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ORIGINAL PAPER

Deciphering the genetic diversity of *Avicennia officinalis* L. across the salinity gradient in the Sundarbans mangrove forest of Bangladesh

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Abstract Mangroves adaptive plasticity in the changing environmental conditions is of vital importance for conservation management. Genetic diversity of mangrove brings about adaptive plasticity, enabling a species to cope with different habitat conditions. The Sundarbans of Bangladesh is the largest coastal wetland and mangrove forest with diversified habitat conditions that support a wide diversity of flora and fauna. *Avicennia officinalis* L. is the pioneer species in mangrove succession in the low-salinity, medium-salinity and high-salinity zones of the Sundarbans. Adopting RAPD-PCR analysis, the genetic diversity of *A. officinalis* was studied to explore the ecotypes of this species in the Sundarbans. The genetic distances of *A. officinalis* between the low-salinity and medium-salinity zones (0.50) and between the low-salinity and high-salinity zones (0.52) are significantly ($p < 0.05$) higher than that between the medium-salinity and

high-salinity zones (0.09). The expected heterozygosity of *A. officinalis* of medium-salinity (0.54 ± 0.14) and high-salinity zones (0.55 ± 0.10) are higher than that of low-salinity zone (0.37 ± 0.12). The genetic diversity of *A. officinalis* of medium-salinity (0.5417 ± 0.3167) and high-salinity (0.5458 ± 0.3189) zones is significantly ($p < 0.05$) higher than that of low-salinity zone (0.3750 ± 0.2313). This genetic diversity of *A. officinalis* bears significant ecological consequences. *A. officinalis* growing in the low-salinity zone is the low salt-adapted ecotype while that growing in the medium-salinity and high-salinity zones is the high salt-adapted ecotype in the Sundarbans. These two ecotypes of *A. officinalis* are of both in-situ and ex-situ conservation importance for the Sundarbans and coastal plantations of Bangladesh to face the climate change induced salinity regime changes in the future.

Keywords Conservation · Ecotype · Genetic distance · Mangrove · Salinity zone · Sundarbans

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Introduction

The Sundarbans, situated in the southwestern coast of Bangladesh ($21^{\circ} 30' - 22^{\circ} 30' \text{ N}$, $89^{\circ} 00' - 89^{\circ} 55' \text{ E}$), is the world's largest (6017 km^2) coastal wetland ecosystem that contains a pool of mangrove species

(Minar et al. 2013; Alam et al. 2018a). Salinity is the most influential ecological factor in this forest (Siddiqi 2001; Alam et al. 2018a). The salinity regimes within the Sundarbans vary with different seasons in the year and also with the frequency and duration of tidal inundation (Siddiqi 2001). The salinity in the Sundarbans remains low during the monsoon season and increases towards the premonsoon (dry period) season. Again, salinity is the lowest in the eastern part and highest in the western part of the Sundarbans (Naskar and Bakshi 1987; Hussain and Acharya 1994; Nasrin et al. 2019). These variations in the salinity regimes result in distinct ecological zonations of the Sundarbans into low-salinity zone (LSZ) (0.5–5 ppt) (parts per thousand), medium-salinity zone (MSZ) (5–18 ppt), and high-salinity zone (HSZ) (18–30 ppt), respectively (Siddiqi 2001; Alam et al. 2018a). Because of these differences in salinity, the availability of essential soil nutrients (nitrogen, phosphorus and potassium) for mangroves in these three salinity zones are apparently variable, even during the different seasons in the year (Alam et al. 2019; Nasrin et al. 2019). For instance, the available form of nitrogen, phosphorus and potassium in soil in the monsoon and post monsoon seasons is higher and decreases significantly in the premonsoon season. Specifically, the available form of nutrients in the MSZ and HSZ in the premonsoon season is significantly lower than that of LSZ in the Sundarbans (Alam et al. 2019). In addition, increasing salinity also reduces the availability of water for plant uptake (Waisel 1972; Saenger 2002). Consequently, the habitat conditions in terms of the availability of nutrients and water for the mangroves in these three salinity zones are different due to the differences in the salinity stress (Siddiqi 2001; Mahmood 2015; Alam et al. 2019). As a result, mangrove species distribution and dominance in the three salinity zones of the Sundarbans varies greatly (Siddiqi 2001; Mahmood et al. 2014). For example, *Heritiera fomes* Buch.-Ham., *Excoecaria agallocha* L., and *Ceriops decandra* (Griff) Ding Hou are the dominant species in the LSZ, MSZ, and HSZ, respectively (Siddiqi 2001; Alam et al. 2018a).

Despite this ecological divergence, *Avicennia officinalis* L. grows gregariously in the three salinity zones of the Sundarbans (Mahmood 2015; Alam et al. 2018b). Being a pioneer species, *Avicennia officinalis* plays significant ecological role in the mangrove succession of the Sundarbans and also in the Indo-

Pacific regions (Spalding et al. 1997; Alam et al. 2017, 2018b). Moreover, this species is mostly planted on the high-saline substrates in the coastal regions of Bangladesh ((Alam et al. 2018a). As *A. officinalis* exhibits a wider salt adaptability in the three ecological habitats in the Sundarbans (Alam et al. 2019), one valuable question pertaining to its greater ecological distribution and salt adaptability bears asking: Are there different ecotypes of *A. officinalis* in the Sundarbans mangrove forest of Bangladesh?

