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Molecular characterization of *Avicennia marina* from the Gujarat Coast

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Original Article

Abstract

Mangrove plants are the most segregated plants, available only in coastal regions around the globe. *Avicennia marina* is the most abundant species luxuriously growing on the coast of Gujarat. The present study has endeavored to understand the intra-species diversity of this plant along the coast of Gujarat. A total of 28 plant samples from the farthest reachable locations of Gujarat coast were collected. All the samples were representative of distinct locations of Gulf of Kachchh and Gulf of Khambhat. These samples were screened against 38 ISSR markers reported for molecular characterization of mangrove plants. All the 38 ISSR markers were found to be polymorphic in nature, thus showed 100% polymorphism. The Un-weighted Pair Group Arithmetic Mean Method (UPGMA) cluster analysis grouped all the 28 *A. marina* into 3 groups, with groups showing locational isolation. The groups can be classified as parent group and its variants. The ISSR marker analysis showed very high level of genetic variation within the population, as revealed by the low average values of Nei's genetic diversity *(H)* of 0.1866, Shanon's Diversity Index (***I***) of 0.3249 and high Percentage of Polymorphic Bands (PPB) of 99.45%. The results showed that the diversification of this species has occurred simultaneously along the Gujarat coast, which probably makes it the most luxuriously propagating species.

Keywords: *molecular characterization, ISSR, mangroves, Avicennia marina*

Introduction

A. marina is an important mangrove species with a wide geographical and climatic distribution which suggests that large amounts of genetic diversity are available for conservation and breeding programs (Maguire *et al.*, 2002). *A. marina* has always been the species of choice for afforestation drives, because of its fast growing tendencies, which is contributed by its ability to tolerate range of salinity (Mehta *et al*., 2005) and adaptability to changing climatic conditions. In forest afforestation programs, species mixing is a practice to improve the belowground production of forests, and *A. marina* has been found to contribute the highest towards below ground

biomass in a mix of *Bruguiera gymnorrhiza*, *Ceriops tagal* and *A. marina* (Lang'at *et al.,* 2013).

A. marina, is the fastest growing mangrove species over the coast and covers the major area along Gujarat coast. Study on the Atlantic-East Pacific lineage and the Indo-West Pacific lineage of *Avicennia,* based on morphological and allozyme data, has revealed two monophyletic clades across all species of *Avicennia*, worldwide (Li *et al.,* 2016). *Avicennia* genera of west coast India were subjected to barcoding using matK + ITS2 alongwith additional markers psbK-psbl, rpoC1 and atpF-atpH. atpF-atpH locus to discriminate three species of *Avicennia* genera (Saddhe *et al.,* 2017). Other species taken up for similar studies are *B. gymnorrhiza, Heritiera fomes* and *C. tagal.* RAPD and ISSR marker studies on *B. gymnorrhiza* and *H. fomes* from Sundarbans, India have revealed that *B. gymnorrhiza* had higher degree of polymorphism and hence higher Nei's genetic diversity than *H. fomes* (Dasgupta *et al.,* 2015). Population genetics study of *Ceriops tagal* from Thailand and China revealed extremely low genetic variation and only 47% of the total gene diversity was maintained within populations. A very high level of Nei's genetic identity existed between populations of *C. tagal*, suggesting common ancestry (Ge and Sun, 2001).

A large number of genetic markers like Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR) etc. have been widely used for population genetics. Studies have shown that ISSR markers have been proven to be one of the most stable marker system for organisms (Bhadalkar *et al.*, 2014). Inter-Simple Sequence Repeats (ISSR) are the regions between two SSRs, and are easy to use, low-cost and procedurally less demanding compared to other dominant markers (Ng and Tan, 2015). Microsatellites (SSR) can be found anywhere in the genome, both in protein-coding and noncoding regions. Because of their high mutability, microsatellites are thought to play a significant role in genome evolution by creating and maintaining quantitative genetic variation (Tautz *et al.*, 1986; Kashi *et al.,* 1997). Primers against the microsatellite regions help amplify region between two SSRs, giving newer markers to identify plants at species level. ISSR can be used in cases where other genomic details are not available, and can be used independently.

