Assessment of Genetic Diversity in 31 Species of Mangroves and their Associates through RAPD and AFLP Markers

Arup Kumar Mukherjee[a,*], Laxmikanta Acharya[b], Pratap Chandra Panda[b], and Trilochan Mohapatra[c]

[a] DNA Fingerprinting Laboratory, Division of Plant Biotechnology, Regional Plant Resource Centre, Nayapalli, Bhubaneswar 751 015, Orissa, India. Fax: 91-674-2550274. E-mail: arupmukherjee@yahoo.com

[b] Division of Taxonomy and Conservation, Regional Plant Resource Centre, Nayapalli, Bhubaneswar 751 015, Orissa, India

[c] DNA Fingerprinting and Rice Genome Mapping Laboratory, NRC on Plant Biotechnology, Indian Agricultural Research Institute, New Delhi 110 012, India

* Author for correspondence and reprint requests

Z. Naturforsch. **61c**, 413–420 (2006); received October 28/November 23, 2005

Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers were used to assess the genetic diversity in 31 species of mangroves and mangrove associates. Four AFLP primer combinations resulted in the amplification of 840 bands with an average of 210 bands per primer combination and 11 RAPD primers yielded 319 bands with an average of 29 bands per primer. The percentage of polymorphism detected was too high indicating the high degree of genetic variability in mangrove taxa both at inter- and intra-generic levels. In the dendrogram, species belonging to a particular family/genus, taxa inhabiting similar habitats or having similar adaptations tended to be together. There were exceptions too; as many unrelated species of mangroves form clusters. The intrafamilial classification and inter-relationships of genera in the family Rhizophoraceae could be confirmed by molecular analysis. Both the markers RAPD and AFLP were found equally informative and useful for a better understanding of the genetic variability and genome relationships among mangroves and their associated species.

Key words: Mangroves, Molecular Characterization, Genetic Diversity

Introduction

Mangroves, the characteristic complex plant-communities of tropical and sub-tropical sheltered coastlines, have attracted considerable scientific attention during the last few decades. Like the terrestrial tropical rain forests, mangals have played a significant role in the economy of tropical societies for thousands of years, providing a wide variety of goods and services including wood production, support for commercial and subsistence fisheries, aquaculture, salt production and shoreline and coastal erosion control. Being ecologically interesting, strictly habitat-specific, highly resourceful, inhabiting vulnerable ecosystem and exhibiting peculiar morphological and anatomical adaptations such as vivipary and pneumatophores, mangrove plants have aroused considerable curiosity and have been the subject of taxonomic, phytogeographical, ecological, cytological, physiological and molecular studies (Schwarzbach and Ricklefs, 2000, 2001; Duke *et al.*, 2002; McCoy and Heck, 1976; Lakshmi *et al.*, 1997, 2000; Parani *et al.*,1997a,b, 1998).

The mangrove forests and coastal wetlands are being destroyed worldwide at an alarming rate due to human interference of various kinds and magnitudes resulting in loss of genetic diversity. Recently, molecular data concerning phylogenetic relationships and population structure have been obtained from macro-molecules like proteins and DNA and used for identifying populations with high genetic diversity that could be important sources for restoration and re-vegetation of these unique and important habitats (Parani *et al.*, 1997a; Schwarzbach and Ricklefs, 2001).

The use of molecular markers in mangrove research has been reviewed by Schwarzbach and Ricklefs (2001), who described at length how protein and DNA markers have been helpful in the assessment of genetic diversity and in establishing inter-relationships and phylogeny among a number of mangrove taxa. As rightly pointed out then,

0939–5075/2006/0500–0413 $ 06.00 © 2006 Verlag der Zeitschrift für Naturforschung, Tübingen · http://www.znaturforsch.com · D

indirect detection methods of DNA sequence difference like AFLPs (amplified fragment length polymorphisms) and SSRs (simple sequence repeats) were not applied in mangrove plant research till 2001 and there was a single report on the use of ISSR (inter simple sequence repeat) for estimation of genetic diversity in a mangrove species *e.g. Aegiceras corniculatum*. Parani *et al.* (1998) used RAPD and RFLP data to analyze genomic relationships of 16 mangrove species of India. Recently, Maguire *et al.* (2002) have used AFLP and SSR markers for studying the intraspecific variability in *Avicennia marina* (Forsk.) Vierh., which is the only published account on application of an AFLP marker to mangroves. However, no report is yet available on the use of RAPDs and AFLPs in combination for establishing the genomic relationships among species of mangroves and their associates, in general. In the present study, the AFLP technique has been used for the first time for assessment of genetic diversity in 31 species of mangroves and mangrove associates in conjunction with RAPD.