The ecotypes are two or more ecologically divergent groups of a species (Raszick and Song 2016). Some species have different ecotypes that are well-adapted both in the coastal and terrestrial environments (Shan et al. 2008). The ecotypes or habitat types (Turesson 1922) refer to the genetic subdivisions of a species that are well adapted to different physical environments (Kimmins 1987). Therefore, the ecotypes are the different ecological sub-units resulting from the genotypic responses of a species to different habitat conditions (Turrill 2006; Raszick and Song 2016). Further, the ecotypes vary in morphology and physiology (Turrill 2006). Such variations in morphological and physiological traits within a species lead to the development of adaptive phenotypic plasticity which enables a species to persist in various ecological habitats (Mettler and Gregg 1969).

Alam et al. (2018a) studied the adaptive phenotypic plasticity with respect to the variation in propagule morphology and propagule growth initiation traits of *A. officinalis* across the salinity gradient in the Sundarbans of Bangladesh. The phenotypic plasticity is the way by which plants can cope with different environmental conditions induced by climate change (Frank et al. 2017). Alam et al. (2018b) assayed the influences of different maternal origins of *A. officinalis* seedlings on their plasticity in salt adaptability with respect to various growth characteristics and nutrient accumulation in different seedling parts. Alam et al. (2019) studied the physiologically adaptive plasticity in nutrient resorption efficiency of *A. officinalis* growing naturally in the three salinity zones of the Sundarbans. All these studies covered adaptive plasticity in propagule morphology, leaf anatomy, and different physical and physiological traits of *A. officinalis* growing in the three distinct salinity zones in the Sundarbans of Bangladesh. Here, we were interested to study the genetic diversity of this species to assay whether there are variations within *A.*

officinalis at the genetic level in the three salinity zones in the Sundarbans, thereby exploring the ecotypes of this species.

Genetic diversity represents the heritable variation within and between the populations of a species (Fairbanks and Andersen 1999; Rao and Hodgkin 2002; Hughes et al. 2008). The genetic variability of populations is of vital importance from the adaptation perspective (Kimmins 1987). The genetic variability enables successful survival of a species with environmental changes (Xiong 2006). The evolutionary divergence i.e., phylogenetics (Xiong 2006) leads to the genetic subdivision of a species into ecotypes which are well adapted to different physical environments (Turesson 1922; Kimmins 1987). Therefore, genetic diversity study by using molecular marker is important to find out the ecotypic differences within a plant species.

The genetic structure, breeding systems and gene flow within and among the populations of a species can be explored through genetic diversity assessment that helps in developing in-situ and ex-situ conservation measures for that species (Jena et al. 2014). Unfortunately, the scientific study on the mangrove genetic resources, particularly, mangrove tree species is still scanty (Hazarika et al. 2013). Only a few studies on the genetic diversity of mangroves have been conducted. Das et al. (2014) studied the phylogenetic relationship among the three species of mangrove genus *Avicennia* in the Indian Sundarbans by applying random amplified polymorphic DNA (RAPD) markers. Hazarika et al. (2013) studied genetic diversity in the three populations of *Avicennia marina* (Forssk.) Vierh along the east coast of India by RAPD markers. However, the genetic diversity of *A. officinalis* growing in the three salinity zones of the Sundarbans of Bangladesh has not yet been studied by using any molecular marker.

Using molecular marker is known to be an effective method of evaluating the genetic diversity and genetic structure of a plant species (Li et al. 2017). The phylogenetic analyses by using molecular marker are important to determine phylogenetic relatedness among the different populations of a species (Elmouni et al. 2017). The genetic diversity can be studied by applying different DNA based molecular markers. Among them, RAPD is simple and easily applicable (Ashish et al. 2011; Hazarika et al. 2013). Therefore, by using RAPD markers, we attempted to

assay the genetic diversity of *A. officinalis* growing in the three ecological habitats in the Sundarbans of Bangladesh for the first time. Based on the genetic diversity of *A. officinalis*, and considering the findings of Alam et al. (2018a), Alam et al. (2018b) and Alam et al. (2019), we attempted to explore whether there are different ecotypes of this species in the Sundarbans of Bangladesh.

