Genomic Relations among Two Non-mangrove and Nine Mangrove Species of Indian Rhizophoraceae

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

[a] DNA Fingerprinting Laboratory, Regional Plant Resource Centre, Nayapalli, Bhubaneswar 751 015, Orissa, India. Fax: 91-674-2550274. Email: arupmukherjee@yahoo.com
[b] Taxonomy and Conservation Division, 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
[§] Present address: INSA Senior Scientist, GMR Research Foundation, 4-123/E Swaroop Nagar, Padmavathi Colony, Uppal Kalan Municipality, Hyderabad 500039

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 572–578 (2004); received February 12/April 7, 2004

Random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) markers were used to study the genomic relationship among 11 members of Indian Rhizophoraceae represented by nine true mangroves and two non-mangrove species. The AFLP and RAPD bands were scored and analyzed for genetic similarities and cluster analysis was done which separated the 11 species studied into two main groups, the true mangroves and the non-mangroves. The polymorphism observed for these markers showed a high degree of genetic diversity among the constituent taxa of the family. The phylogenetic relationship inferred from molecular marker systems supported the traditional taxonomic classification of the family Rhizophoraceae based on morphological characters at the levels of tribe, phylogeny and delimitation of genera and species, except the intra-generic classification of the genus *Bruguiera* and the placement of *Rhizophora* in the family Rhizophoraceae.

Key words: AFLP, RAPD, Rhizophoraceae

Introduction

Molecular markers like random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP) have extensively been used to quantify accurately the inter- and intra-specific and inter-generic variability in different plant groups and crop varieties (Chalmers *et al.*, 1994; Lin *et al.*, 1996; Russell *et al.*, 1997; Garcia-Mas *et al.*, 2000; Purba *et al.*, 2000; Pejic *et al.*, 1998; Sharma *et al.*, 2000; Maki and Horie, 1999; Singh *et al.*, 1999; Mukherjee *et al.*, 2003). The molecular markers are not influenced by the external environment unlike that of morphological markers and hence accurately testify the genetic relationship between and among plant groups. Molecular markers like AFLP and/or RAPD have been used to study the inter- and intra-specific variations in some mangrove species (Parani *et al.*, 1996, 1997, 1998, 2000; Mukherjee *et al.*, 2003). A number of attempts have been made to study the inter- and intra-generic relations among them (Lakshmi *et al.*, 2002; Abeysinghe *et al.*, 2000) using RAPD alone or with other markers but there is no report so far using AFLP for studying genetic relations among Indian Rhizophoraceae.

Hence the present research was conducted with the objective to study the genomic relationship among nine mangrove and two non-mangrove species of Indian Rhizophoraceae which are in the verge of extinction by using molecular markers and to authenticate the formation of the tribes Rhizophoreae and Lignotidae as has been done by Henslow (1879).

Materials and Methods

Plant material

For the present study 9 mangroves and 2 non-mangrove representatives of the family Rhizophoraceae were included. The names, their ecological status, distribution and place 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 of equal quantity were collected for isolation of genomic DNA.

0939–5075/2004/0700–0572 $ 06.00 © 2004 Verlag der Zeitschrift für Naturforschung, Tübingen · http://www.znaturforsch.com · D

Table I. Details of the species of Rhizophoraceae used in the present study.

