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Random amplified polymorphic DNA (RAPD) finger prints evidencing high genetic variability among marine angiosperms of India

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*In India 14 seagrass species can be found with monospecific genera (*Enhalus, Thalassia *and* Syringodium*), Cymodocea with two species and* Halophila *and* Halodule *represented by more than two taxonomically complex species. Considering this, the present study was made to understand the level and pattern of genetic variability among these species collected from Tamilnadu coast, India. Random amplified polymorphic DNA (RAPD) analysis was used to evaluate the level of polymorphism existing between the species. Out of the 12 primers tested, 10 primers amplified 415 DNA fragments with an average of 41.5 fragments per primer. Of the total 415 amplified fragments only 123 (29.7%) were monomorphic and the remaining 292 (70.3%) were polymorphic for Indian seagrass species. Among the 10 primers used four are identified as the key primers capable of distinguishing all the Indian seagrasses with a high degree of polymorphism and bringing representative polymorphic alleles in all the tested seagrasses. From the present investigation, this study shows that the RAPD marker technique can be used not only as a tool to analyse genetic diversity but also to resolve the taxonomic uncertainties existing in the Indian seagrasses. The efficiency of these primers in bringing out the genetic polymorphism or homogeneity among different populations of the* Halophila *and* Halodule *complex still has to be tested before recommending these primers as an identification tool for Indian seagrasses.*

Keywords: Marine angiosperm, genetic variability, RAPD, India

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INTRODUCTION

The PCR-based RAPD marker technique is widely used for distinguishing genetic variation of populations at the individual level since it detects mutations in coding and non-coding regions of genomes (Kirsten *et al.*, 1998), genetic mapping (Chalmers *et al.*, 2001) and for identification of markers linked with desired traits (Bai *et al.*, 2003). Assessment of the genetic variability of seagrasses has been attempted using a variety of techniques including isozyme analyses (McMillan & Williams, 1980; McMillan, 1981, 1982; Laushman, 1993; Capiomont *et al.*, 1996; Reusch, 2001), RAPD analysis (De Heij & Nienhuis, 1992; Kirsten *et al.*, 1998; Procaccini *et al.*, 1999; Angel, 2002; Jover *et al.*, 2003; Micheli *et al.*, 2005), AFLP (Waycott & Barnes, 2001), and microsatellites (Randall *et al.*, 1994; Davis *et al.*, 1999; Reusch, 2002; Reynolds *et al.*, 2012). Isozyme analysis revealed only low diversity even over large geographic distances as it is less sensitive to genomic changes than the DNA markers, because they sample functional enzymes in coding regions that are relatively conserved evolutionarily (Nei, 1987); whereas DNA fingerprinting provides better resolution of genetic relationships as it assesses more loci than directly assaying the genome (Avise, 1994). RAPD, microsatellites and AFLP typically show higher levels of genetic variation than isozymes and are generally inexpensive to implement and require significantly less development time than most other methods for detecting genetic variation. Co-dominant nature and ensuring reproducibility (Clark & Lanigan, 1993; Lambert & Millar, 1995; Grosberg *et al.*, 1996) are the only limitations of RAPD techniques.

RAPD markers have been successfully used to assess the pattern of genetic diversity and the genetic structure of rare plants and help in the conservation of endangered plants (Dong *et al.*, 2010). The technique also helps track the long-term evolutionary history of species (shifts in distribution, habitat fragmentation and population isolation), mutation, genetic drift, mating system, gene flow and selection (Schaal *et al.*, 1998). Patterns of genetic diversity in seagrasses are also influenced by other factors such as the spatial structure, age and maturity of the meadows, which affect the recruitment potential of seedlings, pollen and vegetative propagules. Despite all these points, RAPD has been successfully used to assess genetic diversity of seagrasses, this includes intra-population variability in *Posidonia australis* (Waycott,

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Table 1. Geographic location of the study areas.

S. No.	Station name	Geographic location	Seagrass species
1	Marakkaanam	12°13′96.7″N 79°58′77.2″E	*Halophila ovalis* subsp. *ramamurthiana*
2	Parangipettai	11°29′30.5″N 79°46′30.6″E	*Halophila beccarii* Asch.
3	Mallipattinam	10°15′76.1″N 79°19′24.1″E	*Cymodocea serrulata* (R. Br.) Asch. & Magnus
4	Manora	10°15′93.3″N 79°18′60.3″E	*Halodule pinifolia* (Miki) Hartog., *Syringodium isoetifolium* (Asch.) Dandy
5	Sambaipattinam	10°12′79.0″N 79°16′95.2″E	*Enhalus acoroides* (L.F.) Royle., *Halodule wrightii* Asch.
6	Kattumaavadi	10°07′79.5″N 79°14′93.4″E	*Thalassia hemprichii* (Ehrenb.) Asch. *Halophila ovata* Gaud., *Halophila decipiens* Ostenf., *Halodule uninervis* (Forsk.) Asch.
7	Paasipattinam	9°48′30″N 79°04′71″E	*Halophila ovalis* (R. Br.) Hook. *f.*, *Halophila stipulacea* (Forsk) Asch.
8	Chinnapaalam	9°16′14.5″N 79°12′55.2°E	*Cymodocea rotundata* Ehrenb. & Hempr.ex. Asch.