Materials and methods

Plant material collection

The dominant patches of *A. officinalis* in the low-salinity (22 ° 22' 25.0"N, 89 ° 44'35.7"E), medium-salinity (22 ° 28'06.1"N, 89 ° 30'52.2"E), and high-salinity (22 ° 12'18.6"N, 89 ° 11'45.5"E) zones of the Sundarbans (Fig. 1) were identified by following the Overseas Development Agency (ODA) vegetation map, and the salinity map of the Sundarbans in 2015 (Alam et al. 2018a). For assaying the genetic diversity of *A. officinalis*, the plant samples were collected from the same maternal trees as were collected by Alam et al. (2018a), Alam et al. (2018b), and Alam et al. (2019) in order to maintain consistency in research. The young leaves were collected from eight maternal trees of *A. officinalis* from each of the three salinity zones in December, 2017. The leaf samples of each of the maternal trees were kept in separate polythene bags and were labeled accordingly. The leaf samples collected from the eight maternal trees from the low-salinity zone were labeled as LS1–LS8. Similarly, the leaf samples collected from the medium-salinity zone were labeled as MS1–MS8. Likewise, the leaf samples collected from the high-salinity zone were labeled as HS1–HS8.

gDNA isolation and quantification

The leaf samples were lyophilized at – 80 °C for 24 h. 20 mg of each of the 24 lyophilized leaf samples of three saline zones were kept separately in liquid nitrogen, and then ground into powder by using mortar and pestle. The genomic DNA of each of the leaf samples of *A. officinalis* collected from each of the three salinity zones was isolated by using Gene-jet plant genomic DNA purification mini kit # K0791 and its protocol (Thermo Scientific, Lithuania) in the plant

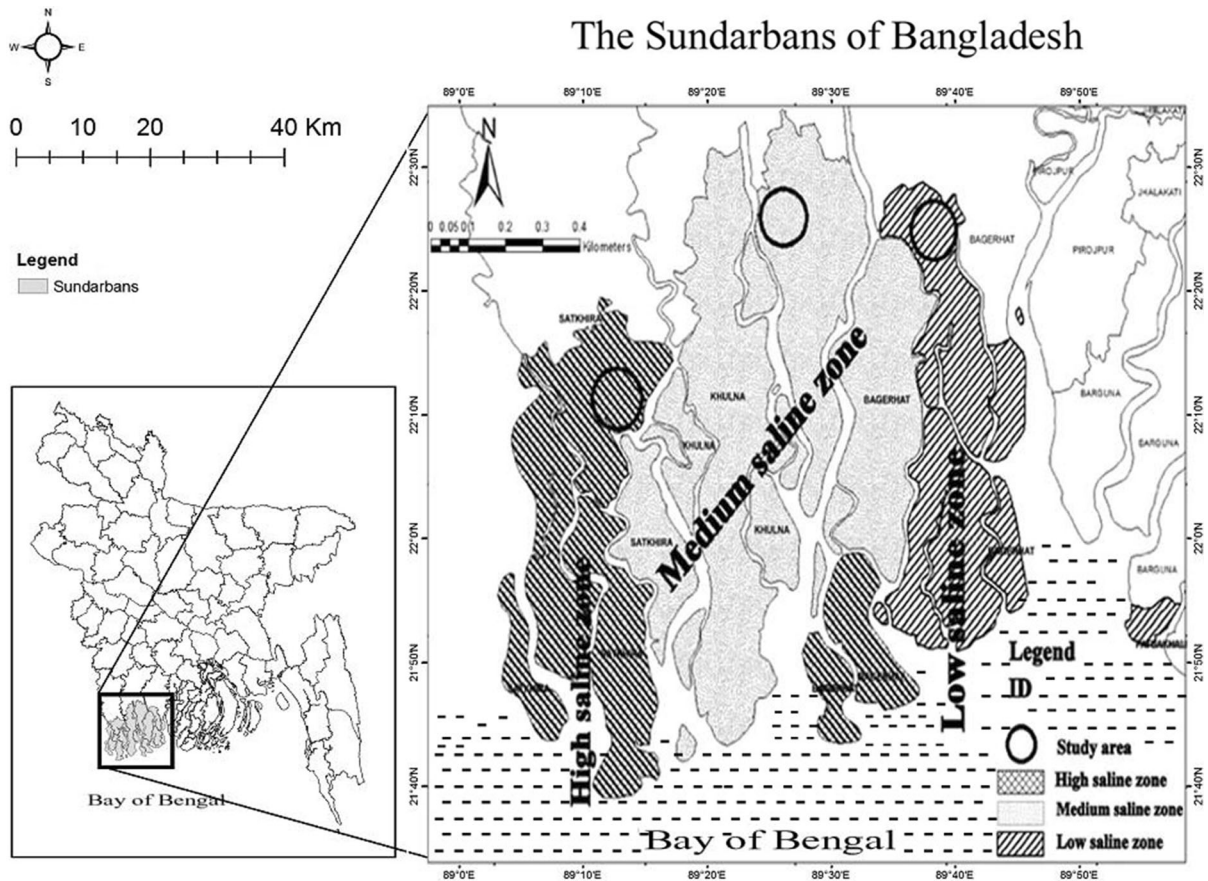


Fig. 1 Plant sample collection areas in the three salinity zones of the Sundarbans (Alam et al. 2018b). The licence # 4465250506454

genetics laboratory of Biotechnology & Genetic Engineering Discipline of Khulna University, Bangladesh in December, 2017. DNA concentration was quantified by using spectrophotometer (Multiskan GO, Thermo scientific) at 260 nm. The purified DNA samples were then stored at -20°C .