Tribe (Henshaw, 1879)	Species	Status	Place(s) of collection
Rhizophoreae	*Bruguiera cylindrica* (L.) Bl.	True mangrove	Bhitarkanika, Orissa
	B. gymnorrhiza (L.) Lamk. (*B. conjugata* Merr.)	True mangrove	Bhitarkanika, Orissa
	B. parviflora Wt. & Arn. ex Griff.	True mangrove	Bhitarkanika/Mahanadi Delta, Orissa
	B. sexangula (Lour.) Poir.	True mangrove	Bhitarkanika, Orissa
	Ceriops tagal (Perr.) C. B. Robinson (*C. candolleana* Arn.)	True mangrove	Bhitarkanika/Mahanadi Delta, Orissa.
	Kandelia candel (L.) Druce (*K. rheedii* Wt. & Arn.)	True mangrove	Bhitarkanika/Mahanadi Delta, Orissa.
	Rhizophora apiculata Bl.	True mangrove	Bhitarkanika, Orissa
	R. mucronata Lamk.	True mangrove	Bhitarkanika/Mahanadi Delta, Orissa
	R. stylosa Griff.	True mangrove	Andaman Islands
Lignotideae	*Cassipourea ceylanica* (Gaertn.) Alston (*Weihea ceylanica* Gaertn.)	Terrestrial/ non-mangrove	Badakuda Island, Chilika Lake, Orissa
	Carallia brachiata (Lour.) Merr. (*C. integerrima* DC.)	Terrestrial/non-mangrove	Bhubaneswar, Orissa

Genomic DNA isolation

Genomic DNA was isolated from freshly collected leaves using the CTAB (cetyl trimethyl ammonium bromide) 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/isoamyl-alcohol (25:24:1 v/v/v) and subsequently three washes with chloroform/isoamyl-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, paletted, dried *in vacuo* and dissolved in 10 mM Tris [tris(hydroxymethyl)aminomethane]/1 mM EDTA ($T_{10}E_1$) buffer. Quantification of DNA was accomplished by analyzing the purified DNA on 0.8% agarose gel alongside diluted uncut lambda DNA as standard. DNA was diluted in $T_{10}E_1$ buffer to the required concentration for different PCR techniques.

RAPD analysis

For RAPD analysis, PCR amplification of 25 ng of genomic DNA was carried out using 11 standard decamer oligonucleotide primers (Operon Tech., Alameda, CA, USA). The primers were OPM01, OPM06, OPM09, OPN04, OPN05, OPP01, OPP02, OPQ01, OPQ20, OPT07 and OPT08. Each amplification reaction mixture of 25 μl contained the template DNA, 2.5 μl of 10X assay buffer (100 mM Tris-Cl, pH 8.3, 0.5 M KCl and 0.01% gelatin), 1.5 mM $MgCl_2$, each of 200 μM dNTPs, 20 ng of primer and 0.5 U Taq DNA polymerase (Bangalore Genei Pvt. Ltd, Bangalore, India). The amplification was carried out in a thermal cycler (Perkin Elmer, Model 9600). The first cycle consisted of denaturation of the 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 next 42 cycles the period of denaturation was reduced to 1 min while the primer annealing and primer extension time remained the same as in the first cycle. The last cycle consisted of only primer extension (72 °C) for 7 min.

PCR products were separated on 1.5% agarose gel containing ethidium bromide solution (@0.5 μg/ml of gel solution). The size of the amplicons was determined using size standards (100 bp ladderplus or DNA ladder mix; MBI Fermentas, Graiciuno, Vilnius, Lithuania). DNA fragments were visualized under UV light and photographed. To test the reproducibility the reactions were repeated at least twice.

AFLP analysis

AFLP analysis was done following the modified method of Vos *et al.* (1995) and according to the protocol supplied with the 'AFLP Core Reagent

Kit' and the 'AFLP Starter Primer Kit' of Life Technologies. The amplified products were analyzed in pre-warmed 5% acrylamide electrophoresis gels. Gels were run at 55 W for approx. 2 h and then transferred to Whatman filter paper (No. 1) and dried under vacuum (BioRad, Model 583). AFLP products were revealed by exposure to X-ray films (Kodak-BioMax MR) for 4–7 d. The primer combinations used for the present study were EACT/MCAG, EACT/MCAT, EACT/MCTA, EAGC/MCTG and EAGC/ MCTT.

