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Genetic variability in *Porphyra* C. Ag. along the coast of Maharashtra

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The coast of Maharashtra (India), between Mumbai in North and Redi in South covering 720 km stretch, was visited frequently during the monsoon period for the collection of *Porphyra*. Morphological observations on the collected material revealed existence of morphometric diversity of greater magnitude. These populations were, therefore, subjected to molecular analysis using RAPD markers to understand the extent of genetic variability. For the study, 17 morphotypes gathered from 12 locations were used. RAPD analysis was done using 60 primers, of which 24 showed reproducible results. The primers amplified a total of 480 fragments, of which 412 were polymorphic, indicating high frequency of polymorphism.

Keywords: Maharashtra, morphotype, *Porphyra*, RAPD, Rhodophyta

Porphyra, the red seaweed, is one of the most commercially important crops. In India, it is not yet systematically studied nor commercially cultivated. *Porphyra* grows abundantly during rainy season along the coast of Maharashtra, India. It appears that *Porphyra* is very difficult to identify due to tremendous morphological variability within and among species[1]. To overcome this problem, it is considered essential to assess the genotypic characters to see whether the variability in the given population is due to genetic components or environmental parameters. It will also confirm the species identified by classical taxonomy.

RAPD has been used in the identification and differentiation of many types of seaweeds, such as, *Ceramium*[2], *Hizikia*[3], *Sargassum*[4], *Chara*[5], *Ulva*[6] and *Gracilaria*[7]. Moreover, Dutcher and Kapraun[8] used RAPD markers for the identification of genetic variation in three species of *Porphyra.* Genetic diversity and phylogenetic relationships in *Porphyra* species have also been analysed using the same technique by Huh *et al*[9], Song *et al*[10] and Weng *et al* in 2005[11]. Since no such studies were carried out in Indian context, the present study was undertaken to assess the genetic variability in *Porphyra* along the coast of Maharashtra by using RAPD markers.

Specimens of *Porphyra* were collected from 17 different locations along the coast of Maharashtra, India and named accordingly (Table 1). Principle of random sampling was followed during the collections. The collections were carried out from July to September 2008-09. Care was taken that minimum 50 plants of *Porphyra* were collected during each visit.

DNA extraction was carried out by using live material of *Porphyra*. The samples collected from field were first washed with seawater then wrapped with cheese cloth and stored in deep freezer. At the time of extraction *Porphyra* blades were washed two

Table 1—Morphotypes of *Porphyra*

No.	Name based on location	Abbreviations
1	Malvan morph I	i
2	Malvan form II	l
3	Redi morph I	h
4	Redi morph II	o
5	Ratnagiri morph I	e
6	Ratnagiri morph II	m
7	Ratnagiri morph III	c
8	Vayangani morph	a
9	Nandivade form	b
10	Purnagad morph	d
11	Borya morph	j
12	Hedavi morph	p
13	Velaneshwar morph	k
14	Palshet morph	n
15	Panchanadi morph	f
16	Harnai morph	g
17	Shekhadi morph	Q

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to three times with tap water and then 2 to 3 times with autoclaved distilled water. DNA extraction was carried out by rapid extraction method[12] with few modifications. PCR amplification was performed as described by Williams *et al*[13] with a set of 20 oligonucleotides of OPA, OPD and OPC series synthesized by Operon Technologies. The reaction mixture (20 µL) contained the following ingredients. Taq buffer (10×) 2.5 µL, $MgCl_2$ (2.5 m*M*) 1 µL, dNTPs (2 m*M*) 1 µL, 10-mers oligo primers (5 *p*moles) 1 µL, DNA template 1.5 µL, Taq DNA polymerase (3 U) 0.33 µL and distilled water 12.67 µL. Each reaction was overlaid with 100 µL of mineral oil to prevent evaporation. The amplification was carried out in PCR machine Gene Amp. for 35 cycles with a program: 94°C for 5 min, ½ min at 94°C, ½ min at 37°C, ½ min at 72°C, followed by a final extension at 72°C for 5 min and final hold at 4°C. The PCR products were loaded on 1.2 % agarose gel for electrophoresis in 1× TAE buffer at 50 V for 3 h and stained with ethidium bromide. After the electrophoresis was completed the agarose gel were documented by the gel documented system of BIOVIS.

All the amplified bands were scored visually. Different fragments produced with each primer were numbered sequentially and the presence or absence of the band in each sample was recorded in binary matrix as 1 if present, or 0 if absent. The matrix was analyzed using MVSP software. The data were used to calculate similarity index using the formula of Nei and Li[14], which was then subjected to the cluster analysis using UPGMA (unweighted pair group method with arithmetic mean algorithm) and construction of dendrogram.