RAPD-PCR analysis

Eight oligonucleotide random primers (Table 1) were used separately for RAPD-PCR analysis with all of the 24 samples in order to analyze genetic diversity among *A. officinalis* populations in the three saline zones of the Sundarbans. Using each primer, 24 Samples were prepared by mixing dNTPs (10 mM each), Taq DNA polymerase (5 U/ μl), $10\times$ reaction buffer, MgCl_2 oligonucleotide primer, template DNA, and milli Q water. Subsequently, the prepared samples were kept in the 24 slots of professional thermo cycler

Table1 Names, nucleotide sequence and T_m of the primers

Primer name	Nucleotide sequence	T_m ($^{\circ}\text{C}$)
OPA 18	5'AGGTGACCGT3'	36.2
OPA 11	5'CAATCGCCGT3'	36.7
OPA 15	5'TTCCGAACCC3'	34.2
OPA 16	5'AGCCAGCGAA3'	38.3
OPA 09	5'GGGTAACGCC3'	37.4
OPA 05	5'AGGGGTCTTG3'	32.6
OPA 03	5'AGTCAGCCAC3'	34.3
OPC 06	5'GAACGGACTC3'	33.5

(Biometra). The sequences of temperature in RAPD-PCR were set as lead temperature at 105°C , DNA denaturing at 94°C for 30 s, annealing of primers and DNA at 27°C for 30 s, and expansion at 72°C for 2 min. Starting the thermo cycler, a total of 37 cycles

were run. Then, the machine was held at 72 °C for 7 min. This process was done 8 times for the selected 8 random primers.

Gel electrophoresis and gel documentation

For each gel run, 1% of agarose gel was prepared by using 1% TAE (Tris HCl, Glacial Acetic Acid, EDTA) buffer. The gel was then kept in gel electrophoresis (compact XS/S, Biometra) containing 1% TAE buffer. The PCR products were then loaded separately in each of the slots of the agarose gel. An electric field (70 amp.) was created in the gel electrophoresis and run for 45 min for PCR products of each primer. 1 Kb (1000 bp) DNA ladder was used as a marker. After gel electrophoresis of each gel, image was taken by using gel documentation system (gel doc) (UV star BDA compact, Biometra.)

Data analysis

Molecular data were generated from the eight images produced from the RAPD-PCR using eight oligonucleotide random primers. Data were analyzed by using phylogenetic analysis using parsimony (PAUP) version 4.0 software to produce UPGMA (un-weighted pair group method with arithmetic means) tree in order to assay the phylogenies (phylogenesis) of *A. officinalis* in the three saline zones of Sundarbans. Again, the molecular data were analyzed by using Arlequin version 3.5.1.2 in order to calculate the fixation index (F_{ST}), the genetic (molecular) variation (%) within and among the three populations, gene diversity over loci, expected heterozygosity (H_{EXP}) of different populations, and pair-wise comparisons of genetic (molecular) distance in fixation index (F_{ST}) based on the number of different alleles over loci among the populations of *A. officinalis* in the low-salinity, medium-salinity and high-salinity zones of the Sundarbans. One-way ANOVA and unpaired t-test (two tailed) were performed by using SAS (6.12.0.1) statistical software to quantify whether the genetic variations and genetic distances among the three populations of *A. officinalis* of LSZ, MSZ and HSZ of the Sundarbans are significant at the 0.05 significance level.

Results

The RAPD-PCR analysis of the DNA samples with oligonucleotide random primers showed that they formed RAPD-bands at different base-pairs levels (Fig. 2). Six of the DNA samples of the low-salinity zone formed monomorphic band when run with 5'AGGTGACCGT3' random primer while only two DNA samples of the medium-salinity zone and two DNA samples of the high-salinity zone form monomorphic band when run with the same primer (Table 2). The DNA of LS1–LS8 samples of *A. officinalis* of the low-salinity zone formed a monophyletic group (Fig. 3). On the other hand, the DNA of MS1–MS8 samples of this species of the medium-salinity zone and the DNA of HS1–HS8 samples of the high-salinity zone formed a greater paraphyletic group of taxa (Fig. 3). The fixation index (F_{ST}) for the three populations of *A. officinalis* in the low-salinity, medium-salinity and high salinity zones was 0.41454 (Table 3) which indicated that these are isolated populations of this species. However, there was some exchange of genetic material (gene flow) between the populations of medium-salinity and high-salinity zones (Fig. 3). There were significant ($p < 0.05$) genetic variations among the three populations (41.45%) and within the population (58.55%) (Table 3) of *A. officinalis* in the Sundarbans. The genetic diversity over loci of genomic DNA of *A. officinalis* of low-salinity, medium-salinity, and high-salinity zones were 0.3750 ± 0.2313 , 0.5417 ± 0.3167 and 0.5458 ± 0.3189 , respectively (Table 4). Expected heterozygosity (H_{EXP}) of *A. officinalis* in the low-salinity, medium-salinity and high-salinity zones were 0.37 ± 0.12 , 0.54 ± 0.14 and 0.55 ± 0.10 , respectively (Table 4). Pair-wise genetic (molecular) distance between the low-salinity and medium-salinity, low-salinity and high-salinity and between the medium-salinity and high-salinity zones were 0.50, 0.52 and 0.09, respectively (Table 5). The genetic distances of *A. officinalis* between the LSZ and MSZ, and between the LSZ and HSZ were significant ($p < 0.05$) while the genetic distance between the MSZ and HSZ was not significant in the Sundarbans ($p > 0.05$).