Data analysis

The banding patterns obtained from RAPD and AFLP autoradiographs were scored as present (1) or absent (0). All bands (polymorphic and monomorphic) were taken into account in similarity calculation to avoid over-estimation of the distance (Gherardi *et al.*, 1998). Jaccard's coefficient of similarity was measured and a dendrogram based on similarity coefficients was generated by using the un-weighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and the SAHN clustering. Similarity matrices obtained with RAPD primers and AFLP primers were compared with the combined similarity matrices using all the RAPD and AFLP primers using the Mantel test (Mantel, 1967). All analyses were done using the computer package NTSYS-PC (Rohlf, 1997).

Results

RAPD analysis

A total of 11 primers were used for RAPD analysis with the 11 members of Rhizophoraceae. This resulted in all the primers showing polymorphic bands. The total number of bands amplified was 187 and all were polymorphic either at generic or specific level.

The size of the amplicons amplified was from 150 to 3000 base pairs. Maximum loci were amplified with the primer OPQ20 (24) where as minimum amplification was observed with the primer OPM06 (12). Detailed RAPD banding pattern is represented in Table II.

AFLP analysis

The 11 members of Rhizophoraceae were analyzed with 5 AFLP primer combinations, all of which showed high degrees of polymorphism. A total number of 572 loci were amplified (114.4 loci per primer), out of which 5 loci were monomorphic and 118 loci were found to be species-specific. 563 (98.42%) were found with a polymorphic pattern among the members of Rhizophoraceae studied. The number of polymorphic bands per total number of primer combinations used was 112.6, on an average. Details of the banding pattern are given in Table III. A representation of AFLP pattern obtained for all genera with one of the primer combinations, EcoR1ACT/Mse1-CAG is shown in Fig. 1.

Genetic similarity

The genetic similarity index was calculated using Jaccard's coefficient among all the genera and species and the UPGMA method allowed the clustering of the genera and species (Fig. 2). Very similar clustering patterns were obtained with the results of RAPD, AFLP and both RAPD and AFLP combined together. All the clustering patterns showed grouping of the 9 mangrove members and the two non-mangroves to two different clusters. The spe-

Table II. Banding pattern as revealed from RAPD in Rhiziophoraceae.

Name of primer	Primer sequence	Total no. of loci amplified	Range of amplicons (bp)
OPM01	5′GTTGGTGGCT3′	16	3000–150
OPM06	5′CTGGGCAACT3′	12	2500–500
OPM09	5′GTCTTGCGGA3′	17	3000–400
OPN04	5′GACCGACCCA3′	14	4000–200
OPN05	5′ACTGAACGCC3′	13	2500–500
OPP01	5′GTAGCACTCC3′	20	4000–300
OPP02	5′TCGGCACGCA3′	16	3000–500
OPQ01	5′GGGACGATGG3′	20	3000–200
OPQ20	5′TCGCCCAGTC3′	24	4000–200
OPT07	5′GGCAGGCTGT3′	17	2500–250
OPT08	5′AACGGCGACA3′	18	2500–200

Table III. Banding pattern as revealed from AFLP in Rhizophoraceae.

Name of primer combination	Total no. of loci amplified	Monomorphic loci	Polymorphic loci	Unique loci
EACT/MCAG	160	2	158	31′
EACT/MCAT	118	1	117	23
EACT/MCTA	88	6	82	12
EAGC/MCTG	96	0	96	32
EAGC/MCTT	110	0	110	20
Total	572	9	563	118

cies of the same genus like *B. cylindrica*, *B. gymnorrhiza*, *B. parviflora* and *B. sexangula* as well as *R. apiculata*, *R. mucronata* and *R. stylosa* came under the same groups. However, the grouping of *C. tagal* and *K. candel* was different with RAPD markers and AFLP markers.

