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GENETIC VARIABILITY ASSESSMENT OF *Sonneratia apetala* (BUCH. -HAM.) IN THE SUNDARBANS OF BANGLADESH

Shamima Nasrin[1]*, Mahmood Hossain[1], Md. Rabiul Alam[1] and Md. Masudur Rahman[2]

[1]Forestry and Wood Technology Discipline, Khulna University, Khulna-9208, Bangladesh;

[2]Bangladesh Forest Research Institute, Chittagong, Bangladesh

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Abstract

Sundarbans is the single largest tract of natural mangrove forest in the world, which is situated in the south western coast of Bangladesh. This forest defends the south-western coastal region of the country from natural calamities, like cyclones, flood, tidal surges, tsunami etc. Sundarbans is the natural habitat for many mangrove plants. *Sonneratia apetala* exists in the low saline zone (LS), medium saline zone (MS) and in the high saline zone (HS) as a pioneer tree species of the Sundarbans. Genetic variability of *S. apetala* in these three saline zones were examined through the adoption of RAPD-PCR molecular marker. The expected heterozygosity of *S. apetala* in MS and HS (0.75±0.05 and 0.76±0.06, respectively) was greater than that of LS (0.60±0.13). Again, the average gene diversity over loci of this species in the MS and HS (0.59±0.37 and 0.62±0.38, respectively) was greater than that of LS (0.51±0.32). The greater heterozygosity and genetic diversity of *S. apetala* establishing in the MS and HS are the causes of its higher salt adaptability than that of growing in the LS in the Sundarbans. Due to higher genetic diversity and salt adaptability, *S. apetala* could persist in the increasing salinities in the Sundarbans. Moreover, *S. apetala* seedlings derived from the MS and HS zones of the Sundarbans can be planted in the high saline substrates in the coastal regions of Bangladesh, hence got added advantages for the coastal afforestation programs of Bangladesh.

Keywords: Adaptive variability, environmental variability, genetic diversity, saline zone

Introduction

The coastal region of Bangladesh expands as long as 711 km (Minar et al., 2013), which is located at the mouth of funnel shaped Bay of Bengal (Rahman & Biswas, 2011). Because of the geographical location, this coastal region is extremely prone to natural calamities such as tidal surges, intrusion of salt water, tropical cyclones, tsunami, etc. which are repeatedly originating from the Bay of Bengal (Alam et al., 2017). The Sundarbans, the world's largest natural mangrove vegetation (Mahmood, 2015), as well as the coastal mangrove plantations provide protection to the coastal regions of Bangladesh against those natural calamities (Alam et al., 2018a). Since mangrove forests fringe into the coastal areas (Sereneski-Lima et al., 2021), it experiences various stressful conditions such as salinity, high rate of sedimentation, anaerobic conditions, etc. (Saenger, 2002).

The mangroves are capable to adapt to the repeatedly occurring biotic and abiotic stresses. This adaptability is imperative for the survival, establishment as well as reproduction of mangroves (Tomlinson, 1986; Hutchings & Saenger, 1987). Siddiqi (2001) and Hogarth (1999) stated that mangrove plants are well adapted to the ecosystems in which they grow naturally. However, the variations in mangrove environments in terms of salinity, nutrient, sedimentation, frequency and duration of tidal inundation (Saenger, 2002) result in the environmental gradients that cause eco-physiological, morphological, physiological, anatomical and genetic differences in mangrove plants, even within the same species, growing in different environmental conditions (Alam et al., 2020). Plants exhibit variability in adaptive responses with respect to their morphological and physiological characteristics to cope with different physical environments (West-Eberhard, 1989; Alam et al., 2018a; Nasrin et al., 2019; Alam et al., 2020).