Materials and Methods

Plant material

For the present study, 31 species of mangroves and their associates were included. Their correct botanical names with synonyms, if any, families to which each of them belong to, their ecological status, and locality of collection are given in Table I. Ten individuals of each species except *R. stylosa* (5 individuals) were randomly selected and fresh and young leaf samples were collected for isolation of genomic DNA.

Genomic DNA isolation

The genomic DNA was isolated from freshly collected leaves using the CTAB method (Saghai-Maroof *et al.*, 1984) with some modifications. The crude DNA was purified by giving RNAseA treatment (@ 60 μg RNaseA for 1 ml of crude DNA solution) followed by three washes with phenol/chloroform/iso-amyl-alcohol (25:24:1 v/v/v) and subsequently three washes with chloroform/iso-amyl-alcohol (24:1 v/v). The upper aqueous phase was separated after centrifugation and mixed with 1/10 volume of 3 M sodium acetate. DNA was precipitated by adding 2.5 volumes of chilled absolute ethanol, pelleted, dried in vacuum and dissolved in $T_{10}E_1$ buffer. Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gels alongside diluted uncut lambda DNA as standard. DNA was diluted in $T_{10}E_1$ buffer to the required concentration for different PCR analyses.

RAPD analysis

For RAPD analysis, PCR amplification of 25 ng of genomic DNA was carried out using 11 standard decamer oligonucleotide primers, *i.e.* OPM01, OPM06, OPM09, OPN05, OPP01, OPQ01, OPQ20, OPS07, OPT04, OPT07 and OPT08 (Operon Tech. Alameda, USA). The RAPD analysis was performed as per the standard method of Williams *et al.* (1990). Each amplification reaction mixture of 25 μl contained 20 ng of template DNA, 2.5 μl of 10X assay buffer (100 mM Tris-HCl, pH 8.3, 0.5 M KCl and 0.01% gelatin), 1.5 mM $MgCl_2$, 200 μM each of dNTPs, 20 ng of primer and 0.5 U TaqDNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India). The amplification was carried out in a thermal cycler (Perkin Elmer, Model 9600, USA). The first cycle consisted of denaturation of template DNA at 94 °C for 5 min, primer annealing at 37 °C for 1 min and primer extension at 72 °C for 2 min. In the subsequent 42 cycles, the period of denaturation was reduced to 1 min while the primer annealing and primer extension time were maintained same as in the first cycle. The last cycle consisted of only primer extension at 72 °C for 7 min. PCR products were separated on a 1.5% agarose gel containing ethidium bromide (@ 0.5 μg/ml of gel solution). The size of the amplicons was determined using size standards (100 bp DNA ladder plus or DNA ladder mix, MBI Fermentas, Graiciuno, Vilnius, Lithuania). DNA fragments were visualized under UV light and photographed using a polaroid photographic system (FOTODYNE Incorporated 950 Walnut Ridge Drive Hartland, WI, USA).

AFLP analysis

AFLP analysis was performed following the modified method of Vos *et al.* (1995) and as per the protocol supplied with the 'AFLP Core Reagent Kit' and the 'AFLP Starter Primer Kit' of Life Technologies (Grand Island, New York, USA). The amplified products were analyzed in prewarmed 5% acrylamide electrophoresis gels. Gels were run at 55 W for approx. 2 h, transferred to Whatman filter paper (No. 1) and dried under vac-

Table I. Details of the species taken for the present study.