Correlation within the markers

A comparison of the data obtained with RAPD and AFLP was made with the combined data of AFLP and RAPD. The values of Mantel test correlation showed a good fit of the data using any of the marker types. The correlation value was 0.9304 between AFLP and RAPD, 0.9580 between RAPD and RAPD + AFLP and 0.9962 between AFLP and RAPD + AFLP. These data indicate that the genetic index obtained with each of the marker types in each of the genus/species shared a good correlation, and thus the results are comparable.

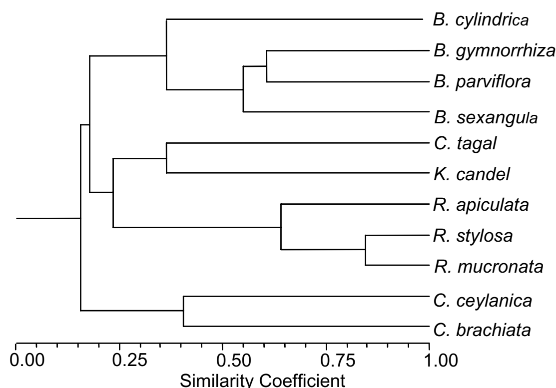

Fig. 2. Dendrograms obtained on the eleven members of Rhizophoraceae with RAPDs, AFLPs and RAPDs + AFLPs.

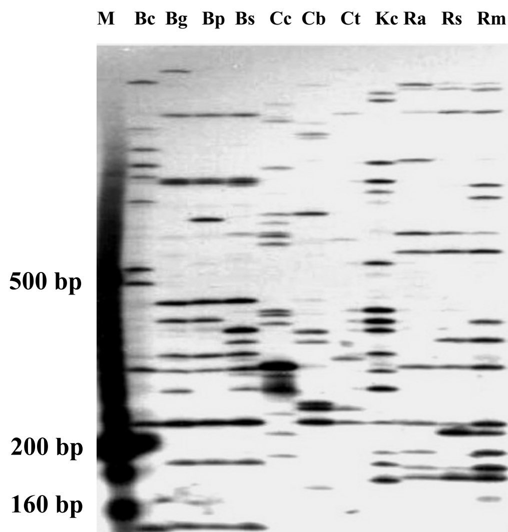

Fig. 1. A part of the AFLP autoradiogram obtained by the primer combination EACT/MCAG; M, 20bp ladder; Bc, *Bruguiera cylindrica*; Bg, *B. gymnorrhiza*; Bp, *B. parviflora*; Bs, *B. sexangula*; Cc, *Cassipourea ceylanica*; Cb, *Carallia brachiata*; Ct, *Ceriops tagal*; Kc, *Kandelia candel*; Ra, *Rhizophora apiculata*; Rs, *R. stylosa*; Rm, *R. mucronata*.

Discussion

Rhizophoraceae, a small pan tropical family of 15 genera and about 120 species is distributed mainly in the Old World (Mabberley, 1997). Henslow (1879) recognized three tribes under Rhizophoraceae. While all the four-mangrove genera *viz. Rhizophora*, *Ceriops*, *Kandelia* and *Bruguiera* were placed under the tribe Rhizophoreae, the genera *Carallia*, *Cassipourea* and *Blepharistemma* along with other predominantly terrestrial genera were grouped under the tribe Lignotideae. Of the 40 species of true mangroves, 30 species are known to occur in the Indian sub-continent.

Eleven members of Rhizophoraceae from different genus, species and ecological habitats were

Table IV. Jaccard's similarity coefficient derived from AFLP and RAPD pooled data.