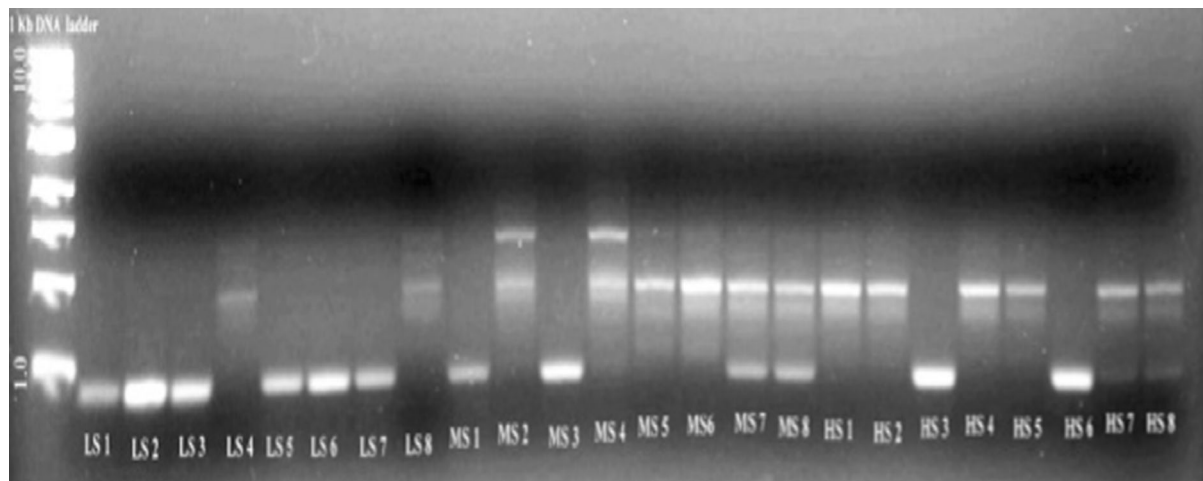


Fig. 2 RAPD bands of *A. officinalis* with 5'AGGTGACCGT3' primer: LS1–LS8 represents samples of low salinity zone; MS1–MS8 represents samples of medium salinity zone; HS1–HS8

represents samples of high salinity zone of the Sundarbans. 10.0 denotes 1000 bp; 1.0 denotes 100 bp

Discussion

Significance of mangroves genetic diversity

Genetic diversity within and among the different populations of a species is extremely important for the successful survival of that species in different environmental conditions (Xiong 2006). It also has significant ecological implications. Genetic diversity within a population of a species influences growth, productivity and stability at the population level as well as inter-specific interactions at the community level in different environmental conditions (Hughes et al. 2008). Mangroves are the most threatened and vulnerable ecosystems globally to climate change consequences (Krauss et al. 2013; Sun et al. 2017). In particular, the Sundarbans mangrove ecosystem is under serious threat due to global climate change (Erwin 2009). It is evident that the salinity in the Sundarbans of Bangladesh is increasing due to sea-level rise as a consequence of global climate change which, in turn, is changing the habitat patterns for the mangroves (Alam et al. 2018b). In such a situation, sustenance of the Sundarbans vegetation is under serious threat (Basar 2012). To cope with the adverse environmental conditions created by climate change, the genetic diversity of mangrove species is extremely important for the sustainability of the Sundarbans. As *A. officinalis* is ecologically significant mangrove species, we, therefore, studied the genetic diversity of

A. officinalis growing in the three ecological habitats, i.e., in the low-salinity, medium salinity and high-salinity zones in the Sundarbans of Bangladesh.

Genetic diversity of *Avicennia officinalis* in the Sundarbans

A greater allelic diversity in the RAPD banding patterns of the DNA of *A. officinalis* of the three salinity zones was observed when run with oligonucleotide random primers. The monophyletic group of taxa originated from a common ancestor in the low-salinity zone can be termed as a clade. Conversely, the paraphyletic group of taxa originated from the medium-salinity and high-salinity zones indicated their distant relationship. Hazarika et al. (2013) found genetic diversity among and within the three populations (i.e., Parangipttai, Kakinada, and West Bengal Sundarbans) of *A. marina* of east coast of India that are advantageous for this species to environmental challenges. Das et al. (2011) found 23.6% genetic variations among the populations and 53.5% genetic variations within the population of *E. agallocha* in the east coast of India. In this study, the three populations of *A. officinalis* have been distributed in the low-salinity, medium-salinity and high-salinity zones in the Sundarbans of Bangladesh. The greater genetic variations of *A. officinalis* among the populations (41.45%) and within the population (58.55%) may confer greater advantage of this species in the face of

Table 2 RAPD bands of *A. officinalis* in the three saline zones of the Sundarbans