Species	Family	Place(s) of collection	Status
Avicennia alba Bl.	Avicenniaceae	Dangamala*	True mangrove
Acanthus ilicifolius L.	Acanthaceae	Dhamara*	Mangrove associate
Aegiceras corniculatum (L.) Blanco	Myrsinaceae	Kajalpatia*	True mangrove
Aegialitis rotundifolia Roxb.	Plumbaginaceae	Thakurdian*	True mangrove
Caesalpinia bonduc (L.) Roxb.	Caesalpiniaceae	Dangamala*	True mangrove
Cryptocoryne ciliata (Roxb.) Schott	Araceae	Khola*	Mangrove associate
Crinum defixum Kar.-Gawl.	Amaryllidaceae	Khola*	Mangrove associate
Cynometra iripa Kostel.	Caesalpiniaceae	Dangamala*	True mangrove
Cerbera manghas L.	Apocynaceae	Kansaridian*	Back mangal
Derris heterophylla (Willd.) Back. and Bakh.	Fabaceae	Musadian*	Mangrove associate
Derris scandens (Roxb.) Benth.	Fabaceae	Kanika*	Mangrove associate
Excoecaria agallocha L.	Fabaceae	Dangamala*	Mangrove associate
Heritiera fomes Buch.-Ham.	Sterculiaceae	Kharnasi*	True mangrove
Heritiera littoralis Dryand	Sterculiaceae	Kalibhanjadian*	Back mangal
Heritiera macrophylla Wall.	Sterculiaceae	Botanic Garden, R.P.R.C.#, BBSR+, Orissa.	Main land
Pongamia pinnata (L.) Pierre	Fabaceae	Dangamala*	Mangrove associate
Lumnitzera racemosa Willd.	Combretaceae	Ek Kula*	True mangrove
Suaeda maritima Dumort.	Chenopodiaceae	Thakurdian*	Mangrove associate
Tylophora tenius (Bl.) Bijdr.	Asclepiadaceae	Dangamala*	Back mangal
Xylocarpus granatum Koenig	Meliaceae	Kajalpatia*	True mangrove
Bruguiera cylindrica (L.) Bl.	Rhizophoraceae	Dangamala*	True mangrove
B. gymnorrhiza (L.) Lamk.	Rhizophoraceae	Ek Kula*	True mangrove
B. parviflora Wt. and Arn. ex Griff.	Rhizophoraceae	Thakurdian*	True mangrove
B. sexangula (Lour.) Poir.	Rhizophoraceae	Dangamala*	True mangrove
Cassipourea ceylanica (Garden) Aston	Rhizophoraceae	Badakuda Island, Chilika Lake, Orissa	Main land
Carallia brachiata (Lour.) Merr.	Rhizophoraceae	Botanic Garden, R.P.R.C.#, BBSR+, Orissa	Main land
Ceriops tagal (Perr.) Robinson	Rhizophoraceae	Kanika*	True mangrove
Kandelia candel (L.) Druce	Rhizophoraceae	Suajore*	True mangrove
Rhizophora apiculata Bl.	Rhizophoraceae	Mahisamara*	True mangrove
R. stylosa Griff.	Rhizophoraceae	Campbell Bay, Andaman Island	True mangrove
R. mucronata Lamk.	Rhizophoraceae	Dangamala*	True mangrove

* All these samples were collected from different sites of Bhitarkanika wildlife sanctuary, Orissa.
# R.P.R.C., Regional Plant Resource Centre.
+ BBSR, Bhubaneswar.

uum (BioRad Model 583). AFLP products were observed when exposed to X-ray films (Kodak-BioMax MR) for 2–5 d. For the present study, four best primer combinations (EACT/MCAC, EACT/MCTA, EAGC/MCAA and EAGC/MCTT) were selected out of 9 primer combinations used in a previous study involving 3 species of *Heritiera* (Mukherjee *et al*., 2003).

Data analysis

The banding patterns obtained from RAPD gel and AFLP autoradiographs were scored as present (1) or absent (0). The data obtained from both the categories of markers were pooled for different analyses. Jaccard's coefficient of similarity (Jaccard, 1908) was measured and a dendrogram based on similarity coefficients generated by the un-weighted pair group method using arithmetic averages (UPGMA) and SAHN (sequential, agglomerative, hierarchical, and nested) clustering methods (Sneath and Sokal, 1973). Similarity matrices obtained with RAPD and AFLP primers were compared with the combined similarity matrices accomplished by the RAPD and AFLP primers using the Mantel test (Mantel, 1967). Principal co-ordinate analysis (PCA) was also performed for confirmation of the grouping of the taxa. All analyses were done using the computer package NTSYS-PC (Rohlf, 1997).

Results

Molecular markers

In case of RAPD analysis, eleven random decamer primers amplified 319 bands with the fragment range of 4000 to 100 bp (Table II). None of the bands was monomorphic. The number of bands ranged from 22 to 42 in different primers. The average number of bands amplified in the individual primer was 29. The RAPD patterns obtained using primers OPM01 and OPQ01 are shown in Fig. 1.

For AFLP analysis, four primer combinations resulted in the amplification of 840 bands with an average of 210 bands per primer. The number of bands amplified varied from 195 to 217 (Table II)

Table II. Details of molecular analysis in 31 species using AFLPs and RAPDs.

