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Genetic Diversity of *Avicennia marina* (Forsk.) Vierh. Populations in the Persian Gulf by Microsatellite Markers

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Abstract: Genetic variation of four populations of *Avicennia marina* encompassing the Iranian coastal areas of Persian Gulf were studied using five microsatellite loci. The average number of alleles per locus per population ranged from 4 to 4.6, showing no significant difference among the four populations. The observed heterozygosity (H_o), ranging from 0.782 to 0.960 with an average of 0.864, was comparable in the Iranian populations and much higher comparing to the earlier studies on *A. marina* in the worldwide range. Significant to highly significant deviations from Hardy-Weinberg expectations were observed in 10 out of 20 (five loci H four populations) cases. Most of F_{is} values were negative and significantly different from zero, thus suggesting excess of heterozygosity. The microsatellite analysis showed low genetic differentiation among the populations (mean F_{st} = 0.044), which could be explained by the remarkable gene flow (N_m>1) among populations.

Key words: *Avicennia marina*, mangrove, microsatellite, genetic diversity, Persian Gulf

INTRODUCTION

The coastal wetlands of the tropics and the subtropics of the world are characterized by the presence of a unique group of plant species, the mangroves. Despite their unique status as intertidal forests, hosting numerous faunal organisms and providing essential functions and services to tropical and subtropical zones and their populations, mangroves are one of the world's most threatened ecosystems (Triest, 2008). Mangrove forests all over the world are heavily exploited for wood and fishpond operations, as well as other activities. The exploitation of mangroves has resulted in the loss of genetic diversity in mangrove ecosystems (Maguire *et al*., 2000).

Since, the mangrove ecosystems are seriously affected, the conservation and suitable management of mangroves is a major priority in coastal areas of many countries. To better

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design effective management strategies, it is important to understand basic population parameters such as inbreeding, dispersal and regional and local population parameters for key members of the mangrove community (Souza *et al*., 2006).

Avicennia marina (Forsk.) Vierh, as a pioneer tree species of mangrove forest ecosystems, is widely distributed from East Africa and Persian Gulf, throughout Asia to China and Japan, to the Southwestern Pacific, New Zealand and Australia (Giang *et al*., 2003). It can grow and reproduce across a wide range of climatic, saline and tidal conditions. The wide geographical and climatic distribution of *A. marina* indicates that there is a large amount of genetic diversity available, which can be exploited for conservation, breeding programs and afforestration schemes (Maguire *et al*., 2002).

Several studies have been carried out on mangrove species in order to assess genetic diversity using genetic markers such as Random Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphism (RFLP) (Balakrishma, 1995; Parani *et al*., 1997) and recently microsatellite and Amplified Fragment Length Polymorphism (AFLP) (Maguire *et al*., 2000, 2002; Dodd *et al*., 2002; Giang *et al*., 2003). Microsatellite markers are short tandem repeats of mono- to tetra-nucleotide repeats, which are assumed to be randomly distributed in the nuclear genome. Such sequence repeats are relatively abundant and have high mutation rates in comparison to other markers, which make them useful for various types of population studies (Lowe *et al*., 2004).

In this study, four populations of *A. marina* encompassing the coastal areas of Persian Gulf were studied using microsatellite markers. The aim of this study was to determine the genetic variation within and between *A. marina* populations in Iran.

MATERIALS AND METHODS

Sample Collection and DNA Isolation

A total of 44 individuals, representing four natural populations, were sampled over the entire Iranian range of *A. marina*; namely Khamir, Qeshm, Tiab and Jask located in 25° 41′ to 27° 5′ North latitude and 55° 28′ to 57° 48′ East longitude (Fig. 1). Leaf materials from each population were collected between June and July 2007. Total genomic DNA was isolated from leaf tissue using a modified CTAB method (Maguire *et al*., 1994).