Table 1. Sample Collection sites for *Avicennia marina*

Sr. No.	Sample Name	Location	Type of Island/Region	GPS Co-ordinates North	GPS Co-ordinates East
1.	LAShs 17413	Sikka, Gulf of Kachchh	Contiguous Island, Gulf of Kachchh	22°26.735′	069°49.825′
2.	LATs 17413	Sikka, Gulf of Kachchh	Contiguous Island, Gulf of Kachchh	22°26.735′	069°49.825′
3.	LAShn 18413	Narara, Gulf of Kachchh	Contiguous Island, Gulf of Kachchh	22°28.028′	069°43.428′
4.	LATn 18413	Narara, Gulf of Kachchh	Contiguous Island, Gulf of Kachchh	22° 28.035′	069°43.421′
5.	LATaj 230114	Ajad	Areal Island, Gulf of Kachchh	22°23.136′	069°19.988′
6.	LAKo 8414	Kori Creek	Creek, Gulf of Kachchh	23°41′19.53″	68°31′43.35″
7.	LAejj1 14514	Ultratech Plant, Nalia	Coast, Gulf of Kachchh	23°20′14.442″	068°37′11.131″
8.	LAejj2 14514	Ultratech Plant, Nalia	Coast, Gulf of Kachchh	23°20′15.308″	068°37′10.362″
9.	LAma 30514	Macchad, Purna Creek, Surat	Coast, Gulf of Khambhat	20° 56.713′	072° 51.299′
10.	LAd1 29514	Dumas, Surat	Coast, Gulf of Khambhat	21° 04.814′	072° 42.906′
11.	LAd2 29514	Dumas, Surat	Coast, Gulf of Khambhat	21° 04.813′	072° 42.909′
12.	LAd3 29514	Dumas, Surat	Coast, Gulf of Khambhat	21° 04.811′	072° 42.908′
13.	LAmro 201014	Rozi, Jamnagar	Coast, Gulf of Kachchh	22° 33.625′	070° 02.448′
14.	LAmdm 181014	Dedka-Mundeka	Aereal Island, Gulf of Kachchh	22° 31.820′	069° 56.144
15.	LAmp 181014	Pirotan	Aereal Island, Gulf of Kachchh	22° 35.965′	069°57.789′
16	LAmp1 181014	Pirotan	Aereal Island, Gulf of Kachchh	22° 36.245′	069° 57.413′
17.	LAsmd 6514	Mandroi, Billimora	Coast, Gulf of Khambhat	21° 25.684′	072° 40.198′
18.	LAshz1 7514	Hazira, Surat	Coast, Gulf of Khambhat	21° 08.856′	072° 39.581′
19.	LAsmz1 7514	Mirzapur, Surat	Coast, Gulf of Khambhat	21° 22.385	072° 38.992′
20.	LAekb 20115	Kalumbhar	Aereal Island, Gulf of Kachchh	22° 27.479′	069° 37.798′
21.	LAmpo1 5515	Poshitra	Contiguous Island, Gulf of Kachchh	22°24.174′	069°12.255′
22.	LABh2 21415	Bhaider	Aereal Island, Gulf of Kachchh	22° 28.226′	069° 18.207′
23.	LAmno 30415	Noru	Submerged Island, Gulf of Kachchh	22° 30.752′	069° 21.800′
24.	LAmdh1 14915	Dhani Bet	Aereal Island, Gulf of Kachchh	22° 24.983′	069° 30.946′
25.	LAm1pa 12915	Panero	Aereal Island, Gulf of Kachchh	22°21′15.609″	069°27′28.141″
26.	LAmgk 12915	Gandiyo Kado	Aereal Island, Gulf of Kachchh	22°23′22.741″	069°29′14.297″
27.	LAms 91015	Sanjan	Coast, Gulf of Khambhat	20° 11.979′	072° 47.740′
28.	LAjp17. 201115	Jakhau Port	Coast, Gulf of Kachchh	23° 13.429′	068° 38.179′