Primers	LSZ								MSZ								HSZ							
	LS1	LS2	LS3	LS4	LS5	LS6	LS7	LS8	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8
5'AGGT GACCGT3'	1	1	1	2	1	1	1	2	1	2	1	3	3	3	3	3	2	2	1	2	3	1	3	3
5'CAAT CGCCGT3'	2	3	3	3	3	3	3	3	5	6	5	6	5	6	5	6	5	6	6	6	5	6	5	6
5'TTCCG AACC3'	2	2	2	2	2	1	2	2	4	6	5	6	5	5	4	5	5	6	6	5	6	5	5	5
5'AGCA GCGAA3'	2	3	2	3	3	2	3	3	3	6	5	5	6	5	5	5	6	5	5	5	5	5	5	4
5'GGGTA ACGCC3'	3	2	1	2	2	2	2	2	4	6	6	5	5	5	6	4	4	6	5	5	6	5	6	6
5'AGGG TCTTG3'	4	3	4	1	3	3	3	3	4	5	5	5	4	5	6	5	4	4	5	5	5	4	3	4
5'AGTCAG CCAC3'	2	2	2	2	2	2	1	1	4	5	4	4	4	4	4	4	5	5	4	5	4	5	5	5
5'GAACGG ACTC3'	2	2	2	2	2	2	2	1	5	4	5	4	5	4	4	4	5	5	5	4	3	5	5	4

LSZ represents low-salinity zone; MSZ represents medium-salinity zone; HSZ represents high-salinity zone. LS1–LS8 represents samples from LSZ, MS1–MS8 represents samples from MSZ; HS1–HS8 represents samples from HSZ

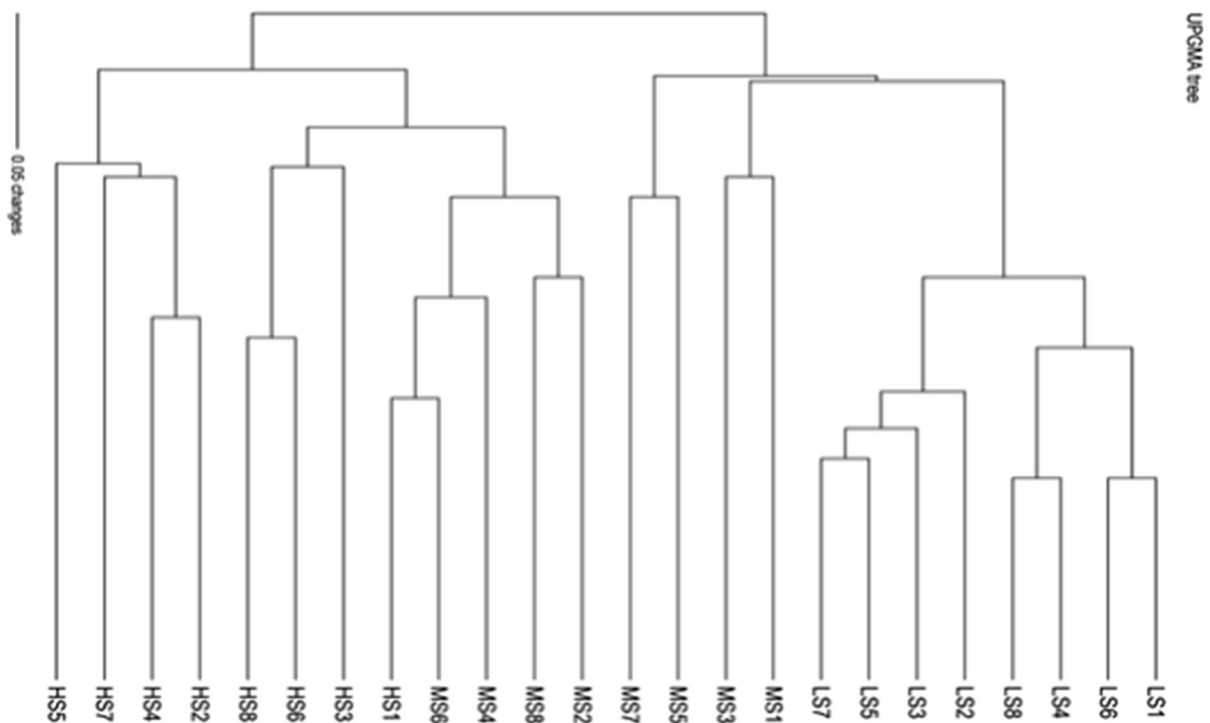


Fig. 3 UPGMA tree of *A. officinalis*. LS1–LS8 represents samples of low salinity zone (LSZ); MS1–MS8 represents samples of medium salinity zone (MSZ); HS1–HS8 represents samples of high salinity zone (HSZ) of the Sundarbans

Table 3 Percentage (%) genetic variation among and within populations, and genetics distance (F_{ST}) of *A. officinalis* populations in the three saline zones of the Sundarbans

Source of variation	df	Sum of squares	Variance components	Percentage (%) of variation
Among populations	2	48.083	1.38072 va	41.45
Within populations	45	87.750	1.95000 vb	58.55
Total	47	135.833	3.33073	
Fixation index	F_{ST}	0.41454		

Significance test: 1023 permutations

Table 4 Gene diversity over loci and expected heterozygosity (H_{EXP}) of *A. officinalis* in the three saline zones of the Sundarbans