RAPD analysis			AFLP analysis	
Primer	Sequence	No. of loci	Primer combination	No. of loci
OPM01	GTTGGTGGCT	23	EACT/MCAC	195
OPM06	CTGGGCAACT	27	EACT/MCTA	212
OPM09	GTCTTGCGGA	23	EAGC/MCAA	217
OPN05	ACTGAACGCC	22	EAGC/MCTT	216
OPP01	GTAGCACTCC	27		
OPQ01	GGGACGATGG	42		
OPQ20	TCGCCCAGTC	23		
OPS07	TCCGATGCTG	33		
OPT04	CACAGAGGGA	38		
OPT07	GGCAGGCTGT	32		
OPT08	AACGGCGACA	29		
TOTAL		319		840

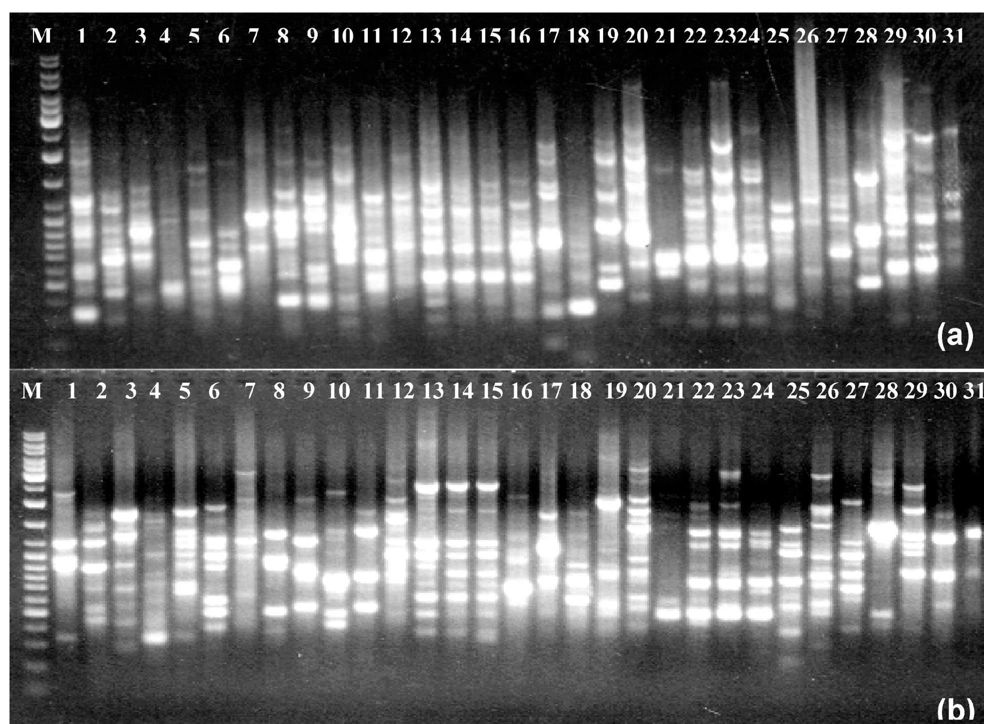

Fig. 1. RAPD pattern of 31 species using (a) OPM01 and (b) OPQ01 primer. M, 100 bp ladder mix (MBI Fermantas); lanes 1 to 31, different species arranged according to Table I.

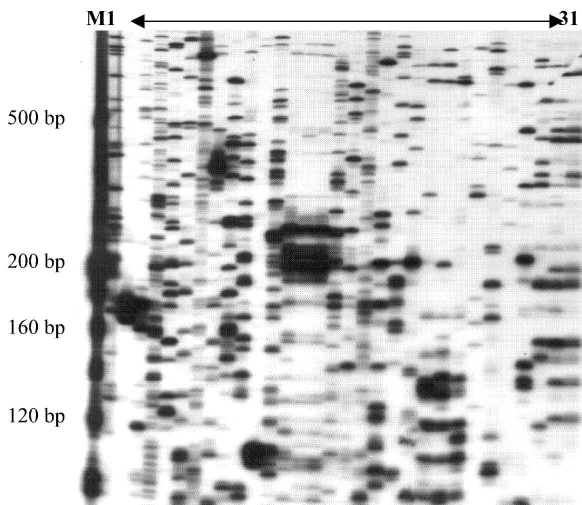

Fig. 2. A part of the AFLP autoradiogram of 31 species obtained by the primer combination EACT/MCAC. M, 20 bp ladder (FMC, USA); lanes 1 to 31, different species arranged according to Table I.

and none of the bands was found to be monomorphic. A representation of AFLP banding pattern of 31 species of mangroves and mangrove associates is shown in the Fig. 2.