In Gujarat mangroves are confined to three regions (a) Indus deltaic region i.e. Kori creek and Sir Creek area, (b) The Gulf of Kutch and (c) The Gulf of Khambhat. Gujarat is reported to have 10 species of mangrove plants, however, inter-population and intra-population diversity of mangroves have not been studied in detail. Hence, the present study was undertaken to document the intra-species diversity of *A. marina* using molecular tools.

Material and methods

Study area and sample collection

The samples of *A. marina* were collected from different regions of the Gujarat coast, and are listed in Table 1.

Genomic DNA Isolation from A. marina

DNA of all the samples were extracted using modified Doyle and Doyle (Doyle and Doyle, 1987) method, where proteinase K was used additionally. 1 gm leaf sample was crushed using Liquid Nitrogen and added to 5 ml pre-heated Lysis Buffer consisting of 0.1M Tris HCl, 0.02M EDTA, 2.5M NaCl, 2% CTAB, 1% PVPP, 1% Proteinase K and 0.1% Beta-mercaptoethanol. The lysis buffer was incubated at 70°C for 1hr. DNA was separated using Phenol: Chloroform: Isoamylalcohol and Chloroform: Isoamylalcohol sequentially, at 6000 rpm for 10 min. DNA was precipitated by standing the solution with equal volume of absolute alcohol at -20°C overnight. The DNA was pelleted by centrifuging at 13000 rpm and washing the pellet using 76% chilled absolute alcohol and 0.5M ammonium acetate. The purified DNA was extracted using 0.5M TE.

PCR amplification and Phylogenetic analysis

ISSR Primers used for the molecular characterization were specific for mangrove species. Thirty eight primers were used and are listed in Table 2. 25μl PCR master mix was prepared by mixing Taq Polymerase (5U/μl): 0.1μl, Taq Polymerase Buffer (10X): 2.5μl, Primer (20μM): 0.5μl, dNTPs (1.25nM): 4μl, MgCl2 (50mM): 1.5μl and Double Distilled Water. 1μl of *A. marina* DNA sample was added to the master mix, after removing the blank.

The PCR cycle was set for 3 min at 94°C; 40 sec at 94°C, 45 sec at Tm and 90 sec at 72°C for 35 cycles; 45 sec at 94°C, 5 min at 72°C; soak at 4°C. The products obtained after PCR amplification were electrophoresed in 1% agarose gel in 1X TBE buffer at 120V for 1hr. The obtained bands were compared (in base pairs bp) with the 100bp molecular marker. Bands with the same migration were considered homologous fragments, independent of their intensity.

Data Interpretation

Distinct, reproducible, well resolved fragments were scored as present (1) or absent (0) for each ISSR reaction and were displayed as part of a binary matrix. The data matrices obtained were analyzed using POPGENE version 1.31 (POPGENE, 1999). Genetic parameters such as percentage of polymorphic bands (PPB), observed number of alleles (Na), the effective number of alleles (Ne), Nei's genetic diversity (H), Shannon's information index (I), total genetic diversity (Ht), genetic diversity within population (Hs), Nei's genetic differentiation index among populations (Gst) and gene flow estimates between populations (Nm) were determined with POPGENE version 1.31. Low values of Shannon Diversity Index indicates more diversity, while higher values indicate less diversity (www.itl.nist.gov). An index value of 1 indicates that all groups have the same frequency. FreeTree, a tool for statistical analysis was used to generate the UPGMA based on Jaccard Coefficient. The Jaccard Coefficient values were used to calculate the Genetic Distance (Genetic Identity). The phylogenetic tree was subjected to Jackknifing in FreeTree and then the tree was created using TreeView (Fig. 1).

