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Genetic Differences and Variations in Two *Porphyra* Species (Bangiales, Rhodophyta)

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Genomic DNA isolated from two *Porphyra* species, *P. tenera* and *P. dentate* from Wando located on the southern coast of Korean peninsula was amplified by PCR reaction. The amplified products were separated by agarose gel electrophoresis (AGE) with decamer primer and stained with ethidium bromide. The eight arbitrarily selected primers OPA-04, OPA-06, OPB-01, OPB-08, OPB-10, OPB-11, OPB-14 and OPC-10 generated the shared loci, polymorphic, and specific loci. The size of DNA bands varies from 100 bp to 2,200 bp. The complexity of the banding patterns varies dramatically between the primers and two *Porphyra* species. A total of 528 loci observed were identified in *P. tenera* and 443 in *P. dentata*: 22 polymorphic loci (4.2%) in *P. tenera* and 30 (6.8%) in *P. dentata*. 154 shared loci observed, the average 19.3 per primer, were identified in *P. tenera* and 143 loci, the average 17.9 per primer, in *P. dentata* species. The number of specific loci in *P. tenera* and *P. dentata* was 73 and 77, respectively. The average bandsharing value was 0.623±0.008 within *P. tenera* and 0.560±0.009 within *P. dentata*. The average bandsharing value between two *Porphyra* species was 0.408±0.004, ranged from 0.305 to 0.564. The dendrogram obtained by the eight primers indicates four genetic clusters. The genetic distance between two *Porphyra* species ranged from 0.076 to 0.627. The individual no. 02 of *P. tenera* was genetically closely related to no. 01 of *P. tenera* (genetic distance=0.082). Especially, two entities between the individual DENTATA no. 21 and DENTATA no. 19 of *P. dentata* showed the longest genetic distance (0.627) in comparison with other individuals used. In this study, RAPD-PCR analysis has revealed the significant genetic distance between two *Porphyra* species pairs ($P<0.001$).

Keywords: Bandsharing value, Dendrogram, Genetic distance, *Porphyra dentata*, *Porphyra tenera*

Introduction

Numerous analytical techniques have been applied to analyze the heredity of organisms such as morphological trait (Orozco-Castillo et al., 1994; Adams, 2000), the allozyme differentiation (Bartish et al., 2000), and various PCR-based molecular techniques including the restriction fragment length polymorphisms (RFLPs) (Kim et al., 1997b; Jaiswal et al., 1998), the amplified fragment length polymorphisms (AFLPs) (Eujayl et al., 1998), and the random amplified polymorphic DNAs (RAPD) (Callejas and Ochando, 1998; Moeller and Schaal, 1999; Esselman et al., 2000; Kim et al., 2000; Yoon and Kim, 2003b; Kim et al., 2004).

Among them, RAPDs are the most frequently used molecular markers for taxonomic and systematic analyses of organisms (Adams, 2000; Bartish et al., 2000). RAPD results also showed that the RAPD technique could be used to identify markers for different cytoplasms used in cytoplasmic male-sterile of sorghum (Jaiswal et al., 1998). Particularly, the polymorphic and/or specific markers specific to the breed, the species, the genus or the geographical population have been applied for the identification of individuals and population, hybrid parentage and for the screening of DNA markers for the marker-assisted selection (MAS) and the genotype-assisted selection (GAS) (Guo et al., 2001). Till now, polymorphic bands generated by RAPD-PCR using arbitrary primers had good merits for detecting DNA similarity and diversity between life organisms (Nebauer et al., 2000; Yoon and Kim, 2001). Generally, RAPD-PCR is one of fast and simple research methods to identify genetic difference and the polymorphism in various organisms, which does not require the prior knowledge of the genomic DNA (Iyengar et al., 2000; Nicolosi et al., 2000).

Porphyra tenera (Kjellman) is an economically important aquacultural species belonging to the family Bangiaceae. *Porphyra dentata* (Kjellman) is only cultivated along the

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western to south coasts of Korea. This *Porphyra* is widely distributed in the western sea, southern sea, and Jeju Island in the Korean Peninsula (Lee and Kang, 2001) as well as in Japan. Dried *Porphyra* has become an important part of the diet of the Korean. The dried, rectangular paper-like sheet of *Porphyra* is the most commonly eaten alga in Korea. Nowadays, *Porphyra* is one of the most delicious algal species among the algae during the four seasons. Especially, the consumption of this alga has increased considerably in home and restaurants specializing in serving food in various ways, such as boiled rice with assorted mixtures such as red pepper spices, vegetables, fried egg, beef and/or sliced raw fish etc. The environmental requirements and the tolerances of the *Porphyra* grown in different geographic sites are not known, as is identification of the species. The color, size and the shape of the *Porphyra* blade vary according to their habitat such as the depth of the water, the salinity, the temperature, the photoperiod and the nutrition etc.