Genomic relationships

The RAPD and AFLP data were pooled and the genetic similarity was calculated using Jaccard's coefficient (Jaccard, 1908). The highest similarity was observed between *Heritiera littoralis* and *H. macrophylla* (0.9083) and the lowest similarity was observed between *Tylophora tenius* and *Aegiceras corniculatum* (0.1201). A similar result was obtained when a cluster diagram was constructed using the UPGMA method and SAHN clustering. The dendrogram showed two major clusters; one with 8 species and the other with rest 23 species of mangroves (Fig. 3). All the members of Rhizophoraceae formed a single sub-cluster and a similar result was observed in case of three species of *Heritiera* (Sterculiaceae). Within the family Rhizophoraceae, the two non-mangrove elements

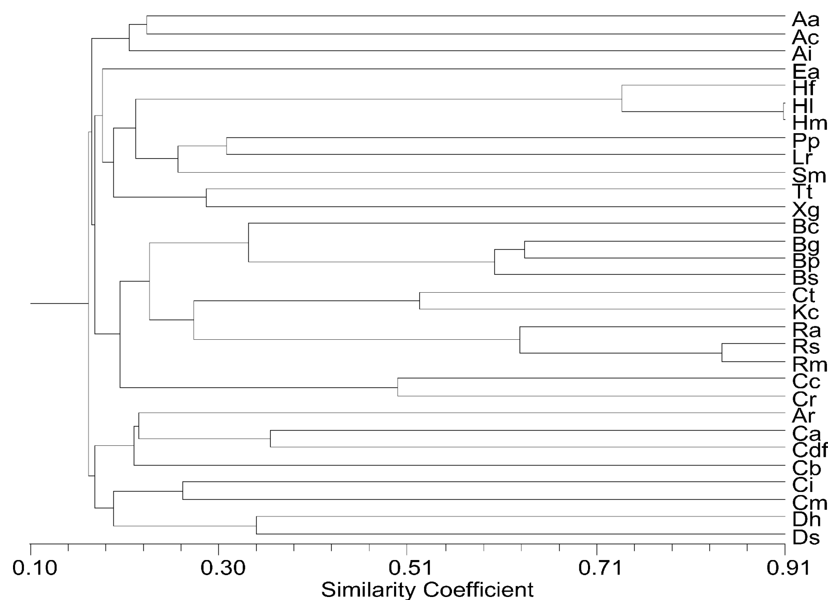

Fig. 3. Dendrogram of the genomic relationship among 31 species using RAPD and AFLP markers. Aa, *Avicennia alba*; Ac, *Aegiceras corniculatum*; Ai, *Acanthus ilicifolius*; Ar, *Aegialitis rotundifolia*; Bc, *Bruguiera cylindrica*; Bg, *B. gymnorrhiza*; Bp, *B. parviflora*; Bs, *B. sexangula*; Ca, *Cryptocoryne ciliata*; Cb, *Caesalpinia bonduc*; Cc, *Cassipourea ceylanica*; Cdf, *Crinum defixum*; Ci, *Cynometra iripa*; Cm, *Cerbera manghas*; Cr, *Carallia brachiata*; Ct, *Ceriops tagal*; Dh, *Derris heterophylla*; Ds, *D. scandens*; Ea, *Excoecaria agallocha*; Hf, *Heritiera fomes*; Hl, *H. littoralis*; Hm, *H. macrophylla*; Kc, *Kandelia candel*; Lr, *Lumnitzera racemosa*; Pp, *Pongamia pinnata*; Ra, *Rhizophora apiculata*; Rm, *R. mucronata*; Rs, *R. stylosa*; Sm, *Suaeda maritima*; Tt, *Tylophora tenius*; Xg, *Xylocarpus granatum*.

got separated from the nine other mangrove Rhizophoraceae, both sharing a common node and making separate sub-clusters. Similarly, two species of *Derris* (Fabaceae) formed a sub-cluster in the dendrogram. Both the monocotyledonous taxa *Cryptocoryne ciliata* and *Crinum defixum* came under one sub-cluster justifying their taxonomic alienation. The result was also confirmed using principal coordinate analysis (Fig. 4).