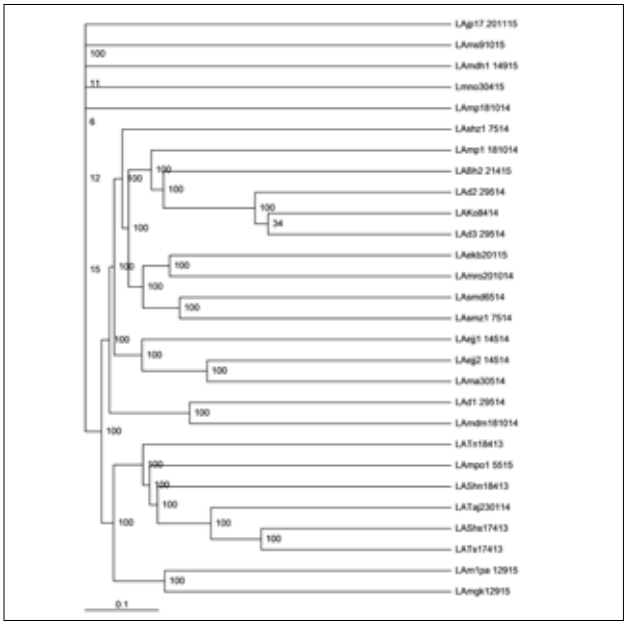

Fig. 1. UPGMA Jackknife tree resulting from ISSR Dataset. Jackknife support values of 1000 replicates are given above the branches.

Results and discussion

PCR Reaction

The gradient PCR reaction was set for all the 28 *A. marina* samples against the 38 primers and annealing temperature standardized for each primer based on number of bands are given in Table 2. The statistical analysis of the PCR results was done using POPGENE version 1.31. The Jaccard Coefficient Index was generated using FreeTree (Table 3).

Genetic Diversity

In this study, genetic diversity was examined in *A. marina*, based on ISSR fingerprinting. In total, 38 primers produced 132

Table 2. Primer List

Sr. No.	Primer Annotation	Optimal Melting Temperature (Tm)	Reference
1	(TG)12(AG)5T(GA)7	84.3	Teixeira S *et al.,* 2003
2	(AC)11	63.4	
3	(CT)11	54.4	
4	(TC)22	75.1	
5	(AG)11	54.4	
6	(GA)11	55.3	
7	(AG)8T	42.5	Tan F *et al.,* 2005
8	(AG)8C	46.8	
9	(GA)8T	42.9	
10	(AG)8CC	52.2	
11	(AG)8TC	45.6	
12	(AC)8CG	60.2	
13	(AC)8TG	53.7	
14	(AC)8T	49.3	Hu *et al.,* 2011
15	(AG)8CT	48.8	
16	(AG)8TT	45.4	
17	(GA)8CC	49.2	
18	(GA)8TC	46.1	
19	(GA)8CG	50.7	
20	(GA)8TG	47.3	
21	TCT (AC)7	46.6	
22	TGT (AC)7	47.7	
23	TTT (AC)7	47	
24	GCG (AC)7	57.9	
25	GGG (AC)7	56.1	
26	GTG (AC)7	50.4	
27	ACA (AC)7	50	
28	AGA (AC)7	48.9	
29	ATA (AC)7	45.5	
30	AAA (TG)7	50.3	
31	ACA (TG)7	51.2	
32	AGA (TG)7	50.1	
33	TAT (TG)7	48.2	
34	TCT (TG)7	51.7	
35	TGT (TG)7	52.8	
36	CAC (TG)7	52.1	
37	CCC (TG)7	57.7	
38	CGC (TG)7	59.4	