1998), high genetic homogeneity in *Cymodocea nodosa* from the Northern Atlantic (Alberto *et al.*, 2001), genetic diversity of *Cymodocea nodosa* and *P. oceanica* populations in the Mediterranean Sea (Procaccini & Mazzella, 1996), low degree of gene flow between populations in *Zostera muelleri* (Jones *et al.*, 2008) and *P. oceanica* from Santa Marinella meadow (Rotini *et al.*, 2011) among others. Furthermore, RAPD markers revealed a decreased genetic diversity in *P. oceanica* along the anthropogenic disturbance gradient, both on a small scale within a meadow and on a large scale in the Mediterranean Sea (Micheli *et al.*, 2005). This gives an indication that these techniques can also be used to assess the health of the seagrass beds at any given time.

Reports are available on the use of molecular markers such as RFLP, AFLP and microsatellites to estimate the genetic diversity of seagrasses, however, these are co-dominant markers, and analyse one locus at a time. RAPD markers are one of the most popular approaches (Martin & Hernandez, 2000) and dominant markers can amplify many loci (Chalmers *et al.*, 2001) at a time. Although the technique was largely used to uncover genetic diversity among populations, gene flow from different populations, etc., researchers alternatively used this technique to investigate the genetic diversity of a single population of different genera. Lucas *et al.* (2012) is the only gene-based study available on Indian seagrasses to date. Studies on Orchidaceae (Lim *et al.*, 1999), Verbenaceae (Viccini *et al.*, 2004) *Musa* spp. (Das *et al.*, 2009), Apocynaceae (Mahmood *et al.*, 2011) and Cyprinidae (Callejas *et al.*, 2004) are the few classic examples. Considering the non-availability of RAPD datasets for Indian seagrasses, the present study aimed to discriminate the genetic variability among the Indian seagrasses by using the RAPD technique and to standardize the technique for population level studies in future.

MATERIALS AND METHODS

Collection and selection of taxa

Young seagrass (*Enhalus acoroides, Thalassia hemprichii, Halophila ovalis, H. ovalis* subsp. *ramamurthiana, H. ovata, H. decipiens, H. stipulacea, H. beccarii, Cymodocea serrulata, C. rotundata, Halodule pinifolia, H. uninervis, H. wrightii* and *Syringodium isoetifolium*) leaves devoid of any epiphytic overgrowth were collected from Tamilnadu coast. Care was taken to collect the samples from a single extension of the rhizome (Table 1). The identification and taxonomic status of the samples were confirmed using the keys for seagrasses (Den Hartog, 1970) and other local keys (Ramamurthy *et al.*, 1992; Kannan & Thangaradjou, 2006). Samples were washed thoroughly in seawater to remove debris and sediments and rinsed with double distilled water. Then the whole leaf material was cut and immersed in NaCl/CTAB solution (Storchova *et al.*, 2000) until the extraction of genomic DNA.

PCR analysis by using RAPD markers

Fresh leaves (100 mg) of seagrass sample were extracted for Genomic DNA by using Plant Genomic DNA Minispin kit (Chromous Biotech, Code: RKT07/08; Bangalore, India) according to the protocol supplied by the manufacturer. Twelve decamer RAPD primers of two series (A and K) obtained from Carl Roth GmbH, Karlsruhe, Germany, were used for DNA amplification (Table 2). The PCR conditions were optimized for DNA concentration, the PCR reaction was performed in a Mastercycler gradient PCR (Eppendorf, Germany) with Red dye PCR master mix (GeNei, Cat:105908; Bangalore) in a total volume of 1× concentration of 12.5 µl reaction mixture by adopting the following PCR

Table 2. List of primers selected from Kit A and K used for the study.

S. No.	Name of primer	Primer sequence
1	3003A	5′-CAG CAC CCA C-′3
2	3004A	5′-TCT GTG CTG G-′3
3	3006A	5′-AGC CAG CGA A-′3
4	3009A	5′-CAA ACG TCG G-′3
5	3010A	5′-GTT GCG ATC C-′3
6	5852A	5′-TGC CGA GCT G-′3
7	5854A	5′-AAT CGG GCT G-′3
8	5858A	5′-GTG ACG TAG G-′3
9	5860A	5′-GTG ATC GCA G-′3
10	8053K	5′-TGG CCC TCA C-′3
11	8055K	5′-AAG TGC GAC C-′3
12	8061K	5′-GTG TCG CGA G-′3

program: 5 min 94 °C, 40 cycles of 1 min at 94 °C, 1 min at 36 °C, 2 min at 72 °C, followed by 5 min of the final extension at 72 °C. The success of the PCR reactions was monitored by running 12.5 µl of PCR reaction on 1% agarose (Himedia, Mumbai) in 1× TAE buffer (40 mM Tris-acetate, 1 mM EDTA) gel (ethidium bromide concentration 0.1 µg per 1 ml of gel) with 50 V applied voltage. DNA bands on the gel were visualized under UV light using Royal Intas Gel1x imager gel doc (Brandenburg, Germany).