*Corresponding author: <Nasrin.shamima@outlook.com >

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The physical environments in the Sundarbans mangrove forest are variable owing to the variation in salinity, like, low saline zone (LS) (0.5-5 psu), medium saline zone (MS) (5-18 psu) and high saline zone (HS) (18-30 psu) (Hossain, 2015; Mahmood, 2015; Alam et al., 2018a; Nasrin et al., 2019). Though mangrove species compositions in these three saline zones are different, *S. apetala* is distributed along the salinity gradient and performs as a pioneer species in the phyto-successional processes in the Sundarbans (Siddiqi, 2001). This species grows well along the salinity gradient (from 0.5 to 30 psu) in the Sundarbans and possesses a wider salt adaptability along the salinity cline in the Sundarbans. Specifically, this species grown in MS and HS is more salt adaptive with respect to nutrient re-translocation than that grown in LS (Nasrin et al, 2019). Again, the seeds of *S. apetala* produced in the MS and HS germinate faster and farther vigorously at higher salinities than such of LS (Nasrin et al., 2020). Moreover, the seedlings of the species produced in the MS and HS grow satisfactorily at high salinities compared with such of LS (Nasrin et al., 2021). Therefore, there exists adaptive variability in *S. apetala* that is thriving in the different saline zones of Sundarbans (Nasrin et al., 2021).

Alam et al. (2018a, b) and Alam et al. (2019) in their several studies found that *A. officinalis* thriving in MS and HS is physiologically, morphologically and anatomically better salt adaptive that such of LS in the Sundarbans. *Avicennia officinalis* growing in MS and HS is genetically more diverse than such of LS, and that was proved to be the reason of higher salt adaptability of the species in the MS and HS (Alam et al., 2020). Having this information and considering the adaptive variability of *S. apetala,* we attempted to find out the cause(s) of this variability in salt adaptability of *S. apetala* in the different saline zones of Sundarbans by studying the genetic variability of this species.

The heterozygosity and quantitative genetic diversity of mangroves enables them be able to survive in the adverse mangrove environments (Reed & Frankham, 2002). Genetic diversity is crucial to overcome the stressful environmental conditions, like, mangrove ecosystems (Alam et al., 2020). Genetic diversity in a species also helps develop acclimatization mechanisms that allow mangroves to cope up with the changing environmental stresses (Munne-Bosch & Alegre, 2013). Alam et al. (2020) proved that the genetic diversity within *A. officinalis* generates adaptive plasticity in the species that makes this species be able to withstand variable habitat conditions caused by variations in salinity in the Sundarbans. Considering the ecological significance of *S. apetala* in mangrove succession, present study was carried out on genetic variability of *S. apetala* flourishing in different saline conditions in the Sundarbans by adopting RAPD marker with PCR.

Materials and Methods

Leaf sample collection

With a view to collecting leaf samples of *S. apetala* from the three salinity zones of Sundarbans, the pre-selected maternal trees of the species (Figure 1) were chosen. The tender leaf samples of *S. apetala* from the pre-selected six maternal trees of each saline zone of Sundarbans were collected separately, viz., LS (N 22° 37'13.37″ and E 89° 64'53.3″), MS (N 22° 37'13.37″ and E 89° 64'53.3″) and HS (N 22° 17' 19.7 ″ and E 89° 19'50.8″) in January, 2018. The leaf samples of each of the maternal trees were preserved separately and were labeled from LS1 to LS6 for the LS; MS1 to MS6 for the MS; and from HS1 to HS6 for the HS of Sundarbans.

Isolation of genomic deoxyribonucleic acid (gDNA) of S. apetala

The genomic DNA of each leaf sample of *S. apetala* was extracted separately by using Gene-jet plant genomic DNA purification mini kit # K0791 (Thermo Scientific) in plant genetics laboratory of Bio-technology and Genetic Engineering Discipline of Khulna University, Bangladesh during January, 2018. All of the leaf samples were kept in a refrigerator for lyophilization at -80 °C for 24 hours. Then, 20 mg of each leaf sample was kept into liquid nitrogen and then ground into powder by using pestle and mortar in the laboratory. Subsequently, each powdered sample was transferred in a 1.5 ml centrifuge tube. Then, 350 µL lysis buffer A was added in each sample in the micro centrifuge tube. It was vortexed thoroughly for 15 seconds. After that, 50 µL lysis buffer B and 20 µL RNase A were added to each sample, and they were mixed thoroughly by vortexing for 1.5 minutes. The samples were thereafter incubated for 15 minutes at 55 °C. 130 µL precipitation buffer was poured in each sample and kept in ice for 5 minutes. All the samples were then centrifuged by using NF800R, NUve at 14000 rpm for 5 minutes according to Alam et al. (2020)