Table 4. Total Bands for 38 Primers

Primer	Total No. of Bands	Monomorphic Bands	Polymorphic Bands	% Poly-morphism	Occurrence in No. of Plants
Primer 1	2	0	2	100	3
Primer 2	4	0	4	100	4
Primer 3	1	0	1	100	3
Primer 4	1	0	1	100	2
Primer 5	6	0	6	100	10
Primer 6	6	0	6	100	8
Primer 7	8	0	8	100	6
Primer 8	5	0	5	100	8
Primer 9	8	0	8	100	6
Primer 10	8	0	8	100	7
Primer 11	4	0	4	100	5
Primer 12	3	0	3	100	2
Primer 13	3	0	3	100	1
Primer 14	3	0	3	100	5
Primer 15	8	0	8	100	9
Primer 16	3	0	3	100	4
Primer 17	4	0	4	100	4
Primer 18	5	0	5	100	7
Primer 19	1	0	1	100	2
Primer 20	5	0	5	100	4
Primer 21	5	0	5	100	12
Primer 22	6	0	6	100	18
Primer 23	3	0	3	100	6
Primer 24	5	0	5	100	9
Primer 25	3	0	3	100	7
Primer 26	6	0	6	100	6
Primer 27	9	0	9	100	8
Primer 28	7	0	7	100	6
Primer 29	7	0	7	100	4
Primer 30	8	0	8	100	5
Primer 31	6	0	6	100	7
Primer 32	5	0	5	100	10
Primer 33	3	0	3	100	15
Primer 34	3	0	3	100	5
Primer 35	3	0	3	100	11
Primer 36	4	0	4	100	13
Primer 37	6	0	6	100	10
Primer 38	4	0	4	100	2

bands, at 187 loci. The percentage of polymorphic bands (PPB) averaged to 99.45, where the polymorphic loci was divided by the total number (polymorphic and monomorphic) of loci (P=npj/ntotal). The observed number of alleles (Na) was found to be 1.9945 and effective number of alleles (Ne) was found to be 1.2485, which coincide with the PPB data. Primer 22 gave the highest number of reactions while Primer 13 gave the lowest number of reactions, with the 28 plant samples (Table 4). POPGENE results showed that the Shannon's Information Index was 0.3249, which shows a high level of diversity, which is also supported by PPB data.

Genetic diversity measures are estimated over several loci that are presumed to be a random samples of the genome. Nei's diversity index is based on two causes of diversity generation, i.e. genetic drift and mutation. Genetic drift is random change in allele frequencies in a population from generation to generation due to finite population size. Mutation is a structural change in the DNA sequence due to loss or addition of one or more base-pairs. Heteorzygosity is lost due to the loss of alleles caused by genetic drift or mutation, and the loss occurs at a low pace. Generally, species with small geographic ranges tend to maintain less genetic diversity than geographically widespread species (Hamrick and Godt, 1989; Luan *et al.,* 2006). High genetic diversity is maintained in plants due to number of factors (Zawko *et al*., 2001), such as recent reduction of population size plus insufficient time for isolation, or extensive recurrent gene

Table 3. Pairwise genetic identity (above diagonal) and genetic distance (below diagonal) using Jaccard Coefficient

