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Genetic diversity in three populations of *Avicennia marina* along the eastcoast of India by RAPD markers

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Abstract

Genetic diversity was analysed in three populations of the mangrove species, *Avicennia marina* by using random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR). Ten random decamer primers were used to score the diversity from three locations of eastcoast of India: Parangipettai (Tamil Nadu), Kakkinada (Andhra Pradesh) and Sundarbans (West Bengal). These primers produced 388 scorable DNA fragments, of which 252 (64.98%) were polymorphic, 182 (46.90%) were monomorphic, and 14 (3.61%) were unique. RAPD banding patterns displayed variations between and within the populations, while, there was no morphological variation.

Key words

Avicennia marina, RAPD marker, Genetic diversity, Polymorphism

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Introduction

Mangroves are the salt tolerant plants of tropical and sub-tropical intertidal regions of the world (Kathiresan and Bingham, 2001). They are highly productive, but extremely sensitive and fragile. Anthropogenic pressure and natural calamities are the potential threats to the mangroves. Discharge of domestic and industrial waste waters are polluting the mangrove habitats (Kathiresan and Qasim, 2005). Therefore, efficient conservation of mangroves is essential in this context of its genetic erosion. However, lack of adequate genetic studies impairs conservation practices of mangrove genetic resources. *Avicennia* represents the largest polymorphic genus of the mangrove. Compared to other taxa, it is well known ecologically, systematically, morphologically and genetically (Arnaud-Haond *et al.,* 2006).

DNA based molecular markers unlike morphological markers are considered as stable and influenced very little by environmental fluctuations (Sudheer *et al*., 2010). DNA-based markers, such as random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) have been widely used in a number of plant groups for a variety of purposes: cultivar identification, diversity studies, parentage determination, developing breeding programmes and conservation strategies. In previous studies, various molecular markers such as allozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites (SSR), Inter-simple sequence repeats (ISSR), and DNA sequence are used (Ashish *et al*., 2011; Giang *et al*., 2003; Dodd *et al.,* 2002; Maguire *et al.,* 2000;). Most of the taxonomical studies on *Avicennia* species are only based on the morphological variations of the vegetative and reproductive organs. But with the advent of recent molecular technologies where genes and its products are directly examined, it is quite clear that the morphological characteristics cannot be a reliable indicators for genetic variation or taxonomic differences, owing to their tendency to be highly influenced by environmental factors (Giang *et al*., 2003). Despite the great interest in conservation and management of mangrove species, relatively few genetic studies have been conducted for their intrinsic value as a genetic resource. Hence, the present study was undertaken to assess the genetic diversity among *Avicennia marina* populations in three different

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states namely Tamil Nadu, Andhra Pradesh and West Bengal along the east coast of India by using ten RAPD primers.

Materials and Methods

Young leaves of *Avicennia marina* (Forsk) Vierh were collected from a population of randomly selected five mature trees of three locations: Parangipettai, TamilNadu (11º 49' N latitude and 79º 46' E longitude), Kakinada, Andhra Pradesh (16º 56' N latitude and 82º 13' E longitude) and Sundarbans, West Bengal (21º 31' N latitude and 88º 37' E longitude).

DNA was isolated following the CTAB protocol described by Saghai-Maroof *et al.* (1984), with minor modifications. Briefly, 1.0 g of leaf tissue was ground to powder under chilled condition. The powdered tissue was suspended in 2 ml of grinding buffer or suspension buffer (pH 8.0) and incubated at 60°C for 30 min in water bath. The suspension was centrifuged at 6000 rpm for 10 min at room temperature. This process was repeated twice by re-suspending the pellet in the same buffer, and the supernatant was discarded. The pellet was suspended in 2 ml of extraction buffer. The suspension was incubated at 60°C for 45 min in water bath followed by chloroform: isoamylalcohol (24:1) extraction. The sample was kept at -20 °C for overnight. Next day it was centrifuged at 13000 rpm. The upper aqueous layer was transferred in to a new eppendorf tube, washed with 70% ethanol and centrifuged at 13000 rpm to get the DNA pellet. The quality of the DNA was checked in 0.8% Agarose gel. Also the DNA concentration and quality was quantified with a spectrophotometer (NanoDrop Technologies Inc., Centreville, DE) at 260 and 280 nm.