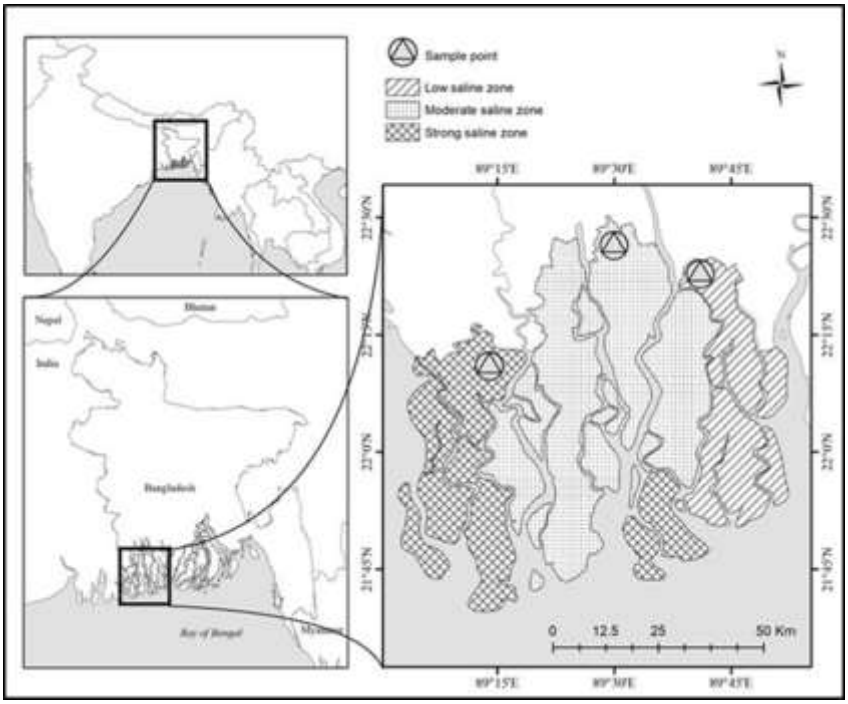

Figure 1. Map showing the study sites in the Sundarbans (Nasrin et al., 2020).

Some 500 µL supernatant form each sample was collected and poured into another micro centrifuge tube. 400 µL plant gDNA binding solution and 400 µL 96% ethanol were added to each supernatant sample and mixed up thoroughly. Half of the mixture (650 µL) was transferred in a spin column and then centrifuged for 1 minute at 8000 rpm. The flow-through was removed. Subsequently, the remaining half of that mixture was poured in the spin column and then centrifuged again for 1 minute at 6000 rpm. 500 µL washing buffer 1 was pour in the spin column and then centrifuged for 1 minute at 8000 rpm. The flow-through was removed. After that, 500 µL washing buffer 11 was instilled in the spin column and then centrifuged for 3 minutes at 14000 rpm. Collection tube was removed. Then, the spin column was shifted in a new sterile 1.5 mL centrifuge tube.100 µL elution buffer was instilled in the spin column and kept for 5 minutes at room temperature. Spin column was then centrifuged for 1 minute at 10000 rpm. Similarly, the second step elution was carried out. Purified DNA samples were kept in a refrigerator at -20 °C for further use.

RAPD-PCR analysis

Six random primers were used for each sample for amplifications of the DNA nucleotide sequences for examining the genetic variability of a species (Alam et al., 2020). For RAPD-PCR, each sample was made by admixing Taq DNA polymerase (5 U/µl), dNTPs (10 mM each), $MgCl_2$, 10 X reaction buffer, random primer, milli Q water and template DNA. The samples were instilled in the slots of Biometra professional thermos-cycler. Temperature sequences in the PCR were fixed as lead temperature at 105 °C, DNA denaturing temperature at 94 °C, annealing temperature at 26 °C, and expansion temperature at 72 °C with 42 cycles. Finally, the thermos-cycler was held for 7 minutes at 72 °C. the process was followed for each random primer.

Gel electrophoresis

Agarose gel was made up with 1% TAE (Tris HCl, EDTA, Glacial Acetic Acid) buffer. Gel was instilled in the Biometra gel electrophoresis containing 1% TAE buffer solution. PCR product was instilled in the slots of the gel. 75 ampere electric field was applied for gel electrophoresis and run for 50 minutes.