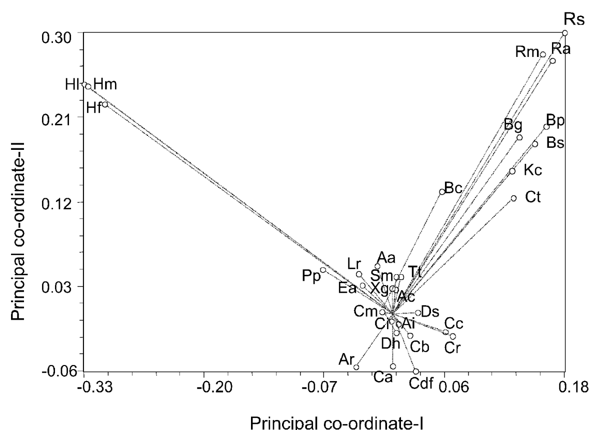

Fig. 4. Principal co-ordinate analysis showing grouping of different taxa of mangroves and their associates (the abbreviations are as used in Fig. 3).

Discussion

Thirty-one species of mangroves and their associates were fingerprinted using RAPD and AFLP markers. Eleven RAPD markers produced 319 bands at an average of 29 bands per primer in contrast to 19.80 bands per primer reported by Parani *et al.* (1998). This difference in banding pattern could be due to the selection of primers as well as the plant materials. In the present investigation, we observed that four AFLP primer sets detected a total of 840 bands with the number of bands ranging from 195 to 217 (mean = 210) per primer but in an earlier study Maguire *et al.* (2002) observed 306 bands per primer while studying the intra-specific variability in *Avicennia marina*. This difference in banding pattern is again attributed to the selection of primer combinations as well as plant materials.

AFLP technology being highly reproducible due to its stringent amplification procedure (Folkertsma *et al.,* 1996; Brown, 1996), we used AFLP markers for the first time to analyze the genetic variability and establish phylogenetic relationships among different genera and species of mangroves, which form the components of a very complex ecosystem.

Four AFLP primer combinations amplified 840 bands, all of which were polymorphic in nature. Eleven RAPD primers also produced 319 polymorphic bands. Russell *et al.* (1997) and Garcia-Mas *et al.* (2000) reported similar results with AFLP and RAPD markers while evaluating the genetic diversity in barley and melon respectively.

In the present investigation, it was not possible to segregate the mangroves, mangrove associates and non-mangroves from the species studied unlike the findings of Parani *et al.* (1998). This could be due to the selection of more taxa from a single genus/family irrespective of their habitat conditions and the use of a different marker system *i.e.* AFLP instead of RFLP where random primer sets were selected without any prior information of their amplification pattern. While analyzing the genomic relationships of 16 mangrove species using RAPD and RFLP data, Parani *et al.* (1998) used the probes originating from mangroves only and also targeted the chloroplast DNA (cpDNA) regions which are inherited maternally and are highly conserved (Harris and Ingram, 1991; Palmer, 1987). Schwarzbach and Ricklefs (2001) observed that the results of Parani *et al.* (1998) differ strikingly from previously published large-scale phylogenetic studies using molecular and non-molecular data. They attributed the lack of congruence, in this particular case, to the small number of taxa studied, use of extremely variable and homoplastic characters of RAPD and RFLP and identification of lineages by cluster analysis (UPGMA), which does not allow for unequal rates of evolution. In another work, Parani *et al.* (2000) studied the molecular phylogeny of 24 species of mangroves and mangrove associates by analysis of *trnS-psbC* and *rbcL* gene regions through RAPD and RFLP markers. However, in the present study, we have taken entire genomic DNA and also four AFLP primer combinations from the ones used in our earlier study on phylogeny of 11 members of Rhizophoraceae (Mukherjee *et al.*, 2004).

The dendrogram showed grouping of taxa belonging to a particular genus or family under the same cluster. Eleven members of the family Rhizophoraceae comprising of nine mangroves and two terrestrial non-mangrove representatives (Ta-

ble I) formed a single sub-cluster justifying their systematic position. The non-mangroves were further segregated from the mangrove Rhizophoraceae (tribe Rhizophoreae) in the sub-cluster supporting the intra-familial division into tribes (Tobe and Raven, 1988). The genus *Bruguiera* (4 species) and *Rhizophora* (3 species) also formed separate clades comprising species of their own. The members of the genus *Rhizophora*, *Ceriops* and *Kandelia* showed closer affinity than *Bruguiera* which maintained a distant place in the tribe. The isolated position of *Bruguiera* from other Rhizophoreae was also evident in the phylogenetic tree constructed by Schwarzbach and Ricklefs (2001) on the basis of chloroplast DNA, nuclear ribosomal DNA and morphology. Of the Rhizophoreae, the genera *Ceriops* and *Kandelia* had closer relationship between them and shared a common node in the dendrogram. Three species of *Heritiera* also came under a single cluster and *H. littoralis* showed more similarity with *H. macrophylla,* an observation made earlier by Mukherjee *et al.* (2003). Similar observations were made with respect to the two species of *Derris* (*D. heterophylla* and *D. scandens*) and between the two monocotyledonous taxa *Cryptocoryne ciliata* and *Crinum defixum*.