Data analysis of amplified profiles

The electropherograms were manually converted into binary matrices reflecting the presence (1) or absence (0) of resulting alleles. The matrices were assessed by FreeTree software, ver. 9.1 using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) construction method. The similarity coefficient was calculated according to Nei and Li (1979) and the pairwise genetic distance according to Nei (1978). On the basis of the genetic distance among individuals, calculated as the average taxonomic distance using the similarity for interval data routine and principal coordinates analysis (PCoA) was performed with the average taxonomic distance in NTSYSpc 2.11L software (Rohlf, 2000). The original matrix was resampled with 500 times bootstrap and a consensus tree was generated. All dendrograms were displayed and printed using TreeView software ver. 1.6 (Page, 1996). The percentage polymorphism was calculated by using the following statistics.

Per cent polymorphism = Number of polymorphic bands/ Total number of bands × 100

RESULTS

Seagrass species (14 belonging to six genera) from Tamilnadu coast, India were analysed using 12 RAPD primers, in order to select a set of RAPD primers which produces reliable and reproducible fingerprints for the Indian seagrasses. After the initial screening 10 primers were retained for assessment of the genetic diversity between the seagrass species while the primers 3004A and 3006A were left without any further analysis as they failed to show any significant genetic differences among different species. The reproducibility of all these markers was checked by performing at least two experiments per RAPD primer, in the event of inconsistency in results then the experiment was repeated again for consistency. The results from each RAPD product were assumed to represent a single locus and data were scored as the presence of these bands and compared with samples of different species. These 10 primers amplified 415 DNA fragments with an average of 41.5 fragments per primer. Of the total 415 amplified fragments only 123 (29.7%) were monomorphic and the remaining 292 (70.3%) were polymorphic (Table 3) in Indian seagrass species.

The maximum number of bands produced by a single primer 5852A was 62 ranging 500 to 2500 bp while the minimum of 25 bands with 500 to 2500 bp were amplified by the primer 8053K. The number of RAPD bands amplified by the remaining primers ranged between 32 and 55 fragments and alleles ranged from ~300 to 5000 bp in length (Figures 1 and 2). A high percentage of polymorphism was

Table 3. The total number of polymorphic bands recorded for each of the 14 seagrass species, using 10 RAPD primers.

No. of primer	Total no. of bands													Total bands	Polymorphic bands	Percentage of polymorphism (%)	Base pair range (bp)
	EA	TH	HOO	HO	HOR	HD	HS	HB	CS	CR	HP	HU	HW	SI			
3003A	1	2	4	5	2	4	2	1	3	3	2	3	3	2	37	33	89	700–2000
3009A	2	2	2	2	2	2	3	0	1	0	6	4	3	3	32	24	75	800–5000
3010A	3	3	4	1	7	5	4	4	2	3	3	2	4	4	49	40	81.6	700–3000
5852A	5	3	4	4	2	5	3	4	5	7	5	5	5	5	62	34	54.8	500–2500
5854A	5	2	4	7	6	3	4	2	5	5	2	4	4	2	55	28	50.9	300–2500
5858A	3	2	2	2	3	2	4	1	3	3	5	5	4	4	43	35	81.3	700–3000
5860A	3	0	3	4	2	3	2	2	4	5	2	3	4	4	41	25	60.9	300–3000
8053K	4	0	2	1	0	0	1	5	2	3	2	1	2	2	25	17	68	500–2500
8055K	2	1	3	4	1	3	2	2	3	3	3	2	4	1	34	28	82.3	700–2500
8061K	4	3	5	2	3	5	1	2	3	3	2	1	2	1	37	28	75.6	700–5000
Total bands	32	18	33	32	28	32	26	23	31	35	32	30	35	28	415	292	70.3	

EA, *Enhalus acoroides*; TH, *Thalassia hemprichii*; HOO, *Halophila ovalis* subsp. *ovalis*; HO, *Halophila ovata*; HOR, *Halophila ovalis* subsp. *ramamurthiana*; HD, *Halophila decipiens*; HB, *Halophila beccarii*; CS, *Cymodocea serrulata*; CR, *Cymodocea rotundata*; HP, *Halodule pinifolia*; HU, *Halodule uninervis*; HW, *Halodule wrightii*; SI, *Syringodium isoetifolium*.