	B. cylindrica	*B. gymnorrhiza*	*B. parviflora*	*B. sexangula*	*C. ceylanica*	*C. brachiata*	*C. tagal*	*K. candel*	*R. apiculata*	*R. stylosa*	*R. mucronata*
B. cylindrica	100.00										
B. gymnorrhiza	41.67	100.00									
B. parviflora	41.08	63.02	100.00								
B. sexangula	39.68	56.03	53.50	100.00							
C. ceylanica	13.90	16.79	15.23	19.85	100.00						
C. brachiata	15.69	16.63	15.38	19.66	41.19	100.00					
C. tagal	18.93	21.41	17.63	19.89	13.87	20.65	100.00				
K. candel	18.18	24.35	23.55	24.14	13.66	12.77	32.50	100.00			
R. apiculata	20.26	22.05	17.86	21.71	17.84	14.99	24.59	29.30	100.00		
R. stylosa	20.85	21.80	19.40	22.89	17.38	15.87	22.65	26.53	63.41	100.00	
R. mucronata	23.83	22.90	19.55	24.49	18.54	16.19	25.00	29.51	66.88	83.16	100.00

fingerprinted using RAPD and AFLP markers. The genetic similarity values obtained with both the markers were broadly similar separating the two non-mangrove genera from the rest of the 9 mangrove genera. In both the marker systems species of the same genus like *Bruguiera cylindrica*, *B. gymnorrhiza*, *B. parviflora* and *B. sexangula* and *Rhizophora apiculata*, *R. mucronata* and *R. stylosa* came under the same sub-cluster justifying their inclusion under the same generic name. From Jaccard's similarity (Table IV) it was revealed that among the taxa studied, *R. mucronata* and *R. stylosa* are most closely related being similar at 83.16% level and *Kandelia candel* and *Carallia brachiata* are widely separated with similarity of just 12.77%. Taxonomists have divided the four *Bruguiera* species into two groups, *B. gymnorrhiza* and *B. sexangula* under one group and *B. cylindrica* and *B. parviflora* in the other on the basis of morphological and ecological characteristics. However, in the present study it was observed that *B. cylindrica* is separated out from the rest of the species. It was interesting that in the dendrogram (Fig. 2) *B. gymnorrhiza* and *B. parviflora* share the same node instead of *B. sexangula* and *B. gymnorrhiza* as treated by the conventional taxonomists. Our findings conform the earlier observation of Abeysinghe *et al.* (2000), which shows that *B. sexangula* and *B. gymnorrhiza* are completely different entities without the possibility of interspecific hybridization. Our observation also supported the observation of Ding Hou (1958) who reported that such grouping did not exist when two new species were added to the genus.

Since introduction (Williams *et al.*, 1990), the RAPD marker is being extensively used for measuring genetic relationship in many plant species. The easiness and low expenses of the method, which only requires PCR techniques, has led the researchers to use it for different types of studies like inter- and intra-specific genetic variability among and between plant species (Mailer *et al.*, 1994; Khan *et al.*, 2000; Parani *et al.*, 1998; Mengistu *et al.*, 2000; Maki and Horie, 1999). In an earlier report, working with 22 species of mangroves, Parani *et al.* (1998) obtained 20.22 bands per primer. Analysing 3 species of *Rhizophora* including a natural hybrid (*Rhizophora* × *lamarkii*), Parani *et al.* (1997) got 5.08 bands per primer. However, we observed 17 bands per primer. This variation in banding pattern is due to the selection of plant samples as well as primers. In the present study, 11 members of Rhizophoraceae from six different genera were included and also the primers were selected after screening 50 primers in two species of *Rhizophora.* The best 11 primers were selected for the present study producing polymorphic bands.

We introduced AFLP markers for the first time to measure the genetic variability among different species/genera of Rhizophoraceae, which inhabit a very complex ecosystem. The UPGMA analysis gave similar results with the RAPD, AFLP and the combined markers. The clusters were similar and two main groups were found as expected: one