Ten oligonucleotide primers purchased from GeNei (Bangalore) were used to analyse genetic diversity and identify species-specific markers in three populations of *A. marina*. The amplification reaction was carried out after standardization in our laboratory. Amplifications were performed in a 25-µl reaction volume containing 10 mM Tris-HCl, pH 8.3, 2.5mM $MgCl_2$, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.2 µM of each primer, 1 U of *Taq* DNA polymerase and 25 ng of template DNA. RAPD-PCR was performed in a thermalcycler (Tech Gene) for 40 cycles consisting of denaturation at 94°C for 30 sec, annealing at 45°C for 30 sec and extension at 72°C for 60 sec. The final extension was carried out at the same temperature for 5 min. The primer sequence details are given in Table 1. The amplified product was checked in 1.5% of agarose gel electrophoresis.

Genetic similarity/distance between the three populations were estimated using PopGene Software (Version 1.31, Yeh *et al.* 1999). Nei and Li's (1979) genetic similarity (GS) among the three populations was computed and converted by PopGene into genetic distance (GD) according to Hillis and Mortiz's (1990) formula : GD = 1-GS. The GS reflects the proportion of the bands shared between individuals and values range from 1 when present to 0 when absent.

Phylogenetic relationship based on genetic distance values generated from RAPD data among the three populations was made and dendrogram was plotted, following unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

Results and Discussion

The technical simplicity of the RAPD technique has facilitated its use in the analysis of genetic relationship in several genera (Barbosa *et al*., 2010). In the present study, Random amplified polymorphic DNA (RAPD) marker was used to assess the genetic diversity in three populations of *Avicennia marina* from Parangipettai, Kakinada and Sundarbans. There were 33 private bands found in three *A.*

Table 1 : Comparison of genetic diversity among three populations of *Avicennia marina*

Primer name	Population					
	Parangipettai		Kakinada		Sundarbans	
	Total no. of bands	Size range (bp)	Total no. of bands	Size range (bp)	Total no. of bands	Size range (bp)
Apl1	6	289-1539	15	376-1539	16	361-1482
Apl 2	18	239-1606	14	447-1495	3	698-1495
Apl 3	5	61-1000	16	186-1553	9	269-1447
Apl 4	6	318-1228	11	214-1360	17	38-1314
Apl 5	6	261-1188	8	270-668	13	217-915
Apl 6	11	50-1340	5	120-1186	8	345-2290
Apl 7	18	268-1693	20	231-1410	8	374-1180
Apl 8	4	604-1439	4	874-1439	14	226-1297
Apl 9	11	312-1525	9	345-1525	14	155-1424
Apl 10	14	522-1422	15	522-1510	12	456-1909

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Table 2 : Nei's (1978) genetic identity (above diagonal) and genetic distance (below diagonal) of three *Avicennia marina* populations

Population	Parangipettai	Kakinada	Sundarbans
Parangipettai	*******	0.8438	0.8246
Kakinada	0.1699	******	0.8453
Sundarbans	0.1928	0.1680	*****

marina populations. Ten private alleles in Parangipettai population, twelve in Kakinada population and nine private alleles in Sundarbans population were observed. The band size and other details are given in Table 1.

Nei's (1979) unbiased genetic distances and genetic similarity between three *A. marina* populations are given in Table 2. The genetic distance between Parangipettai and Kakinada population was 0.1699 based on the RAPD data. The genetic distance between Parangipettai and Sundarbans population was 0.1928, whereas, it was 0.1680 between Kakinada and Sundarbans population. The genetic diversity within and between the three populations of *A. marina* is given in Table 3. The genetic diversity (H) of Kakinada population was more (0.2068 ± 0.1869) than other two populations. In the dendrogram the cluster values indicated distinct relationship between the three populations of *A. marina*. This result reveals an interesting fact that the greater levels of genetic variation within species and populations are advantageous with respect to the environmental and anthropogenic challenges (Said and Ehsan, 2010).