Of 60 oligonucleotide primers used for RAPD analysis, 24 primers yielded good amplification. Overall 480 fragments were generated; of which 412 fragments were polymorphic (85.333%) and 68 were monomorphic (14.666 %). The size of amplified fragments ranged between 281 to 2322 bp. The number of amplified fragments for each primer ranged from 3 to 49. The amplified DNA fragments were scored for computer analysis based on Nei and Li's similarity coefficient[14] and a dendrogram was constructed using MVSP software.

The similarity indices of all the genotype ranged from 0.304 to 0.800 (Table 2). The seventeen genotypes analyzed were divided in to two clusters. The first cluster comprises of two sub-clusters, sub-cluster I and II. The sub-cluster I consists of two morphotypes, namely, e (Ratnagiri morph I) and j (Borya morph) with a similarity coefficient 0.690; the sub-cluster II also comprises of two morphotypes, h (Redi morph I) and i (Malvan morph I) with a similarity coefficient 0.711. These two sub-clusters join with each other with a similarity coefficient 0.619 to form node 6. The morphotype a (Vayangani morph) joins to the sub-cluster I and sub-cluster II with a similarity coefficient 0.374 to form cluster I (Fig. 1).

The cluster II also comprises of two sub-clusters, sub-cluster III and sub-cluster IV. The sub-cluster III further consists of two groups, group I and group II. The group I is further divided into sub-group A and B. The sub-group A includes two morphotypes, namely, c (Ratnagiri morph III) and d (Purnagad morph) with the highest similarity coefficient 0.800. The morphotypes k (Velaneshwar morph) and f (Panchanadi morph) show 0.694 similarity coefficient and form sub-group B. Finally, sub-group A and B join with a similarity coefficient 0.645 to form node 5. The morphotype g (Harnai morph) joins to the node 5 with similarity coefficient 0.568 to form node 8; morphotype b (Nandivade morph) ultimately joins to node 8 with a similarity coefficient 0.431 and forms group I. The Group II consists of three morphotypes, namely, m (Ratnagiri morph II), p (Hedavi morph) and q (Shekhadi morph). Of which morphotype p joins to morphotype q with a similarity coefficient 0.565 and to these morphotypes joins to form node 9;

Table 2—Pair-wise comparison of 17 morphotypes of *Porphyra*

Node	Group 1	Group 2	Similarity index	Objects in group
1	c	d	0.800	2
2	h	i	0.711	2
3	f	k	0.694	2
4	e	j	0.690	2
5	Node 1	Node 3	0.645	4
6	Node 4	Node 2	0.619	4
7	l	o	0.579	2
8	Node 5	g	0.568	5
9	p	q	0.565	2
10	Node 7	n	0.562	3
11	b	Node 8	0.431	6
12	m	Node 9	0.416	3
13	Node 11	Node 12	0.406	9
14	a	Node 6	0.374	5
15	Node 13	Node 10	0.315	12
16	Node 14	Node 15	0.304	17

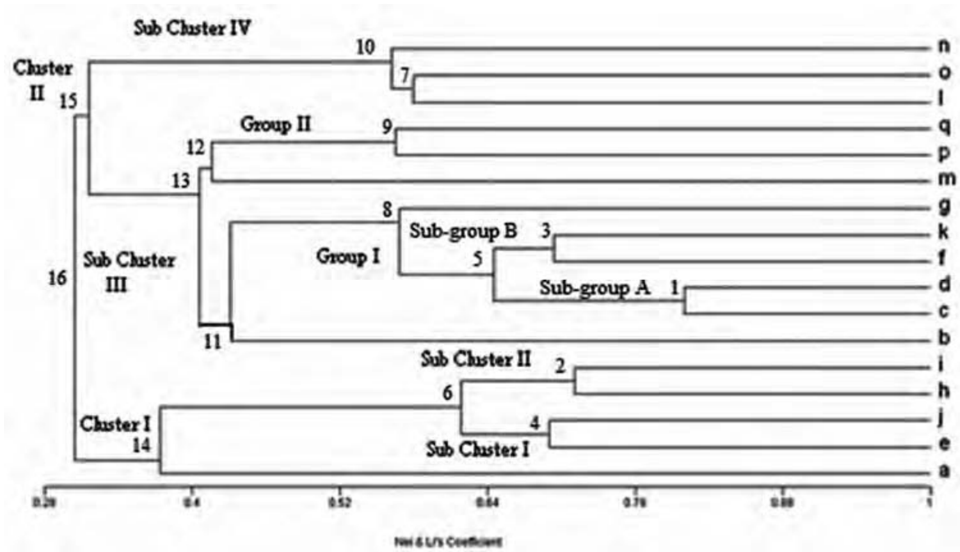

Fig. 1—Dendrogram showing relationship between 17 morphotypes of *Porphyra* using Nei and Lie's coefficient.

while morphotype m adds to node 9 with a similarity coefficient 0.416.