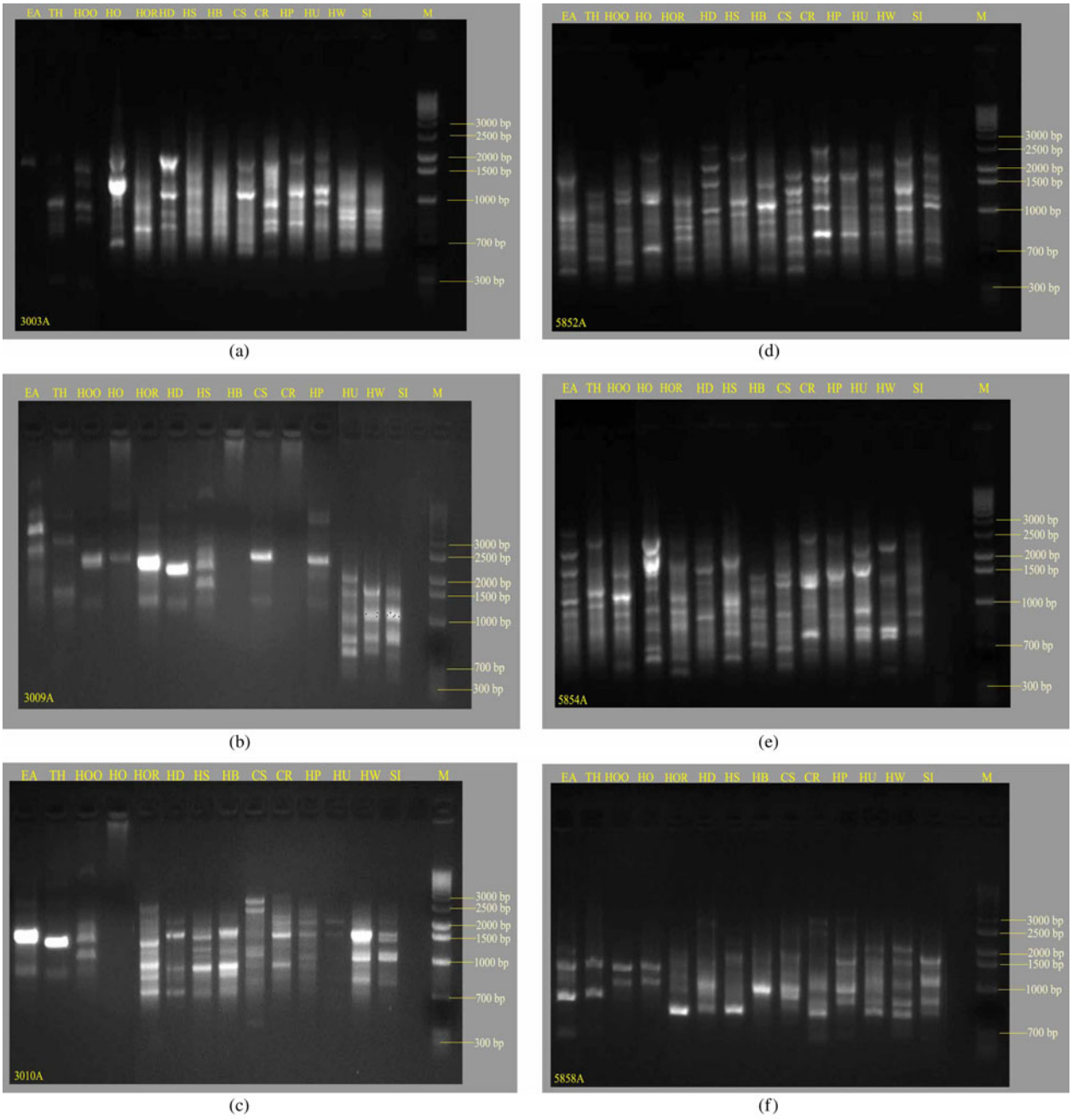

Fig. 1. The electropherogram of RAPD profiles for marine angiosperms of India using different primers: (A) 3003A, (B) 3009A, (C) 3010A, (D) 5852A, (E) 5854A, (F) 5858A.

observed with primers 3003A, 8055K, 3010A and 5858A with 89, 82.3, 81.6 and 81.3%, respectively.

The unbiased pairwise genetic distance showed that the lower values were obtained at intraspecies level of genera *Halophila, Cymodocea* and *Halodule* as 0.5, 0.66 and 0.61, respectively. However, higher genetic distances were observed between intergeneric levels (Table 4) for all seagrass genera. Species of *Halophila ovalis* subsp. *ovalis, H. ovata* and *H. ovalis* subsp. *ramamurthina* showing subtle morphological variations recorded a genetically distinct relation with a genetic distance of 0.584, 0.557 and 0.5, respectively.