Though taxonomically distantly placed, *Avicennia alba*, *Aegiceras corniculatum* and *Acanthus ilicifolius* shared a common node. The affinity of *Aegiceras* and *Avicennia* has been earlier reported by Parani *et al.* (1998) using RAPD and RFLP markers. All the three species *viz. Acanthus ilicifolius*, *Aegiceras corniculatum* and *Avicennia alba* have salt excreting glands on their leaves in common and it is likely that all of them might have evolved some genetic similarity to adapt to the stressful mangrove environment. However, non-inclusion of *Aegialitis rotundifolia*, another prominent salt excreting species, in this group is not understandable. There is also no suitable explanation for the relatedness observed between *Xylocarpus granatum* and *Tylophora tenius.*

It was interesting to note that all the species inhabiting less salinity regimes like *Pongamia pinnata*, *Xylocarpus granatum*, *Lumnitzera racemosa*, *Suaeda maritima* formed a cluster in the dendrogram. Once again, inclusion of the three species of *Heritiera* in the above clade does not appear justifiable.

A fairly high '*r*' value (0.81) obtained on comparison of AFLP and RAPD data indicated that both the markers are comparable in the present investigation. This was in conformity with the earlier result of Garcia-Mas *et al.* (2000). The comparison of individual AFLP primers with that of the pooled data showed that only two primers namely EACT/MCTA and EAGC/MCAT were sufficient enough for genetic characterization of all the 31 species now investigated.

As commonly conceived, mangroves are a heterogeneous assemblage of diverse groups of plants with independently derived lineages and the species are defined ecologically by their occurrence in tidal swamp forests and physiologically by their ability to withstand high salt concentrations and low soil aeration (Schwarzbach and Ricklefs, 2001). Even with the knowledge that mangroves are polyphyletic in origin, the inter-relationships of many constituent taxa have been the matter of controversy over years. According to Schwarzbach and McDade (2002), strongly convergent evolution of many characters to the stressful mangrove environment may be the most important factor contributing to this confusion. As a matter of fact, a number of common morphological characters is observed among unrelated mangrove species but such similarities are not to be found between mangroves and their closest terrestrial relatives.

The limitations of RAPD and AFLP data in the present study emphasize the need for phylogenetic work based on conserved DNA sequences involving more numbers of mangrove species. Though the work is of preliminary nature, the molecular data shall provide information on genetic diversity of the mangroves and have impact on conservation and management of this important group of plants.

Acknowledgements

The authors acknowledge the financial assistance from the Department of Environment and Forest, Govt. of Orissa, India and wish to thank the Director of the institute for facilities.

Brown J. K. M. (1996), The choice of molecular markers methods for population genetic studies of plant pathogens. New Phytol. **133**,183–195.

Duke N. C., Lo E. Y. Y., and Sun M. (2002), Global distribution and discontinuities of mangroves emerging patterns in the evolution of *Rhizophora*. Trees **16**, 65–69.

Folkertsma R. T., van der Voort J. N. A. M. R., de Groot K. E., van Zandvoort P. M., Schots A., Gommers F. J., Helder J., and Bakker J. (1996), Gene pool similarities of potato cyst nematode populations assessed by AFLP analysis. Mol. Plant-Microbe In. **9**, 47–54.

Garcia-Mas J., Oliver M., Gomez-Paniagua H., and de Vicente M. C. (2000), Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. Theor. Appl. Genet. **101**, 860–864.

Harris S. A. and Ingram R. (1991), Chloroplast DNA and biosytematics: the effects of intraspecific diversity and plastid transmission. Taxon **40**, 393–412.

Jaccard P. (1908), Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**, 223–270.

Lakshmi M., Rajalakshmi S., Parani M., Anuratha C. S., and Parida A. (1997), Molecular phylogeny of mangroves. I. Use of molecular markers in assessing the intra-specific gene variability in the mangrove species, *Acanthus ilicifolius* Linn. (Acanthaceae). Theor. Appl. Genet. **94**, 1121–1127.