As the *Porphyra* cultivation industry is increasing considerably, the understanding of the genetics of this alga species to evaluate exactly the patent genetic effects induced by *Porphyra* production operations. However, little information is known about the genetics of *Porphyra* in Korea. Particularly, the clustering analysis of the genetic distance between genera/species/populations of various fishes and invertebrates from the different geographic sites has been performed using RAPD-PCR (Tassanakajon et al., 1998; Kim et al., 2000; Klinbunga et al., 2000b; Yoon and Park, 2002). The genetic variation, the species-specific markers and the region-specific markers in seaweeds/marine algae/marine plants have been assessed by molecular biological methods (Hong et al., 1997; Kim et al., 1997a; Kim et al., 1997b).

Here, to elucidate the genetic distances and the differences in two species, *P. tenera* and *P. dentata*, we performed the clustering analysis and the genetic diversity.

Materials and Methods

Sample collection and extraction of genomic DNA

Porphyra tenera and *P. dentata* were obtained from Wando located in the southern coast of Korean peninsula. The conchocelis of them cultured in our laboratory was collected, placed in the sterile tubes on ice immediately, and stored at -40°C until needed. The RAPD-PCR analysis was performed on the conchocelis extract of 22 individuals using eight arbitrarily selected primers of different decamer primers. The extraction of genomic DNA was performed under conditions as described (Yoon and Kim, 2003b). After several washing, the lysis buffer I (155 mM NH_4Cl; 10 mM $KHCO_3$; 1 mM EDTA) was added to samples, and the mixture tubes were gently inverted. The precipitates obtained were centrifuged and resuspended with lysis buffer II (10 mM Tris-HCl, pH 8.0; 10 mM EDTA; 100 mM NaCl; 0.5% SDS) and added 15 μl proteinase K solution (10 mg/ml). After incubation, there was added 300 μl of 3 M NaCl and gently pipetted for a few of min. Added not phenol, 600 μl of chloroform were added to the mixture and then inverted. DNA from the lysates was extracted by adding ice-cold 70% ethanol and centrifuged at 19,621 g for 5 min. The DNA pellets were incubation-dried for more than 2 hrs, held at -40°C until analysis and then dissolved in the pure water (JABA KOREA, Korea). The concentration of the extracted genomic DNA was measured with the absorbance ratio at 260 nm by a spectrophotometer (Shimadzu, Australia; Beckman DU 600 series, UK).

The oligonucleotides decamer primers, the molecular markers and the amplification stipulation

The decamer primer chosen arbitrarily were purchased from Operon Technologies, USA. The G+C content of the primers is between 60~70%. Eight primers, OPA-04 (5'-AATCGGGCTG-3'), OPA-06 (5'-TGGTCCCTGAC-3'), OPB-01 (5'-GTTTCGCTCC-3'), OPB-08 (5'-GTCCACACGG-3'), OPB-10 (5'-CTGCTGGGAC-3'), OPB-11 (5'-GTAGACCCGT-3'), OPB-14 (5'-TCCGCTCTGG-3') and OPC-10 (5'-TGTCTGGGTG-3') were shown to generate the shared loci, polymorphic loci and specific loci that can be scored clearly and reproducibly. We thus used the primers to identify the genetic polymorphism, diversity and similarity of two *Porphyra* species. RAPD-PCR was performed using two Programmable DNA Thermal Cyclers (Perkin Elmer Cetus, USA; MJ Research, Inc., USA). The DNA amplification was performed in 25 μl containing 10 ng of template DNA, 20 μl premix (Super-Bio Co., Korea) and the 1.0 unit primer. Amplification products were separated by 1.4% agarose (VentechBio, Korea) gel electrophoresis with TBE (90 mM Tris, pH 8.5; 90 mM borate; 2.5 mM EDTA). The 100 bp DNA Ladder (Bioneer Co., Korea) was used as DNA molecular weight marker. The agarose gels electrophoresed were stained with ethidium bromide. The bands were illuminated with ultraviolet ray and then photographed by photoman direct copy system (PECA products, USA).