Microsatellite Analysis

Primer sequences specific for five microsatellite loci described by Maguire *et al*. (2000) were used in this study (Table 1). The Polymerase Chain Reaction (PCR) conditions were optimized for the five microsatellite loci as necessary to produce scorable amplification products. The PCR was performed in a 20 μL reaction volume containing 100 ng of template DNA, 10 pmol of each primer, 400 μM each of the dNTPs, 1 U of Taq DNA polymerase (Cinnagen, Iran), 1.5 mM $MgCl_2$ and 1×PCR buffer. The temperature profile consisted of 3 min initial denaturation at 94°C followed by 30 cycles of: 30 sec at 94°C, 45 sec at the annealing temperature (55°C) and 30 sec at 72°C, ending with 5 min at 72°C. The PCR products were separated on 6% polyacrylamide gels stained with silver nitrate.

The recorded microsatellite genotypes were used as input data for the GenAlex software version 6 package (Peakall and Smouse, 2006) in order to calculate allele and genotype frequencies, observed (H_o) and expected (H_e) heterozygosities and to test for deviations from Hardy-Weinberg Equilibrium (HWE). The phylogenetic relationship among the four populations was estimated from Nei's standard genetic distance (D) and genetic similarity index (I) (Nei, 1972). Genetic differentiation between populations was also evaluated by the

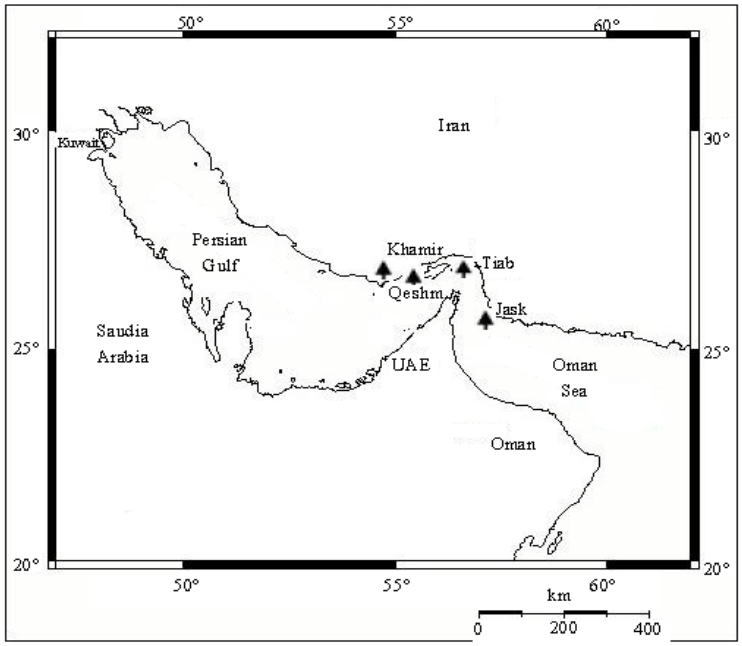

Fig. 1: Map showing sampling locations of four populations of *A. marina* in the Persian Gulf

Table 1: The five microsatellite loci used in this study

Locus	Repeat unit	Primer sequence (5′-3′)
M3	$(TG)_{15}$	GGTTCCTGCAAGTATGTCAACACCCTC
		ACCTTCGATTCCTCCCCGAATGC
M40	$(AG)_{32}$	CCCATAGATGACGGCAATCTTATGATCC
		ACCATCCAAAATAAAATAAATCTCCCTCCC
M47	$(CA)_{13}$	TGACACCAAGGGAAATCAACATGCC
		GAACCTAGCGACCAATAGATCATCCTGG
M81	$(CA)_9(CT)_{14}$	GAATGATGATCGGATGTTGCTACTCCTG
		CAATCCCAAAGCCCCAAAAATAATCC
M98	$(CGG)_8$	CCCAAACTCGTTACGATGGATGACTTC
		CTTACAGTTGCGGTAAAATGAGACGTGC

calculation of pairwise estimates of F_{st} values and testing their significance using the FSTAT software (Goudet, 1995). We also estimated an overall inbreeding coefficient (Fis; (Weir and Cockerham, 1984)) for each population and locus, which can measure HWE departures within a population. A UPGMA tree was constructed based on Nei's genetic distance using TFPGA version 1.3 (Miller, 1997).