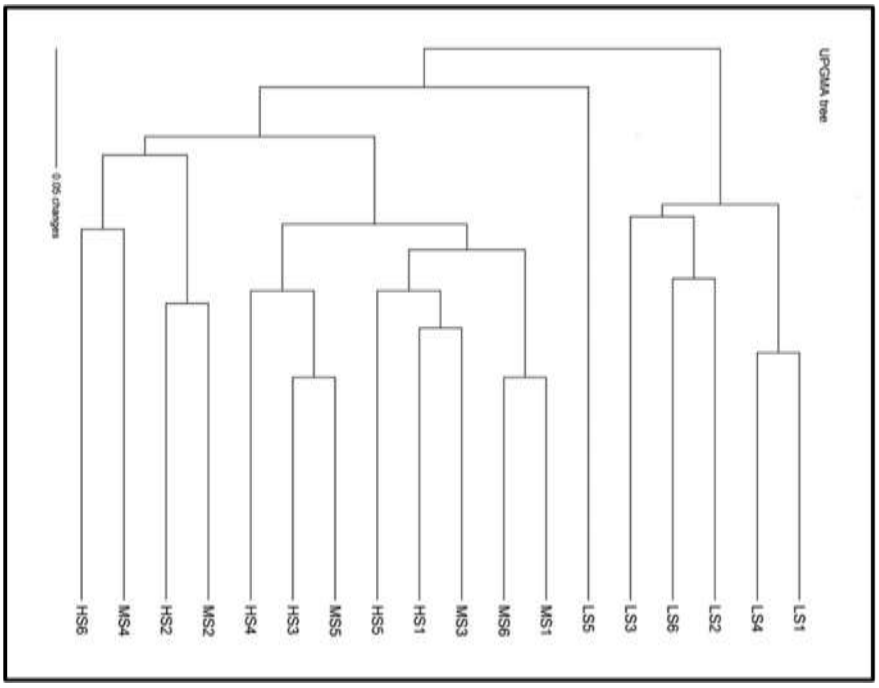

Figure 2. UPGMA tree of *S. apetala* of LS, MS and HS of Sundarbans.

Gel documentation

Image of every gel was taken up by applying gel documentation system (UV star BDA compact, Biometra).

Molecular data analysis

Molecular data were produced from each image. Data were analyzed with the help of PAUP, version 4.0 software to generate UPGMA tree in order to assay the phylogeny of *S. apetala* in different saline zones of Sundarbans. Secondly, the data were analyzed through using Arlequin ver. 3.5.1.2 to examine the expected heterozygosity (H_{EXP}) of different populations, gene diversity over loci and analysis of molecular variance (AMOVA) and to calculate genetic distance matrix for comparison of genetic distance among the populations of *S. apetala* in the Sundarbans.

Results

DNA molecules of LS1, LS2, LS3 LS4 and LS6 of the LS created a monophyletic taxon (Figure 2). While those of LS5 of LS; MS1, MS2, MS3 MS4, MS5 and MS6 of the MS and those of HS1, HS2, HS3 HS4, HS5 and HS6 of the HS formed larger paraphyletic taxon of *S. apetala* (Figure 2).

RAPD-PCR analysis

RAPD-PCR analysis exhibited that the DNA molecules of *S. apetala* of different saline zones produced RAPD-bands at different base-pairs levels (Fig. 3). Each of the DNA samples of LS, MS and HS produced polymorphic band when run with random primers OPE-20 (5´AACGGTGACC3´), OPA-04 (5´AATCGGGCTG3´), OPB-05 (5´TGCGCCCTTC3´), OPC-06 (5´GAACGGACTC3´), OPB-08 (5´GTCCACACGG3´), OPC-17

(5´TTCCCCCCAG3´) while only one DNA sample of the LS produced monomorphic band when run with OPB-08 (5´GTCCACACGG3´) (Table 1).