Zone	Gene diversity over loci (mean $\pm$ s.d.)	H_{EXP} (mean $\pm$ s.d.)
LSZ	0.3750 $\pm$ 0.2313	0.37 $\pm$ 0.12
MSZ	0.5417 $\pm$ 0.3167	0.54 $\pm$ 0.14
HSZ	0.5458 $\pm$ 0.3189	0.55 $\pm$ 0.10

LSZ represents low-salinity zone; MSZ represents medium-salinity zone; HSZ represents high-salinity zone

environmental changes due to salinity increase in the coastal regions of Bangladesh. Dashzeveg et al. (2017)

stated that intra-specific diversity within the population of a species is of paramount importance for the

Table 5 Pairwise genetic distance (F_{ST}) between *A. officinalis* populations in the three saline zones of the Sundarbans

Zone			
Zone	LSZ	MSZ	HSZ
LSZ	0.00		
MSZ	0.50	0.00	
HSZ	0.52	0.09	0.00

Significance level: 0.05

LSZ represents low-salinity zone; MSZ represents medium-salinity zone; HSZ represents high-salinity zone

adaptability and survival of that species under different environmental conditions. Therefore, the greater genetic diversity among and within the populations of *A. officinalis* might be the reason for its wider distribution and survival across the salinity gradient in the low-salinity, medium-salinity and high salinity zones in the Sundarbans of Bangladesh.

The fixation index (F_{ST}) of 0.41454 (Table 3) indicates that the populations of *A. officinalis* in the low-salinity, medium-salinity, and high-salinity zones are isolated. However, the formation of monophyletic group (clade) by the taxa of low-salinity zone made them completely isolated whereas the taxa of medium-salinity and high-salinity zones together formed a greater paraphyletic group with common ancestors at several nodes (Fig. 3). Furthermore, significantly ($p < 0.05$) greater genetic distances between the populations of the low-salinity and medium-salinity zones (0.50) and between the low-salinity and high-salinity zones (0.52) indicate that *A. officinalis* distributed in the low-salinity zone is an isolated population. On the other hand, the comparatively lower and insignificant ($p > 0.05$) genetic distance between the populations of *A. officinalis* in the medium-salinity and high-salinity zones (0.09) indicates that *A. officinalis* distributed in the medium-salinity and high-salinity zones together forms a wider population of this species.

Zabala (1990) observed that the flowering of *A. officinalis* in the Sundarbans commences from March to June. In our field visit into the Sundarbans during the period of 2015–2017, we observed that the flowering of this species growing in the low-salinity zone started in the mid-March, but that in the medium-salinity and high-salinity zones commenced together in the May of the year. *A. officinalis* is cross-pollinated

by insects (Tomlinson 1986; Zabala 1990). The sexual reproduction of this species is dependent on local insects for cross-pollination (Aluri 2013). Since the flowering of *A. officinalis* in the low-salinity zone commences earlier than that of the medium-salinity and high-salinity zones, there may be no gene flow or genetic exchange either between the low-salinity and medium-salinity zones or between the low-salinity and high-salinity zones. This may be the reason for the greater genetic distance between the low-salinity and medium-salinity zones and between the low-salinity and high-salinity zones. It confirmed that *A. officinalis* growing in the low-salinity zone is an isolated population. On the contrary, there might be gene flow or genetic exchange among the individuals of *A. officinalis* growing in the medium-salinity and high-salinity zones because the flowering of this species in these two salinity zones commences at the same time i.e., in May. This might be the intrinsic reason for the lower genetic distance between *A. officinalis* populations of medium-salinity and high-salinity zones. Therefore, it is validated that *A. officinalis* growing in the medium-salinity and high-salinity zones together constituted as a wider population of this species.

Because of the gene flow between the medium-salinity and high-salinity zones, *A. officinalis* growing in these two salinity zones are genetically more diverse (0.5417 ± 0.3167 and 0.5458 ± 0.3189 , respectively) than that growing in the low-salinity zone (0.3750 ± 0.2313) in the Sundarbans. For the similar reason, *A. officinalis* growing in the medium-salinity and high-salinity zones is more heterozygous than that growing in the low-salinity zone. Hazarika et al. (2013) found genetic diversity of *A. marina* populations of Parangipttai, Kakinada, and West Bengal Sundarbans of east coast of India and attributed this diversity to gene flow through cross pollination. Kahrood et al. (2008) found high level of allelic diversity and expected heterozygosity among the different populations of *A. marina* in the three coastlines of Bushehr province of southwest region of Iran, and opined that these diversities may lead to inbreeding coefficient in different populations of this species. Manurung et al. (2017) also found the genetic variations among different populations of *A. marina* in the heavily polluted and non-polluted areas of estuaries of Cilegon Industrial area, Indonesia Das et al. (2014) explored that *A. marina* is genetically

more diverse in the Indian Sundarbans, and suggested for its use in breeding programme. Xiong (2006) stated that the greater genetic diversity of a species would be the source of raw materials for natural selection. *A. officinalis* growing in the medium-salinity and high-salinity zones, being genetically more diverse, will be more fit to adverse environmental conditions due to salinity increase, and obviously enjoy the advantage of natural selection. As a result, *A. officinalis* growing in the medium-salinity and high-salinity zones can survive and will get the opportunity to produce new progeny that will lead to adaptive biological evolution in the future. Xiong (2006) opined that natural selection is the driving force behind the biological evolution.