	LAShs17413	LATs17413	LAShn18413	LATaj23014	LAKo8414	LAejj114514	LAejj214514	LAma30514	LAd129514	LAd229514	LAd329514	LAmro201014	LAmdm181014	LAekb20115	LAmp181014	LAsmd6514	LAshz17514	LAsmz17514	LAmpo15515	LATn18413	LAmp1181014	LABh221415	Lmno30415	LAmdh1149115	LAm1pa129115	LAmgk129115	LAms91015	LAjp17.201115
LAShs17413		0.48148	0.23148	0.28676	0.04211	0.02083	0.01053	0.03093	0.04211	0.04211	0.03158	0.05155	0.04255	0.04082	0	0.06796	0.0396	0.11429	0.17431	0.18182	0.09574	0.03191	0	0	0.08163	0.06796	0	0
LATs17413	0.48148		0.1859	0.40123	0.02027	0.02041	0.0137	0.03401	0.02721	0.02721	0.02041	0.04054	0.02041	0.03356	0	0.05882	0.03974	0.10458	0.19205	0.15753	0.05442	0.01361	0	0	0.06757	0.07285	0	0
LAShn18413	0.23148	0.1859		0.17647	0.04762	0	0	0.02273	0.02326	0.04762	0.04878	0.02174	0.02381	0.02174	0	0.07843	0.06383	0.05085	0.14063	0.19231	0.04348	0.02439	0	0	0.0625	0.01852	0	0
LATaj230114	0.28676	0.40123	0.17647		0	0.03659	0	0	0.01176	0	0	0.03488	0.0119	0.03488	0	0.06593	0.05747	0.08333	0.19792	0.11828	0.01124	0	0	0	0.0814	0.07778	0	0
LAKo8414	0.04211	0.02027	0.04762	0		0	0.16667	0.22222	0.25	0.42857	0.5	0.18182	0	0.18182	0	0.16667	0.14286	0.07692	0.08333	0	0.27273	0.33333	0	0	0	0.05	0	0
LAejj114514	0.02083	0.02041	0	0.03659	0		0.2	0.11111	0.125	0	0	0	0	0.09091	0	0	0.07143	0.08	0.05556	0	0.08333	0	0	0	0.06667	0.11111	0	0
LAejj2 14514	0.01053	0.0137	0	0	0.16667	0.2		0.33333	0	0.16667	0.2	0.11111	0	0.11111	0	0.05882	0	0.08696	0	0	0	0	0	0	0	0	0	0
LAma30514	0.03093	0.03401	0.02273	0	0.22222	0.11111	0.33333		0.1	0.1	0.25	0.16667	0	0.16667	0	0.04762	0	0.11538	0.05263	0.03571	0.07143	0	0	0	0.05882	0.04762	0	0
LAd129514	0.04211	0.02721	0.02326	0.01176	0.25	0.125	0	0.1		0.25	0.125	0.08333	0.28571	0	0	0.10526	0	0.07692	0.02632	0.03704	0.27273	0	0	0	0.0625	0	0	0
LAd2 29514	0.04211	0.02721	0.04762	0	0.42857	0	0.16667	0.1	0.25		0.5	0.18182	0.125	0.08333	0	0.23529	0.06667	0.07692	0.02632	0.03704	0.16667	0.14286	0	0	0.0625	0.10526	0	0
LAd3 29514	0.03158	0.02041	0.04878	0	0.5	0	0.2	0.25	0.125	0.5		0.2	0	0.09091	0	0.17647	0.07143	0.125	0.05556	0.03846	0.08333	0.16667	0	0	0.06667	0.11111	0	0
LAmro201014	0.05155	0.04054	0.02174	0.03488	0.18182	0	0.11111	0.16667	0.08333	0.18182	0.2		0	0.23077	0	0.2	0	0.19231	0.02439	0.03333	0.0625	0	0	0	0	0.04348	0	0
LAmdm181014	0.04255	0.02041	0.02381	0.0119	0	0	0	0	0.28571	0.125	0	0		0	0	0.05263	0	0.08	0	0.03846	0.08333	0	0	0	0.23077	0	0	0
LAekb20115	0.04082	0.03356	0.02174	0.03488	0.18182	0.09091	0.11111	0.16667	0	0.08333	0.09091	0.23077	0		0	0.09091	0.11765	0.14815	0.10526	0.03333	0.13333	0.1	0	0	0.05263	0.09091	0	0
LAmp181014	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0
LAsmd6514	0.06796	0.05882	0.07843	0.06593	0.16667	0	0.05882	0.04762	0.10526	0.23529	0.17647	0.2	0.05263	0.09091	0		0.17391	0.25806	0.08696	0.02632	0.13636	0.05556	0	0	0	0.14286	0	0
LAshz17514	0.0396	0.03974	0.06383	0.05747	0.14286	0.07143	0	0	0	0.06667	0.07143	0	0	0.11765	0	0.17391		0.0625	0.15385	0.0303	0.11111	0.16667	0	0	0.04545	0.08	0	0
LAsmz17514	0.11429	0.10458	0.05085	0.08333	0.07692	0.08	0.08696	0.11538	0.07692	0.07692	0.125	0.19231	0.08	0.14815	0	0.25806	0.0625		0.07547	0.06977	0.06667	0.04	0	0	0.12903	0.21875	0	0
LAmpo15515	0.17431	0.19205	0.14063	0.19792	0.08333	0.05556	0	0.05263	0.02632	0.02632	0.05556	0.02439	0	0.10526	0	0.08696	0.15385	0.07547		0.14	0.075	0.08824	0	0	0.04545	0.13636	0	0
LATn18413	0.18182	0.15753	0.19231	0.11828	0	0	0	0.03571	0.03704	0.03704	0.03846	0.03333	0.03846	0.03333	0	0.02632	0.0303	0.06977	0.14		0.03226	0	0	0	0.16667	0.05405	0	0
LAmp1181014	0.09574	0.05442	0.04348	0.01124	0.27273	0.08333	0	0.07143	0.27273	0.16667	0.08333	0.0625	0.08333	0.13333	0	0.13636	0.11111	0.06667	0.075	0.03226		0.2	0	0	0	0.08696	0	0
LABh221415	0.03191	0.01361	0.02439	0	0.33333	0	0	0	0	0.14286	0.16667	0	0	0.1	0	0.05556	0.16667	0.04	0.08824	0	0.2		0	0	0	0.05556	0	0
Lmno30415	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0	0
LAmdh1149115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0	0	0	0
LAm1pa129115	0.08163	0.06757	0.0625	0.0814	0	0.06667	0	0.05882	0.0625	0.0625	0.06667	0	0.23077	0.05263	0	0	0.04545	0.12903	0.04545	0.16667	0	0	0	0		0.21739	0	0
LAmgk129115	0.06796	0.07285	0.01852	0.07778	0.05	0.11111	0	0.04762	0	0.10526	0.11111	0.04348	0	0.09091	0	0.14286	0.08	0.21875	0.13636	0.05405	0.08696	0.05556	0	0	0.21739		0	0
LAms91015	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0
LAjp17.201115	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