Finally the Group I and Group II form sub-cluster III with similarity coefficient 0.406. The sub-cluster IV comprises three morphotypes, l (Malvan morph II), o (Redi morph II) and n (Palshet morph). The morphotype l and o join with a similarity coefficient 0.579. To these morphotype n joins with a similarity index 0.562 and forms sub-cluster IV. The sub-cluster III and sub-cluster IV join with each other and show similarity index 0.315. Finally, the cluster I and cluster II are merged in a single cluster with the lowest similarity index of 0.304 (Fig. 1).

The present data support the studies of Song *et al*[10] where systematic relationships of four species of *Porphyra*, collected from different regions of the world, were analyzed. They found that the species living in same environmental conditions shared maximum similarity. The same results were observed in the present study. Morphotypes c and d shared maximum similarity coefficient (0.800). In addition, morphotype h and i, morphotype f and k, and morphotype e and j also showed maximum similarity coefficient of 0.711, 0.694 and 0.690, respectively. All these morphotypes belonged to the same environmental and geographical conditions. However, the morphotypes with similarity coefficient below 0.690 were from different environmental and geographical conditions.

The present study also shows that RAPD method proves to be a useful molecular tool for the phylogenetic analysis of the species.

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References

1 Lindstrom S C & Cole K M, An evaluation of species relationship in *Porphyra perforata* complex (Begiales, Rhodophyta) using starch gel electrophoresis, *Hydrobiologia*, 204/205 (1990) 179-183.

2 Ran K S, Yoon H S, Boo S M & Paik S G, Morphological and random amplified polymorphic DNA features in populations of *Ceramium kondoi* (Rhodophyta), *J Plant Biol*, 40 (1997) 27-32.

3 Park J W, Cho Y C, Nam B H, Jin H J, Sohn C H *et al*, RAPD identification of genetic variation in seaweed *Hizikia fusiformis* (Fucales, Phaeophyta), *J Mar Biotechnol*, 6 (1998) 62-64.

4 Wong C L, Gan S Y & Phang S M, Morphology and molecular characterization and differentiation of *Sargassum baccularia* and *S. polycystum* (Phaeophyta), *J Appl Phycol*, 16 (2004) 439-445.

5 Abrol D, Bhatnagar S & Kumar S, Molecular characterization of five species of the genus *Chara* (Division Charophyta) from India, *J Biol Res*, 6 (2006) 213-220.

6 Prasad U, Deshmukhe G, Dwivedi A & Singh S D, Detection of genetic variation in four *Ulva* species based on RAPD technique, *Indian J Mar Sci*, 38 (2009) 52-56.

7 Sim M C, Lim P E, Gan S Y & Phang S M, Identification of random amplified polymorphic DNA (RAPD) markers for

differentiating male from female and saprophytic thalli of *Gracilaria changii* (Rhodophyta), *J Appl Phycol*, 19 (2007) 763-769.

8 Dutcher J A & Kapraun D F, Random amplified polymorphic DNA identification of genetic variation in three species of *Porphyra* (Bangiales, Rhodophyta), *J Appl Phycol*, 6 (1994) 267-273.

9 Huh M K, Lee B K & Lee H Y, Genetic diversity and phylogenetic relationships in five *Porphyra* species reveled by RAPD analysis, *Protistology*, 4 (2006) 245-250.

10 Song L-S, Duan D-L, Li X-H & Li C-X, Use of RAPD for detecting and identifying *Porphyra* (Bangiales, Rhodophyta), *Chin J Oceanol Limnol*, 16 (1998) 237-242.

11 Weng M, Liu B, Jin D, Yang Q, Zhao G *et al*, Identification of 27 *Porphyra* lines (Rhodophyta) by DNA fingerprinting and molecular markers, *J Appl Phycol*, 17 (2005) 91-97.

12 Edwards K, Johnstone C & Thompson C, A simple and rapid method for the preparation of plant genomic DNA for PCR analysis, *Nucleic Acids Res*, 19 (1991) 1349.

13 Williams J G, KubeliK A R, Livak K J, Rafalski J A & Tingey S V, DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, *Nucl Acids Res*, 18 (1990) 6531-6535.

14 Nei M & Li W H, Mathematical model for studying genetic variation in terms of restriction endonucleases, *Proc Natl Acad Sci USA*, 76 (1979) 5269-5273.