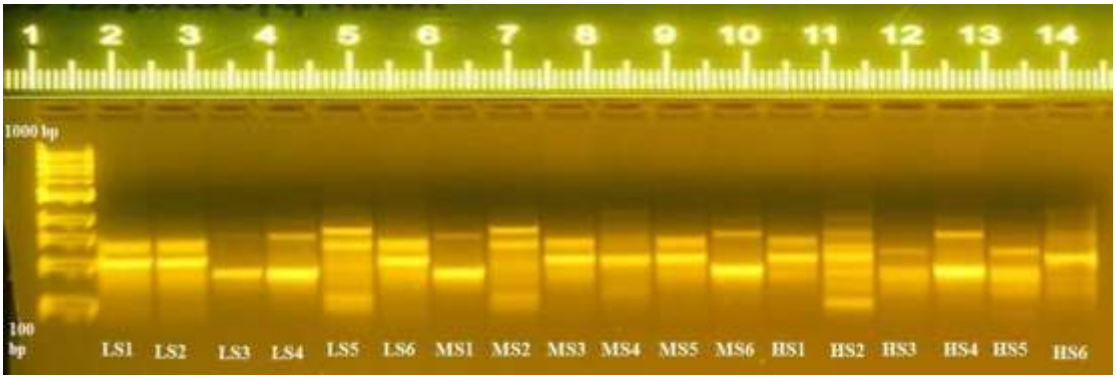

Figure 3. RAPD bands of *S. apetala* of LS, MS and HS of Sundarbans.

Expected heterozygosity (H_{EXP}) of different populations

Expected heterozygosity (H_{EXP}) of *S. apetala* in the LS, MS and and HS were 0.60±0.13, 0.75±0.05, 0.76±0.06, respectively (Table 2).

Average gene diversity over loci

Average gene diversity *S. apetala* in the LS, MS and HS were 0.51±0.32, 0.59±0.37 and 0.62±0.38, respectively (Table 3).

Table 1. RAPD bands of *S. apetala* in different saline zones of Sundarbans.

Primers	LS						MS						HS					
	LS1	LS2	LS3	LS4	LS5	LS6	MS1	MS2	MS3	MS4	MS5	MS6	HS1	HS2	HS3	HS4	HS5	HS6
5´AACGGTGACC3´	2	2	2	3	4	3	2	5	3	5	3	3	3	8	4	3	4	6
5´AATCGGGCTG3´	3	3	3	3	3	3	5	4	5	5	5	4	5	5	5	5	5	5
5´TGCGCCCTTC3´	4	2	3	5	3	4	5	4	4	4	4	5	4	3	6	2	3	2
5´GAACGGACTC3´	3	3	3	3	3	3	7	5	5	6	5	6	7	5	6	6	4	5
5´GTCCACACGG3´	5	1	2	5	3	3	6	5	6	5	5	5	3	6	3	5	3	4
5´TTCCCCCCAG3´	2	4	4	2	3	5	5	4	2	5	3	4	2	4	4	2	3	5

Table 2. Expected heterozygosity (H_{EXP}) of *S. apetala* in different saline zones of Sundarbans.

Saline zone	H_{EXP} (mean±sd)
LS	0.60±0.13
MS	0.75±0.05
HS	0.76±0.06

Table 3. Average gene diversity of *S. apetala* in different saline zones of Sundarbans.

Saline zone	Average gene diversity (mean ± sd)
LS	0.51±0.32
MS	0.60±0.37
HS	0.63±0.38

Table 4. Analysis of molecular variance (AMOVA).

Source of Variation	d.f	Sum of squares	Variance components	Percentage (%) of variation
Among populations	2	20.000	0.51667 va	21.38
Among individuals within Populations	15	57.000	1.90000 vb	78.62
Total	17	77.000	2.41667	
Fixation Index	F_{ST} :	0.21379		

Analysis of molecular variance (AMOVA)

Analysis of molecular variance was performed in order to calculate the genetic variation (Patrick & Shenglin, 2018) within different populations of *A. officinalis* in the Sundarbans. Genetic variation in different populations and within the population of *S. apetala* were 22.38% and 78.62%, respectively (Table 4). The fixation index (F_{ST}) for the species in LS, MS and HS was 0.21 (Table 4).

Genetic distance matrix

Genetics distance matrix is important to examine the strength of genetic structure and to assay the genetic variations within the individual, within the population and among the populations of a species ((Patrick & Shenglin, 2018) Pair-wise genetic distance of S. apetala between LS and MS (0.34) and between LS and HS (0.36) were higher than that of between MS and HS (0.13) (Table 5).

Table 5. Pairwise genetic distance (F_{ST}) matrix of *S. apetala.*

Saline zone	LS	MS	HS
LS	0.00		
MS	0.34	0.00	
HS	0.36	0.13	0.00

Discussion

Genetic diversity provides a species with necessary adaptive mechanisms to the prevailing environmental conditions (Kimmins, 1987). Again, the genetic variability of mangroves generates adaptive plasticity in the species and consequently allows it to cope up with a wider range of physical environments (Alam et al., 2020). Since the saline conditions in Sundarbans fluctuate, the physical environmental conditions in LS, MS and HS of the Sundarbans are different (Nasrin et al., 2019). But, *S. apetala* grows everywhere in the Sundarbans and exhibits wide spectrum of salt adaptive variability (Nasrin et al., 2021). To determine the reason, the genetic diversity of the species flourishing in LS, MS and HS of Sundarbans was studied.