The relationship among the different seagrass species was further subjected by PCoA which recorded the first three coordinates, accounting for 34.65, 23.9 and 19.72% of variance, with a total cumulative variance of 78.27%. In this analysis, the majority of groupings followed the same pattern as depicted in the dendrogram with minor differences such as the genus belonging to *Cymodocea* and *Halodule* also being clustered together in the PCoA. However, the PCoA (Figure 3) showed a separation of the individuals into scattered form and grouping of individual species belonging to the same genus grouped together except *H. beccarii*.

The UPGMA dendrogram (Figure 4) generated by following the Nei's distance matrix revealed clear genetic relationship among the 14 seagrass species. The rooted dendrogram clustered the species within distinct groups according to their genus specificity. Interestingly, the dendrogram formed two distinctive clusters corresponding to Cymodoceaceae

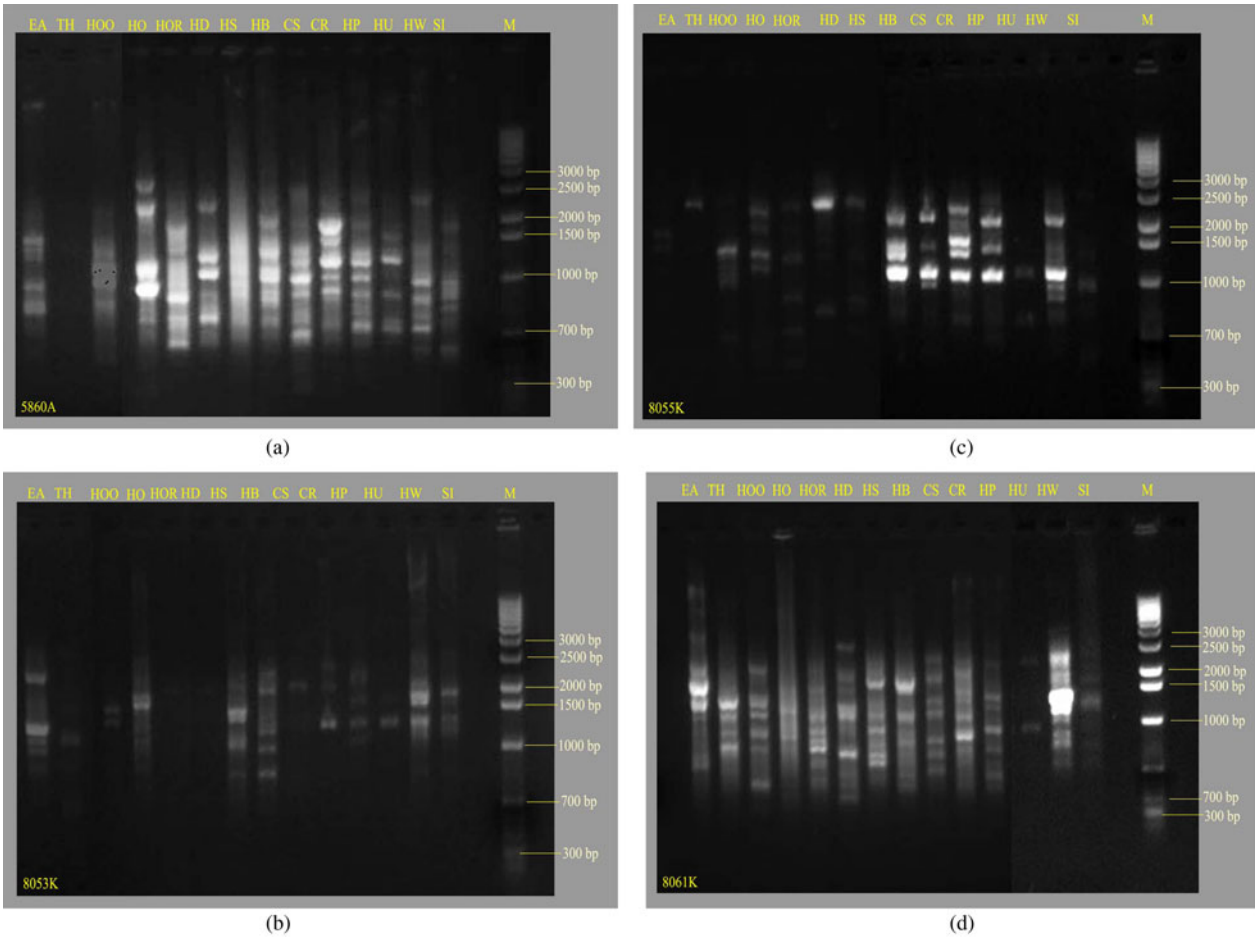

Fig. 2. The electropherogram of RAPD profiles for marine angiosperms of India using different primers: (A) 5860A, (B) 8053K, (C) 8055K, (D) 8061K.

and Hydrocharitaceae families. Cluster I divided into three subclusters viz. (1) *H. ovalis, H. ovata* and *H. ovalis* subsp. *ramamurthiana*, (2) *H. decipiens, H. beccarii* and *H. stipulacea* and (3) *E. acoroides* and *T. hemprichii*. Cluster II divided into two subclusters (1) *Cymodocea* and (2) *Halodule* and *Syringodium* and further subdivided up to species level.