Lakshmi M., Parani M., Ram N., and Parida A. (2000), Molecular phylogeny of mangroves. IV. Intraspecific genetic variation in mangrove species *Excoecaria agallocha* L. (Euphorbiaceae). Genome **43**, 110–115.

Maguire T. L., Peakall R., and Saenger P. (2002), Comparative analysis of genetic diversity in the mangrove species *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae) detected by AFLPs and SSRs. Theor. Appl. Genet. **104**, 388–398.

Mantel N. A. (1967), The detection of disease clustering and a generalized regression approach. Cancer Res. **27**, 209–220.

McCoy E. D. and Heck K. L. Jr. (1976), Biogeography of corals, sea grasses and mangroves, an alternative to the center of origin concept. Syst. Zool. **25**, 201–210.

Mukherjee A. K., Acharya L. K., Mattagajasingh I., Panda P. C., Mohapatra T., and Das P. (2003), Molecular characterization of three *Heritiera* species using AFLP marker. Biol. Plantarum **47**, 445–448.

Mukherjee A. K., Acharya L. K., Panda P. C., Mohapatra T., and Das P. (2004), Genomic relations among two non-mangroves and nine mangroves species of Indian Rhizophoraceae. Z. Naturforsch. **59c**, 572–578.

Palmer J. D. (1987), Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am. Nat. **130**, S6–S29.

Parani M., Lakshmi M., Elango S., Ram N., Anuratha C. S., and Parida A. (1997a), Molecular phylogeny of mangroves. II. Intra and inter-specific variation in *Avicennia* revealed by RAPD and RFLP markers. Genome **40**, 487–495.

Parani M., Rao C. S., Mathan M., Anuratha C. S., Narayanan K. K., and Parida A. (1997b), Molecular phylogeny of mangroves. III. Parentage analysis of *Rhizophora* hybrid using random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) markers. Aquat. Bot. **58**, 165–172.

Parani M., Lakshmi M., Senthilkumar P., Ram N., and Parida A. (1998), Molecular phylogeny of mangroves. V. Analysis of genome relationships in mangrove species using RAPD and RFLP markers. Theor. Appl. Genet. **97**, 617–625.

Parani M., Lakshmi M., Ziegenhagen B., Fladung M., Senthilkumar P., and Parida A. (2000), Molecular phylogeny of mangroves. VII. PCR-RFLP of *trnS-psbC* and *rbcL* gene regions in 24 mangrove and mangrove associate species. Theor. Appl. Genet. **100**, 454–460.

Rohlf F. J. (1997), NTSYS-PC, numerical taxonomy and multivariate analysis system. Exeter Software, New York.

Russell J. R., Fuller J. D., Macaulay M., Hatz B. G., Jahoor A., Powel W., and Waugh R. (1997), Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor. Appl. Genet. **95**, 714–722.

Saghai-Maroof M. A., Soliman K. M., Jerenson R. A., and Allard R. W. (1984), Ribosomal DNA spacer length polymorphism in barley, Mendelian inheritance, chromosomal location and population dynamics. Natl. Acad. Sci. USA **81**, 8014–8018.

Schwarzbach A. E. and Ricklefs R. E. (2000), Systematic affinities of Rhizophoraceae and Anisophylleaceae, and intergeneric relationship within Rhizophoraceae, based on chloroplast DNA, nuclear ribosomal DNA and morphology. Am. J. Bot. **87**, 547–564.

Schwarzbach A. E. and Ricklefs R. E. (2001), The use of molecular data in mangrove plant research. Wetlands Ecol. Manag. **9**, 195–201.

Schwarzbach A. E. and McDade L. A. (2002), Phylogenetic relationship of the mangrove family Avicenniaceae based on chloroplast and nuclear ribosomal DNA sequences. Syst. Bot. **27**, 84–98.

Sneath P. H. A. and Sokal R. (1973), Numerical Taxonomy. Freeman, San Francisco, California.

Tobe H. and Raven P. H. (1988), Seed morphology and anatomy of Rhizophoraceae, inter and intrafamilial relationships. Ann. Mo. Bot. Gard. **73**, 1319–1342.

Vos P., Hogers R., Bleeker M., Reijans M., Van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M., and Zabeau M. (1995), AFLP, a new technique for DNA fingerprinting. Nucleic Acids Res. **23**, 4407–4414.

Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A., and Tingey S. V. (1990), DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**, 6531–6535.