of them contained nine true mangroves and the other one had two non-mangroves. Species having similar physio-chemical, physiological and structural similarities are a result of convergent evolution (Yanney-Ewusine, 1980) form the mangrove ecosystem. This community has a distinct commonality with respect to characteristics like tolerance to salinity, submergence and susceptibility to frost and low temperature. Therefore it was expected that all mangroves would come under the same cluster. The dendrogram using the RAPD markers showed that the *Rhizophora* and *Bruguiera* are very closely related. The genera *Ceriops* and *Kandelia* are very close to *Rhizophora* in morphological characteristics (Banerjee and Rao, 1990). The five AFLP markers not only separated the *Bruguiera* and *Rhizophora* but also brought the *Ceriops* and *Kandelia* very close under a same group. Both the genera formed a group along with the *Rhizophora* spp. as expected because they exhibit morphological similarity and inhabit the same type of habitats (Banerjee and Rao, 1990). The separation of non-mangroves from their mangrove representatives suggests that there may be a different evolutionary process for the mangroves, which may have enabled them to withstand the high salinity and low biological oxygen demand. Both the non-mangrove genera *Carallia* and *Cassipourea* occur wild in the inland forest, share common morphological features and came under one sub-cluster. Analyses of RAPD and AFLP data also show the segregation of both the taxa under a distinct cluster maintaining their taxonomic entity at the level of tribe. These findings justify their placement under the tribe Lignotideae away from Rhizophoreae as observed by Henslow (1879). The four species of *Bruguiera* (*B. cylindrica, B. gymnorrhiza, B. parviflora* and *B. sexangula*) and the three species of *Rhizophra (R. apiculata, R. mucronata* and *R. stylosa*) formed two distinct subclusters justifying their generic status. However, the genetic relationship between *K. candel* and *C. tagal* as determined by RAPD and AFLP markers were different. After pooling the RAPD and AFLP data together it was revealed that *Rhizophora* shared a node at a 25% level of similarity and appeared closer to *Ceriops* and *Kandelia* than *Bruguiera.* In the present study *Ceriops* and *Kandelia* shared a node at a 33% level justifying their generic circumscription to form a cluster with *Rhizophora* at a 27% level, of similarity.

From the dendrogram constructed from the AFLP and RAPD pooled data, it could be observed that among the three species of *Rhizophora*, *R. apiculata* is quite distinct from *R. mucronata* and *R. stylosa* sharing a node at a 78% level of similarity. The inter-specific relationship as revealed by the pooled data does not show unanimity with the previous observation for *Bruguiera.* But the inter-specific relation among the species of *Bruguiera* is not stable when all the species are included and when few species are studied separately. AFLP marker separated the genus *Bruguiera* and *Rhizophora* and brought the genus *Ceriops* and *Kandelia* closer to *Rhizophora.* Further study may be helpful to understand the evolutionary process and phylogeny of this specialized group of plants.

Acknowledgements

The authors are thankful to Dr. U. C. Basak of Regional Plant Resource Centre, Bhubaneswar, Orissa, India for providing some of the plant materials and also to the Department of Environment and Forest Government of Orissa for assisting financially to conduct the present research.

Abeysinghe P. D., Triest L., DeGreef B., Koedam N., and Hettiarachi S. (2000), Genetic and geographic variation of the mangrove tree *Bruguiera* in Sri Lanka. Aquatic Bot. **67**, 131–141.

Banerjee L. K., and Rao T. A. (1990), Mangroves of Orissa Coast and Their Ecology. Bishen Singh Mahendra Pal Singh Publishers, Dehra Dun, India, p. 118.

Chalmers K. J., Newton A. C., Waugh R., and Powell W. (1994), Evaluation of the extent of genetic variation in mahoganies (*Meliaceae*) using RAPD markers. Theor. Appl. Genet. **89**, 504–508.

Ding Hou (1958), Rhizophoraceae. In: Flora Malesiana **1** (van Steenis C. G. G. J.. ed.). Martinus Nijhoff Publishers, Dordrecht, The Netherlands, pp. 429–493.

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.

Gherardi M., Mangin B., Goffinet B., Bonnet D., and Huguet T. (1998), A method to measure genetic distance between allogamous populations of alfalfa (*Medicago sativa*) using RAPD molecular markers. Theor. Appl. Genet. **96**, 406–412.