DISCUSSION

Results herein represent the first successful application of RAPD markers to characterize the genetic variations among the Indian seagrasses, which provide important functions in shallow coastal ecosystems. The RAPD survey of 14 seagrass species (*Enhalus acoroides, Thalassia hemprichii, Halophila ovalis* subsp. *ovalis, H. ovalis* subsp. *ramamurthiana, H. ovata, H. decipiens, H. stipulacea, H. beccarii, Cymodocea serrulata, C. rotundata, Halodule pinifolia, H. uninervis, H. wrightii* and *Syringodium isoetifolium*) demonstrates that the seagrasses display a wide range of genetic diversity between genera and registers close similarity between intraspecies of genus *Halophila, Cymodocea* and *Halodule* which are critical in discriminating the species through conventional means.

Primers used to generate the RAPD profile for each seagrass recorded 70.3% polymorphic loci (292) confirming the uniqueness of genotypes of different seagrasses. Waycott (1995) used RAPD markers to determine genetic variation and clonality with the seagrass *Posidonia australis*, four primers identified 45 loci which includes 56% polymorphic and five RAPD primers used on Florida *T. testudinum* produced 29 polymorphic loci (Kirsten *et al.*, 1998). Micheli *et al.* (2005) have reported 80% of polymorphism within the population of a *P. oceanica* bed at Punta Mesco. However, in the present study some of the primers such as 3003A, 3010A and 5852A are capable of showing more than 30 polymorphic loci in Indian seagrasses. This confirms the efficiency of selected primers in distinguishing the genetic variability within Indian seagrasses.

Among the 10 primers used 3003A, 3010A, 5858A and 8055K are the key primers capable of distinguishing all the Indian seagrasses and proved to be quite powerful in detecting a high degree of polymorphism. However, the present study carried out with single populations of species warrants further tests with different populations so as to check the consistency of the primers in showing the polymorphism. Moreover, polymorphic variation was obtained from different species to show the genetic variability among different species. The polymorphic alleles recorded in all the tested species encourages further work with these primers to finalize the taxonomic tools.

Among the different primers used for the present study primers such as 5852A and 5854A are capable of showing more DNA bands (62 and 55 respectively) than the others. Unfortunately the percentage of polymorphism was very

Table 4. Nei's unbiased pair-wise genetic distance values recorded for 14 Indian seagrass species.

	EA	TH	HOO	HO	HOR	HD	HS	HB	CS	CR	HP	HU	HW	SI
EA	***	0.4	0.4	0.281	0.3	0.187	0.266	0.4	0.126	0.059	0.187	0.161	0.089	0.1
TH		***	0.392	0.28	0.304	0.16	0.173	0.097	0.122	0.075	0.12	0.125	0.075	0.086
HOO			***	0.584	0.557	0.276	0.262	0.321	0.062	0.117	0.123	0.126	0.088	0.065
HO				***	0.5	0.281	0.266	0.218	0.095	0.089	0.031	0.064	0.059	0.033
HOR					***	0.466	0.392	0.313	0.033	0.095	0.1	0.137	0.095	0.107
HD						***	0.5	0.436	0.063	0.059	0.125	0.161	0.179	0.133
HS							***	0.588	0.067	0.063	0.066	0.103	0.095	0.107
HB								***	0.111	0.068	0.072	0.037	0.034	0.039
CS									***	0.666	0.412	0.327	0.393	0.305
CR										***	0.507	0.553	0.457	0.444
HP											***	0.709	0.567	0.4
HU												***	0.615	0.448
HW													***	0.698
SI														***

low (54.8 and 50.9% respectively). Such primers are key to confirming the identity of Indian seagrasses in tracking the origin of the plant samples. Based on the performances of different primers in resolving the genetic differences in seagrasses, it is evident that during the growth stage, the different markers (Mariette *et al.*, 2002) show differing RAPD results for the same species; under these conditions selection of plants and experimental consistency are key in the successful application of RAPD techniques in seagrass taxonomy. The present result found 0.031–0.709 pairwise genetic variability among Indian seagrasses. Waycott & Barnes (2001) clearly stated that the genetic diversity of a seagrass population ranges from zero to high for different species and populations; this warrants the need for testing these primers at population level. Waycott & Barnes (2001) also emphasized that several studies to date are inadequately sampled and this often reduces the interpretability of the existing results. Hence in the present study the utmost care was taken for sampling, Samples were collected based on the following criteria, (1) should be from a single extension, (2) fresh young leaves in first three nodes only collected and (3) leaves attached with epiphytes were removed.