flow (Maguire and Sedgley, 1997, Chiang and Schaal., 2006). Field observations of mangroves have cited that large covers of mangroves including *A. marina* have disappeared due to over-exploitation for firewood purpose, deforestation by industries, disease epidemics or natural calamities. According to population genetic theory, larger populations tend to maintain higher allelelic diversity (Hedrick, 1986; Ellstrand and Elam, 1993). *A. marina* is a proliferant propagating mangrove species and it is also a cross pollinated species. All these factors might have contributed towards maintaining genetic polymorphism in the 3 groups of plants. Habitat destruction, larger population and cross pollination, might have caused this genetic diversity, and this phenomenon would have occurred only recently as indicated by high levels of genetic diversity and the lack of genetic distance (differentiation).

Genetic Distance or Genetic Identity

Genetic analysis using Jaccard Coefficient showed the highest identity of 0.48148 existed between LAShs17413 and LATs17413, while the lowest identity of 0 existed between LAShs17413 and Lmno30415, LAmdh114915, LAms 91015, LAjp17201115 and Lamp181014 (Table 3).

Taking total genetic diversity (HT) as 0.1866 and the genetic diversity within different locational samples (HS) as 0.0000, the genetic diversity between locations (DST) (Rothe and Gunter, 1994), would be:

$DST = HT - HS$
$DST = 0.1866$

The coefficient of Nei's genetic differentiation (GST) = DST/HT = 1 (Achigan-Dako, 2008). Hence, the percentage of genetic diversity is 100%. The low value of 0.3249, of Shannon's Diversity Index (I), also shows a high genetic diversity. In a similar study conducted on *A. marina* (Forsk.) Veirh, in Vietnam, on 6 populations it was found that HS ranged from 0.037 to 0.083 and HT was 0.086 (Giang, 2003).