Henslow G. (1879), Rhizophoraceae. In: Flora of British India, Vol. 2 (Hooker J. D., ed.). L. Reeve & Company, London, pp. 435–443.

Khan S. A., Hussain D., Askari E., McStewart D., Malik K. A., and Zafar Y. (2000), Molecular phylogeny of *Gossipium* species by DNA fingerprinting. Theor. Appl. Genet. **101**, 931–938.

Lakshmi M., Parani M., Ram N., and Parida A. (2002), Molecular Phylogeny Mangroves IX: Molecular marker assisted intra-specific variation and species relationship in the Indian mangrove tribe Rhizophorae. Aquatic Bot. **74**, 201–207.

Lin J. J., Kuo J., Ma J., Saunders J. A., Beard H. S., MacDonald M. H., Kenworthy W., Ude G. N., and Matthews B. F. (1996), Identification of molecular markers in soybean comparing RFLP, RAPD and AFLP DNA mapping techniques. Plant Mol. Biol. Rep. **14**, 156–169.

Mabberley D. J. (1997), The Plant Book, 2nd ed. Cambridge University Press, Cambridge, UK.

Mailer R. J., Scarth R., and Fristenski B. (1994), Discrimination among cultivars of rapeseed (*Brassica napus* L.) using DNA polymorphisms amplified from arbitrary primers. Theor. Appl. Genet. **87**, 697–704.

Maki M. and Horie S. (1999), Random amplified polymorphic DNA (RAPD) markers reveal less genetic variation in the endangered plant *Cerastium fischerianum* var. *molle* than in the widespread conspecific *C. fischerianum* var. *fischerianum* (Caryophyllaceae). Mol. Ecol. **8**, 145–150.

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

Mengistu L. W., Muller-Warrant G. W., and Barker R. E. (2000), Genetic diversity of *Poa annua* in Western Oregon grass seed crops. Theor Appl. Genet. **101**, 70–79.

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

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.

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

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

Parani M., Lakshmi M., Rajalakshmi S., Anuratha C. S., and Parida A. (1996), Genetic polymorphism in Indian mangrove species revealed through molecular markers. In: Recent Advances in Biotechnological Applications of Plant Tissue and Cell Culture (Ravishankar G. A. and Venkatraman L. V., eds.). Oxford and IBH Publishing Co Pvt Ltd, New Delhi and Calcutta, India, pp. 421–428.

Pejic I., Ajmone-Marsan P., Morgante M., Kozumplick V., Castiglioni P., Taramino G., and Motto M. (1998), Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs and AFLPs. Theor. Appl. Genet. **97**, 1248–1255.

Purba A. R., Noyer J. L., Baudouin L., Perrier X., Hamon S., and Lagoda P. J. L. (2000), A new aspect of genetic diversity of Indonesian oil palm (*Elaeis guineensis* Jacq.) revealed by isozyme and AFLP markers and its consequences for breeding. Theor. Appl. Genet. **101**, 956–961.

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., Jorenson R. A., and Allard R. W. (1984), Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA **81**, 8014–8018.

Sharma A., Sharma R., and Machii H. (2000), Assessment of genetic diversity in a *Morus* germplasm collection using fluorescence-based AFLP markers. Theor. Appl. Genet. **101**, 1049–1055.

Singh A., Negi M. S., Rajgopal J., Bhatia S., Tomar U. K., Srivastava P. S., and Lakshmikumaran M. (1999), Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theor. Appl. Genet. **99**, 272–279.

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

Tomilson P. B. (1986), The Botany of Mangroves. Cambridge University Press, Cambridge, UK.

Vos P., Hogers R., Bleeker M., Reijans M., van de Lee T., Hornes M., Fritjers 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 polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**, 6531–6535.

Yanney-Ewusine J. (1980), Elements of Tropical Ecology. Heinemann Educational Books.