Throughout the world the systematic placement of the *Halophila* section has been based on morphology (Eiseman & McMillan, 1980) and isozyme banding (McMillan & Williams, 1980) characteristics. In India, the genus *Halophila* is represented by six species (*Halophila ovalis* subsp. *ovalis, H. ovalis* subsp. *ramamurthiana, H. ovata, H. decipiens, H. stipulacea* and *H. beccarii*). Subtle morphological and vegetative differences (number of cross veins, seeds, shape of the leaf lamina etc.) among *Halophila ovalis, H. ovalis* subsp. *ramamurthiana* and *H. ovata* make identification challenging and often results in misidentification. The present RAPD study showed clear genetic distance between the species (0.584, 0.557 and 0.5, respectively). Similarly, Smith *et al.* (1997) found a genetic similarity of 0.6 and 0.66 for *H. decipiens* and *H. johnsonii* which means a genetic distance of 0.4 and 0.44. This indicates that RAPD markers provide more reliable results than the morphological characters which are able to resolve the distinctions of closely related species (Pau & Othman, 2002). Further in depth studies of these three species with different populations may result in identification of the possible parent species of the subspecies *H. ovalis* subsp. *ramamurthiana* and also help to resolve the relatedness of the species with *H. ovalis* subsp. *ovalis* and *H. ovata.* Recently, Nguyen *et al.* (2013) studied Amplified Fragment Length Polymorphism (AFLP) among the subspecies of *H. ovalis* subsp. *ovalis, H. ovata* and *H. ovalis* subsp. *ramamurthiana* and they concluded that *H. ovalis* subsp. *ramamurthiana* was genetically different between species as well as geography. It is evident that the genetic structure of the plant populations could enable elucidation of the evolutionary linkages, mutation, genetic drift, mating system, gene flow etc. (Slatkin, 1987; Schaal *et al.*, 1998). Even the PCoA and UPGMA dendrogram showed similar pattern of variation between these three species. Other *Halophila* species have distinct vegetative characters, such as serrulate leaf margin and stiff hairs on abaxial side of the leaf (*H. decipiens*), serrulate leaf margin and sheathed leaf base (*H. stipulacea*) and more than 6 leaves per node (*H. beccarii*). The *Halophila* group showed a close similarity in DNA fingerprints among each other and clustered together in a single clade in the dendrogram. It confirms that the RAPD markers are capable of bringing out the interspecies differences within the genera. The PCoA plot also clearly distinguished these species into separate entities comparable to the UPGMA dendrogram.

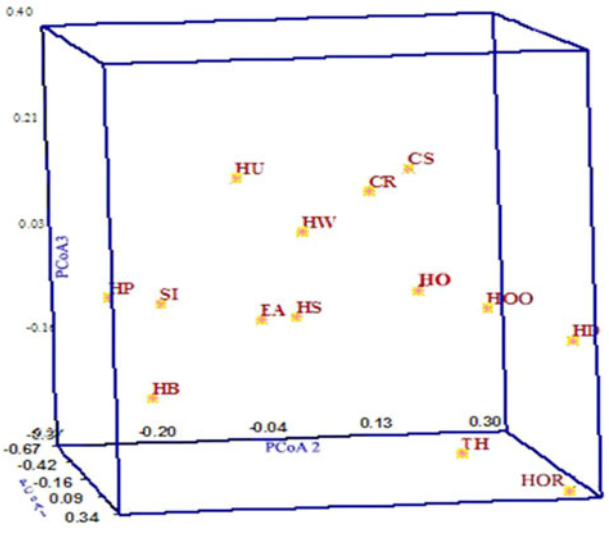

Fig. 3. Principal coordinate analysis of the 14 Indian seagrass species.

The genetic distance between *Enhalus acoroides* and *Thalassia hemprichii* was 0.4, the PCoA plot separated these

