta*), and Alam et al. (2018) for *A. officinalis*.

In the germination experiment, seeds of different saline zones displayed variation in germination traits among the saline treatments. This may arise from acclimatisation of the plants to local environmental conditions (Vandelook et al. 2008). Johnson et al. (1992) demonstrated that species exposed to stressful environmental conditions were physiologically more adaptive to the stressful conditions than species from less-stressful environments. Variations in geographical condition lead to the evolution of different adaptations to the local environment (Savolainen et al. 2007). This type of adaptive responses may help to tolerate the heterogeneous environments across the distribution of a species (Liu et al. 2015).

CONCLUSION

The fruits of *S. apetala* that grow in LSZ were much heavier than MSZ and HSZ. Seed germination traits of this species in terms of FGI, GI and GIT varied among salinity treatments and origin of seeds. Faster and vigorous germination of seeds originated from MSZ and HSZ compared to those from LSZ, at higher saline treatments. This phenomenon indicated that seeds of MSZ and HSZ may have more inherent capacity to adapt with high saline conditions during their germination process. Local environmental variation (i.e salinity gradient of the Sundarbans) may have potential influences on the adaptive population of *S. apetala* in MSZ and HSZ of the Sundarbans.

ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support provided by Research Grants Program (RGP), Khulna University Research Cell, Khulna University. The authors also appreciate the technical support from Forestry and Wood Technology Discipline, Khulna University, Bangladesh.

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