Most RAPD bands of the species of LS, MS and HS are polymorphic (Table 1) and a higher percentage (78.62%) of genetic variation was found among the individuals within each of the populations of *S. apetala.* Alam et al. (2020) also detected the polymorphism and higher genetic variation within the population of *A. officinalis* spreading in the LS, MS and HS of Sundarbans. Therefore, the polymorphism and higher genetic variation were the clear reflections of higher genetic diversity of *S. apetala* across the Sundarbans.

Genetic diversity of *S. apetala* of MS and HS was higher than that of LS (Table 3), and expected heterozygosity of this species of MS and HS was also higher than that of LS (Table 2). As a result, the genetic distance between the populations of the species of MS and HS was narrower than that between LS and MS and between LS and HS. Alam et al. (2020) in their study found higher genetic diversity and expected heterozygosity of *A. officinalis* in MS and HS compared with that in LS. Genetic structure of *A. schaueriana* populations of South America are diversified (Mori et al., 2015). Genetic diversity of different populations of *A. marina* favors the species to cope up with the changing environmental conditions in east coast of India (Hazarika et al., 2013). Sereneski-Lima et al. (2021) also found genetic diversity in *L. racemosa* in the equatorial, tropical and subtropical population in the Pacific region. They argued that the ecological conditions are ascribable to the genetic structure of mangroves.

So, the results demonstrated that the higher genetic diversity and expected heterozygosity of *S. apetala* in MS and HS resulted in larger salt adaptability of the species surviving in MS and HS compared with that of LS. Finally, it

can be inferred that the higher genetic diversity of *S. apetala* is the reason of the species' salt adaptive variability in LS, MS and HS of Sundarbans. Hence, salt adaptability is the outcome of the genetic diversity of *S. apetala.* Moreover, the higher heterozygosity in the populations *S. apetala* of MS and HS allows them to take part in cross pollination. This might result in further increase in genetic diversity within the species.

High genetic diversity favors a plant species in the heterogeneous environments (Alam et al., 2020). Dashzeveg et al. (2017) also demonstrated that intra-species genetic diversity is necessary for its survival in various habitats. Again, Alam et al. (2020) validated that *A. officinalis* can cope up with different saline environments due to its genetic diversity in the LS, MS and HS of Sundarbans. Therefore, it is concluded that the greater genetic diversity among populations and among individuals within populations of *S. apetala* may make this species be capable to survive and grow in the increasing saline conditions in the Sundarbans of Bangladesh.

Bangladesh started coastal afforestation program in 1966, and since then, *S. apetala* has been the principal mangrove planting species in those coastal plantations (Siddiqi, 2001; Nasrin et al., 2021). In the coastal areas of Bangladesh, salinity is increasing owing to sea level rise (Alam et al., 2018a, b). *Sonneratia apetala*, particularly, of MS and HS possesses salt adaptive variability and genetic diversity so that the species will continue to be a major mangrove planting species in the higher saline conditions in the coastal areas of Bangladesh.

Conclusion

Sonneratia apetala of MS and HS is more heterozygous and genetically more diverse than that of LS. The genetic distance of *S. apetala* between MS and HS is less, thereby constituting a larger population of it while the species of LS is genetically more distant from such of MS and HS. Owing to greater heterozygosity and genetic diversity, *S. apetala* of MS and HS is much more adaptive to salinity than that of LS of the Sundarbans of Bangladesh. Considering this, it can be inferred that *S. apetala* maternal trees can give precise evidence of the genetic variability in the three distinct salinity zones. Though the salinity in the Sundarbans is increasing day by day, *S, apetala* of MS and HS zones can persist in its habitats and thereby playing a significant role in the sustainability of the Sundarbans. Again, owing to higher salt adaptability and genetic diversity, *S. apetala* of MS and HS zones can be a reliable source of planting material for restoration of Chokaria Sundarbans and also for the coastal afforestation in the high saline coastal regions of Bangladesh. Hence, the coastal dwellers get protection against frequently arising natural calamities in future.

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Conflict of Interests

The author declares no conflict of interest.

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