In total, 388 bands were generated by amplification with ten RAPD primers. The number of RAPD markers amplified within the populations varied from 117 to 99. Among them, the polymorphic RAPDs varied from 30.74 to 36.75% within the populations. The intrapopulation's polymorphism in Parangipettai was estimated to be 30.74%. This high degree of overall polymorphism within the species could be attributed to its cross-pollinating nature of the mangrove species (Ghosh and Chakraborti, 2011). The present results showed that a significant portion of the polymorphosim was unique to individual populations and therefore, comparisons of the individuals across population resulted in substantial increase in the Percentage of polymorphic loci, indicating genetic divergence between the populations. Since the populations are physically

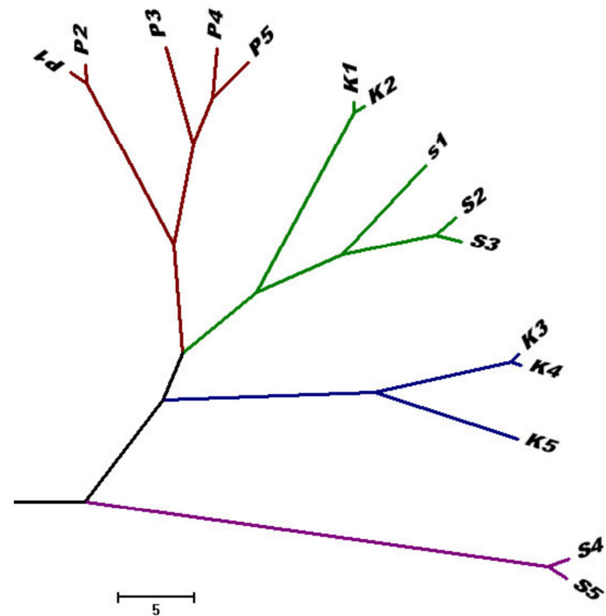

Fig. 1 : Neighbour joining tree (1000 replications) of three *Avicennia marina* populations (P-Parangipettai; K-Kakinada; S-Sundarbans).

isolated, the genetic content of the individuals that originally colonized the locations may be one of the causes of divergence. Selection in the original genetic content and local adaptations might have led to establishment of genotypes unique to different locations and populations. Local adaptation in natural populations and selective advantage of certain characters of original colonizers has been well documented in several species other than mangroves (Burczyk and Chybicki, 2004). The dendrogram constructed after pooling the data from RAPD markers showed a definite pattern of clustering between the populations. Similar variations among *Avicennia* population were observed by using microsatellites along the coast of Iran by Kahrood *et al.* (2008). Although the present study was an initial geographical survey at species level, RAPD markers indicated possible variability among and within populations of *A. marina.* Similar observations were also made by Abeysinghe *et al.* (2000) among *Bruguiera* species. The individuals from the populations inhabiting Kakinada and Sundarbans were found closer to each other than to the individuals from Parangipettai. This may be due to that Parangipettai populations are artificially developed recently,

Table 3 : Overall observed number of alleles (Na), Effective number of alleles (Ne), Nei's (1973) gene diversity (H), Shannon's information index (I), Number of polymorphic loci (NP) and percentage of Polymorphic loci (Pp) in three *Avicennia marina* populations

Population	Na	Ne	H	I	NP	Pp (%)
Parangipettai	0.1387 ± 0.4622	1.2074 ± 0.3339	0.1206 ± 0.1870	0.1777 ± 0.2720	87	30.74
Kakinada	1.3675 ± 0.4830	1.2298 ± 0.3206	0.2068 ± 0.1869	0.2068 ± 0.2757	104	36.75
Sundarbans	1.3675 ± 0.4830	1.2413 ± 0.3410	0.1421 ± 0.1928	0.2102 ± 0.2814	104	36.75
Overall	2.0000 ± 0.0000	1.3278 ± 0.2336	0.2274 ± 0.1122	0.3783 ± 0.1379	——	——-

whereas, populations from Kakinada and Sundarbans are natural ones and age old. The need to understand what determines the patterns of genetic variation is an important issue for mangrove conservation and reforestation (Kahrood *et al*., 2008). However, further genetic studies by using various other molecular markers such as AFLP (amplified fragment length polymorphism), microsatellite, and ITS (internal transcribed spacers) is the need of hour.

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