RESULTS

The genetic variability indices estimated for the four *A. marina* populations are summarized in Table 2. All the five microsatellite loci were polymorphic in all the populations examined and the levels of the polymorphism varied depending on the locus. The only private allele (M81-4) at the population level was observed in Jask. The average number of alleles per locus ranged from 4 in the Khamir population to 4.6 in the Qeshm and Tiab populations, showing no significant difference among the four populations ($p>0.05$). The

Table 2: Variability of five microsatellite loci in four *A. marina* populations from Iran

Locus	Parameters	Qeshm	Khamir	Tiab	Jask
Ca1	A	5	5	5	5
	H_o	1.000	1.000	0.909	1.000
	H_e	0.674	0.780	0.674	0.760
	P	0.268	0.033*	0.060	0.042*
	F_{is}	-0.485	-0.282	-0.350	-0.316
Ca3	A	4	4	4	4
	H_o	0.727	0.900	0.455	0.400
	H_e	0.715	0.685	0.649	0.740
	P	0.162	0.032*	0.114	0.000**
	F_{is}	-0.017	-0.314	0.299	0.459
Ca5	A	5	4	6	5
	H_o	0.909	1.000	1.000	1.000
	H_e	0.789	0.590	0.826	0.720
	P	0.063	0.000**	0.000**	0.000**
	F_{is}	-0.152	-0.695	-0.210	-0.389
Z8145	A	5	3	4	4
	H_o	0.909	1.000	0.727	0.900
	H_e	0.736	0.620	0.715	0.705
	P	0.001**	0.019*	0.162	0.066
	F_{is}	-0.236	-0.613	-0.017	-0.277
Z10362	A	4	4	4	4
	H_o	0.727	0.900	0.818	1.000
	H_e	0.715	0.745	0.682	0.700
	P	0.271	0.109	0.258	0.038*
	F_{is}	-0.017	-0.208	-0.200	-0.429
Average No. of alleles per locus		4.6	4	4.6	4.4
Average H_o		0.855	0.960	0.782	0.860
Average H_e		0.726	0.684	0.709	0.725

A: No. of alleles; H_o: Observed heterozygosity; H_e: Expected heterozygosity; P: p-values of Chi-Square tests for Hardy-Weinberg equilibrium; F_{is}: Fixation index. Statistically significant values are marked with asterisks. *p<0.05, **p= 0.001

differences between populations were not statistically significant (p>) for the average observed heterozygosity (H_o). The average H_o ranged from 0.782 in the Tiab population to 0.960 in the Khamir population. The expected heterozygosity (H_e) was high, ranging from 0.590 to 0.826.

Significant to highly significant deviations from Hardy-Weinberg expectations were observed in 10 out of 20 (five loci H four populations) cases (Table 3). Most of F_{is} values were negative and significantly different from zero, thus suggesting excess of heterozygosity.

The population differentiation (F_{st}) value between Tiab and Khamir populations was the highest (0.100) and significant among the population pair, while the F_{st} value between the Khamir and Qeshm populations (0.021) was the lowest and not significant (Table 3). The estimated gene flow (N_m) value between the Qeshm and Khamir population across all the studied loci was the highest, while the N_m value between Khamir and Tiab populations was the lowest (Table 4).

Genetic distance (D) and genetic similarity index (I) between any two populations are shown in Table 4. The genetic distance was the smallest (0.189) between the Qeshm and Khamir populations, whereas the largest distance (0.482) was between Khamir and Tiab populations. The UPGMA dendrogram constructed on the basis of the Nei's genetic distance showed the four populations allocated into two groups (Fig. 2), that is, one group including the Qeshm and Khamir populations and the other group including the Tiab and Jask populations.