The greater genetic diversity of *A. officinalis* growing in the medium-salinity and high-salinity zones may be the intrinsic reason for its high salt adaptability in comparison to *A. officinalis* with lower genetic diversity in the low-salinity zone. Alam et al. (2018a) found that *A. officinalis* growing in the medium-salinity and high-salinity zones is the most salt adapted phenotype while that growing in the low-salinity zone is the least salt adapted phenotype, with respect to propagule morphology and propagule growth initiation traits. Alam et al. (2018b) also found that the seedlings of *A. officinalis* originated from the medium-salinity and high-salinity zones are more salt adaptive than those originated from the low salinity zone, with respect to different growth characteristics, nutrients, proline, and phenolic compounds accumulation, and osmotic potential. Furthermore, *A. officinalis* growing in the medium-salinity and high-salinity zones is physiologically more adaptive in terms of nutrient resorption efficiency than that growing in the low-salinity zone. Even, the leaf vein density of this species growing in the medium-salinity and high-salinity zones is higher than that growing in the low-salinity zone in order to facilitate nutrient retranslocation under stressful saline conditions (Alam et al. 2019).

Synthesis

Considering the plasticities in propagule morphology, leaf anatomy, propagule growth initiation traits, growth characteristics, nutrient accumulation, different physiological adaptabilities () as well as the genetic diversity and genetic distance in this study, it

can be inferred that *A. officinalis* growing in the low-salinity zone can be defined as the low salt-adapted ecotype while those growing in the medium-salinity and high-salinity zones can be defined as the high salt-adapted ecotype of *A. officinalis* in the Sundarbans of Bangladesh. A species can be differentiated into a number of ecotypes for different habitats (Turrill 2006) having variations in leaf anatomy, seed morphology, growth, physiology and genetics (Waisel 1972; Shin and Kim 2013). In this study, the low salt-adapted ecotype of *A. officinalis* has been adapted and growing well in the low-salinity zone while the high salt-adapted ecotype of *A. officinalis* has been well adapted and growing satisfactorily in the medium-salinity and high-salinity zones in the Sundarbans. These two ecotypes of *A. officinalis* bear ecological and environmental significance in the context of both in-situ and ex-situ conservation in the Sundarbans mangrove ecosystem of Bangladesh.

Adoption of in-situ conservation of high-salt adapted ecotype of *A. officinalis*, being a pioneer species in mangrove succession, is of vital ecological significance for the sustainability of the vegetation cover in the Sundarbans. Furthermore, Bangladesh Forest Department took an initiative of coastal afforestation program with mangrove species in 1966 in order to protect the vulnerable coastal regions against frequently occurring natural catastrophes (Alam et al. 2017). Since then, *A. officinalis* has been the second principal species in those afforestation programs (Alam et al. 2018a). In these afforestation programs, *A. officinalis*, because of its salt-adaptive plasticity, can be of great significance for ex-situ conservation. The propagules and seedlings of *A. officinalis* of low salt-adapted ecotype can be planted in the low saline areas whereas those of high salt-adapted ecotype can be planted as ex-situ conservation in the climate change induced high saline areas of the coastal regions of Bangladesh.

Conclusion

Avicennia officinalis growing in the low-salinity zone formed a monophyletic group while those of medium-salinity and high-salinity zones together formed a paraphyletic group. The genetic distances between the low-salinity and medium salinity zones and between the low-salinity and high salinity zones were higher

than that between the medium-salinity and high-salinity zones. The expected heterozygosity of *A. officinalis* of medium-salinity and high-salinity zones was higher than that of low-salinity zone. The genetic diversity of *A. officinalis* of medium-salinity and high-salinity zones was higher than that of low-salinity zone. Alam et al. (2018a) observed that *A. officinalis* growing in the medium-salinity and high-salinity zones are the most salt adapted phenotype while this species growing in the low-salinity zone is the least salt adapted phenotype in the Sundarbans. Alam et al. (2018b) also demonstrated that the seedlings of *A. officinalis* originated from the medium and high salinity zones are more salt adaptive than those originated from the low salinity zone of the Sundarbans. Furthermore, *A. officinalis* growing in the medium and high salinity zones is eco-physiologically and anatomically more salt adaptive than that growing in the low salinity zone in the Sundarbans (Alam et al. 2019). Therefore, considering the results of the present study and previous studies, it can be postulated that *A. officinalis* growing in the low-salinity zone is the low salt-adapted ecotype while that growing in the medium-salinity and high-salinity zones is the high salt-adapted ecotype of *A. officinalis* in the Sundarbans of Bangladesh. These two ecotypes of *A. officinalis* are of great ecological significance for both in-situ and ex-situ conservation in the face of climate change induced environmental changes in the Sundarbans and in the vulnerable coastal region of Bangladesh as well.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest associated with this article.

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