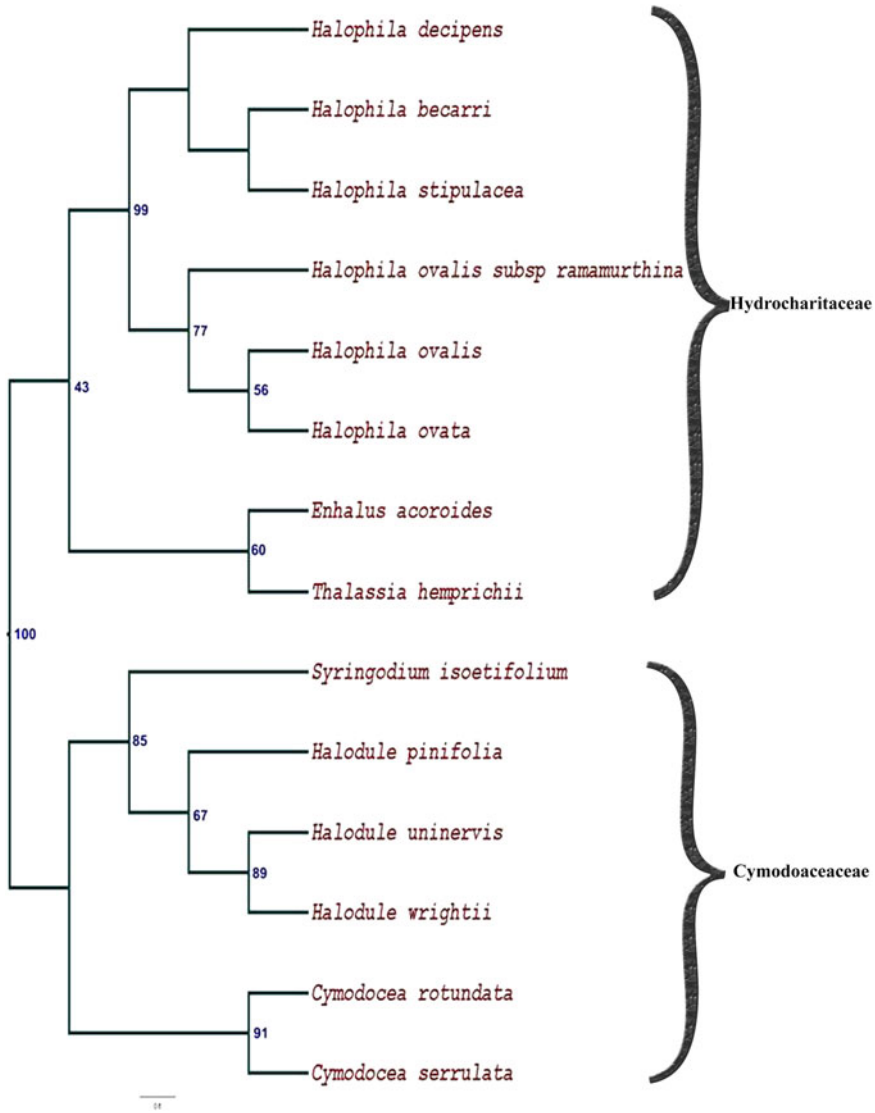

Fig. 4. Dendrogram showing genetic relationships among the 14 Indian seagrass species. The neighbour-joining method (FreeTreeware) was applied to an average 'taxonomic' distance matrix among the individuals.

two genera from each other, and the UPGMA dendrogram grouped these plants in one cluster indicating the relatedness of these two species. Isozyme studies of three Hydrocharitaceae genera also enable to distinguish these species, but *Enhalus* is more similar to *Thalassia* than to *Halophila* (McMillan, 1982). Even the morphological distinctness of *Halophila* having petiolate leaves is a clear character distinguishing the other two genera of the marine Hydrocharitaceae family which are apetiolate in nature.

The sympatric species *C. serrulata* and *C. rotundata* have a close similarity in DNA fingerprinting and shared a common node in UPGMA dendrogram with a genetic distance of 0.666. The heterogeneous RAPD pattern found between the *Cymodocea* species indicates that the leaf morphometrics such as difference in width of the leaf lamina (3–6 mm for *C. rotundata* and 4–10 mm for *C. serrulata*), serrulation in the apex (rarely serrulate in case of *C. rotundata* and serrulate in *C. serrulata*), number of nerves (9–14 for *C. rotundata* and 12–22 for *C. serrulata*) were associated with an increased genetic variation between the species. Alberto *et al.* (2001) also applied RAPD techniques in *C. nodosa* from six populations of the Atlantic sea using 28 random primers and found genetic homogeneity among *C. nodosa* populations of the Atlantic sea, which means the genetic characters of the same species of the same regions are more homogeneous in nature, but this is not common to all species of seagrasses. However, Rotini *et al.* (2011) reported that RAPD showed low genetic variability in *P. oceanica* from Santa Marinella seagrass meadows; whereas, Nguyen *et al.* (2014) analysed genetic diversity of seagrasses based on microsatellite markers and revealed that the genetic variability in the Western Pacific is higher than in the Eastern Indian ocean.

The genetic variation between the *Halodule* species of *H. pinifolia* (no lateral teeth), *H. uninervis* (tridentate leaf tip) and *H. wrightii* (bidentate leaf tip) conventionally can be identified by leaf tip morphology (Den Hartog, 1970) but the fragile nature of leaves means that they easily break away under natural conditions and can result in a taxonomic paradigm. The genetic distance of 0.709, 0.567 and 0.615 recorded for these species gives a clear clue for differentiating the species without any ambiguity.

The high level of similarity at intraspecies level has also shown that all the samples of each species are monophyletic. It can be inferred from the present investigation that the RAPD technique is a useful tool for the analysis of genetic diversity among the Indian seagrasses and can be used as the tool to resolve the taxonomic issues of Indian seagrasses. Primers 3003A, 3010A and 5852A are the key primers that can be used as a tool for identifying the genetic and species variations after testing the primers at different populations of the same species.

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