Table 3: Multilocus N_m (above diagonal) and F_{st} values (below diagonal) between pairs of *A. marina* populations across all loci

Populations	Qeshm	Khamir	Tiab	Jask
Qeshm	------	11.440	8.520	7.179
Khamir	0.021	------	2.252	5.547
Tiab	0.028	0.100*	------	6.320
Jask	0.034	0.043*	0.038	------

*p<0.05

Table 4: Genetic distance (D) (above diagonal) and genetic similarity (I) (below diagonal) between pairs of *A marina* populations

Populations	Qeshm	Khamir	Tiab	Jask
Qeshm	------	0.189	0.221	0.260
Khamir	0.827	------	0.482	0.270
Tiab	0.802	0.618	------	0.264
Jask	0.771	0.763	0.768	------

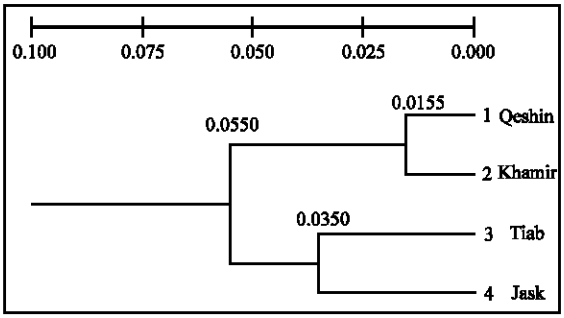

Fig. 2: The UPGMA dendrogram shows genetic distance between four populations allocated into two groups

DISCUSSION

Using five microsatellite loci a total of 24 alleles were detected. The average number of alleles per locus per population ranged from 4 to 4.6, showing the same level of allelic diversity comparing to the values detected earlier for *A. marina* sampled from the worldwide range (Maguire *et al*., 2000).

The observed heterozygosity (H_o) detected over all loci, ranging from 0.782 to 0.960 was comparable in the Iranian populations of *A. marina*. This is much higher than the levels of heterozygosity described earlier, where estimates of H_o ranged from 0.210 (Giang *et al*., 2003) to 0.407 (Maguire *et al*., 2000). Furthermore, *A. marina* populations in Iran were found to be generally outcrossing with no inbreeding, which do not correspond to earlier data (Giang *et al*., 2003; Maguire *et al*., 2000). This is not unexpected as these populations may have not been subjected to repeated bottleneck or founder effects in earlier times, due to episodes of glaciations and transgressions. It has been reported that populations of *A. marina* show reduced levels of polymorphism due to the constant use of foliage for fodder and grazing as well as environmental pollution (Parani *et al*., 1997). Regarding the higher levels of genetic heterozygosity in our study, it could be inferred that the Iranian populations of *A. marina* may have not been severely subjected to environmental impacts compared to other populations of *A. marina* in the entire worldwide range.

Pairwise genetic differentiation (F_{st}) was used to assess genetic differentiation, which is the acquisition of allele frequencies that differ among populations (Daniel and Clark, 1997). The value of F_{st} is a useful measure of genetic differentiation among populations and different values mean different variation degrees. Significant population differentiation was observed between Tiab and Khamir and also between Jask and Khamir populations. In our study, the microsatellite analysis showed low genetic differentiation among the populations (mean F_{st} = 0.044). However, high values of genetic differentiation have been found in worldwide populations of *A. marina* using microsatellite analysis (F_{st} = 0.410, Maguire *et al*., 2000). The low value of F_{st} in *A. marina* in Iran could be explained by the remarkable gene flow (N_m>1) among populations. The ocean-borne propagules accompanied by insect pollination may act as the efficient means of gene flow.

A dendrogram based on genetic distance (Fig. 2) showed two major clusters corresponding to the delineated geographical region. Our results indicate that geographical distance has caused genetic divergence among *A. marina* populations in Iran due to limitation of propagules dispersal.

The data generated in this study provide useful information on the genetic variation and differentiation in the Iranian populations of *A. marina*. Since, the Iranian mangrove forests showed higher genetic variation, suitable management strategies should be considered to avoid the loss of genetic diversity in the Iranian mangrove ecosystems.

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