Gene Flow

Gene flow (Nm) is the number of migrants per generation (Rothe and Gunter, 1994). Low mean private allele frequencies in a species indicate a high rate of gene flow, while high mean frequency of rare allele indicates low rate of gene flow. Nm is calculated as 0.5 (1-GST)/GST = 0.0000, indicating a very low migration rate between the sample. The value of observed number of alleles (Na) is 1.9945 and value of effective number of alleles is 1.2485, which also shows a low gene flow between the individuals of the species.

Slatkin and Barton (1989) compared Wright, S (1943) Genetic Divergence Index (FST) and Nei's (1973) Genetic Diversity Coefficient (GST) and found that they yield roughly comparable estimates under a wide variety of conditions. The current study used Nei's GST to calculate the gene flow (Nm). The gene flow estimates vary considerably, from very low (<0.1) to very high (>10.0) (Govindaraju, 1989 and Hamrick 1987). Nm values greater than one are considered high. Hence, in this case gene flow amongst the species is restricted which might be due to the geographical distance of the sample species from each other.

Cluster Analysis based on the ISSR derived genotyping

Highest Pairwise genetic identity is 0.48148 between LAShs17413 and LATs17413 and lowest identity of value 0 is between LAShs17413 and LAjp17.201115. The phylogenetic analysis was subjected to Jackknife Analysis, with 1000 cycles of resampling. The clusters generated have a branch support value of 100%, which supports the grouping seen in the species. The study revealed that Group 3 plants are the parent plants and the Group 2 and Group 3 are derivatives of these, as also mentioned in the conclusion.

Spatial distribution of the population along the coast of Gujarat

The mangrove plants are distributed along the Gujarat coast in three main groups. The geographical distribution pattern of the three groups can be seen in Fig. 2. In this present study the phylogenetic tree shows three major groups of mangrove trees occuring along the Gujarat coast.

Group 1 plants include: plants from Northern coast of Gulf of Kachchh which are, Kori Creek and Nalia, Southern coast of Gulf of Kachchh which are Rozi port, Jamnagar coast, Aereal Islands of Marine National Park which are Pirotan, Dedeka-Mundeka, Kalumbhar, Bhaider and Gulf of Khambhat which are, Machhad, Dumas, Mandroi, Hazira and Mirzapur.

Group 2 plants include: plants from Continguous Islands of Gulf of Kachchh which are Narara, Sikka and Poshitra and Aereal Islands which are Gandiyo Kado, Ajad and Panero.

Fig. 2. Three groups of *A. marina* at different Sampling Sites.

Group 3 plants include: plants from Northern coast of Gulf of Kachchh which are Jhakhao, Aereal Island which are Noru and Pirotan, Contiguous Island which is Dhani Bet and Gulf of Khambhat which is Sanjan, Umargao.

Predominance of Group 1 variety of mangroves can be seen on the Gujarat coast, which is followed by Group 2 and Group 3. Group 1 plants have the highest migration efficiency. Group 2 is isolated to Gulf of Kachchh only. The plants of Group 3 are the parent lines from which the plants of the other regions have seemingly diversified. The time of divergence of all the varieties from the parent line coincide, according to Jaccard Coefficient. However, plants of Group 3 are also maximally isolated in Gulf of Kachchh and found only in one region of Gulf of Khambhat *i.e*. Sanjan.

Conclusion

A. marina is a dominant mangrove species on the Gujarat coast. The large population, destruction of their ecosystem, geographical isolation and cross pollination has caused genetic diversity in these plants.

A more intense study with more number of samples from geographically isolated locations would reveal the details of gene flow regionally. In this case Gulf of Kachchh and Gulf of Khambhat can be treated as geographically isolated locations. It would give data on the status of genetic variation, i.e. if the genetic exchange is historical or is due to current migration rates. A further study to develop SCAR marker for all the three groups can be undertaken.

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