The data analysis

Only the bands ranged from 100 bp to 2,200 bp that were readily visible were scored for the statistical analysis. The primers that generate minor bands were excluded from the analysis. The bandsharing (BS) value was calculated by the presence/absence of amplified products at the specific positions in the same gel from the RAPD profiles. The values were calculated according to Nei (1987) and Jeffreys and Morton (1987). Comparing two lanes, the BS was calculated as follows:

BS=2 (Nab)/(Na+Nb).

Nab: the number of bands shared by the samples b and a

Na: the total number of bands in a

Nb: the total number of bands in the sample b.

The average of within-species similarity is calculated by the pairwise comparison between individuals within a population. The relatedness among different individuals of *P. tenera* of Wando (TENERA 01~TENERA 11) and *P. dentata* of Wando (DENTATA 12~DENTATA 22) generated according to the bandsharing values and similarity matrix. The hierarchical clustering tree was analyzed by the similarity matrices to generate a dendrogram using pc-package program Systat version 10 (SPSS Inc., Chicago, IL, USA). The genetic difference and Euclidean genetic distance within- and between-species were calculated with hierarchical dendrogram program Systat version 10. All data were expressed as mean±SE. Significance was accepted for all tests at $P<0.001$.

Results and Discussion

The variation within- and between-species, bandsharing values, and the genetic distances

In spite of the variation in the RAPD profiles and the difference in reproducibility, many genetic researchers used the techniques because RAPD-PCR is a relatively rapid, reliable and conveniently useful method to investigate numerous genomic DNAs for the genetic diversity in a population as well as it does not require the prior knowledge of the genome (Orozco-Castillo et al., 1994; Iyengar et al., 2000; Klinbunga et al., 2000a and b). The polymorphisms are determined by the banding patterns of amplified products at the specific positions by primers (Tassanakajon et al., 1998; Nozaki et al., 2000; Yoon and Kim, 2001). Thus, RAPD and/or RAPD-based techniques have been applied to the identification of the genetic characteristic of diverse species of plants and/or seaweeds (Moeller and Schaal, 1999; Adams, 2000; Esselman et al., 2000; Kim and Choi, 2003; Kwon et al., 2004). Genomic DNA isolated from two species of *Porphyra* obtained from Wando Sea was amplified by PCR reaction. The amplified products were separated by AGE with oligonucleotides decamer primer and stained with ethidium bromide.

In this study, eight decamer primers generated a total of 528 loci in *P. tenera* and 443 in *P. dentata* with DNA band size from 100 bp to 2,200 bp, as summarized in Table 1. When the plant universal primer set is used for PCR amplification in 18 genus and 26 species of seaweeds, a single or a few bands of 200~600 bp were generated (Hong et al., 1997). One to eight DNA bands were amplified, ranging from approximately 240 bp to 1,200 bp in seaweed *Hizikia fusiformis* (Park et al., 1998). The total amplified products from the six isolates were: 143 bands from the Chungmu sample, 135 bands from Haenam, 72 bands from Kijang, 120 bands from Pusan, 136 bands from Wando, and 109 bands from Yosu. In sorghum, 17 primers amplified from 4 to 12 DNA bands in the size range 500~2,500 bp (Jaiswal et al., 1998). In pumpkin species including *C. maxima*, *C. moschata*, and *C. pepo*,

Table 1. The number of loci observed, number of shared loci, number of specific loci and number of polymorphic loci generated by RAPD-PCR using 8 random primers in *P. tenera* and *P. dentata* from Wando of Korea

Primer	No. of loci observed per primer		No. of shared loci by each species		No. of specific loci		No. of polymorphic loci	
	P. tenera	*P. dentata*	*P. tenera*	*P. dentata*	*P. tenera*	*P. dentata*	*P. tenera*	*P. dentata*
OPA-04	77 (7.0)	66 (6.0)	33	11	14	2	6	4
OPA-06	55 (5.0)	40 (3.6)	11	11	14	24	1	5
OPB-01	62 (5.6)	56 (5.1)	0	11	21	11	0	2
OPB-08	47 (4.3)	50 (4.5)	22	0	5	15	0	6
OPB-10	109 (9.9)	110 (10.0)	44	22	3	5	9	12
OPB-11	93 (8.5)	69 (6.3)	11	66	8	12	5	1
OPB-14	60 (5.5)	30 (2.7)	22	11	4	8	1	0
OPC-10	25 (2.3)	22 (2.0)	11	11	4	0	0	0
Total no.	528 (48.1)	443 (25.5)	154	143	73	77	22	30
Average no. per primer	66.0	55.4	19.3	17.9	9.1	9.6	2.8	3.8

The average number of loci per lane generated by a primer in *P. tenera* and *P. dentata* is shown in parentheses.

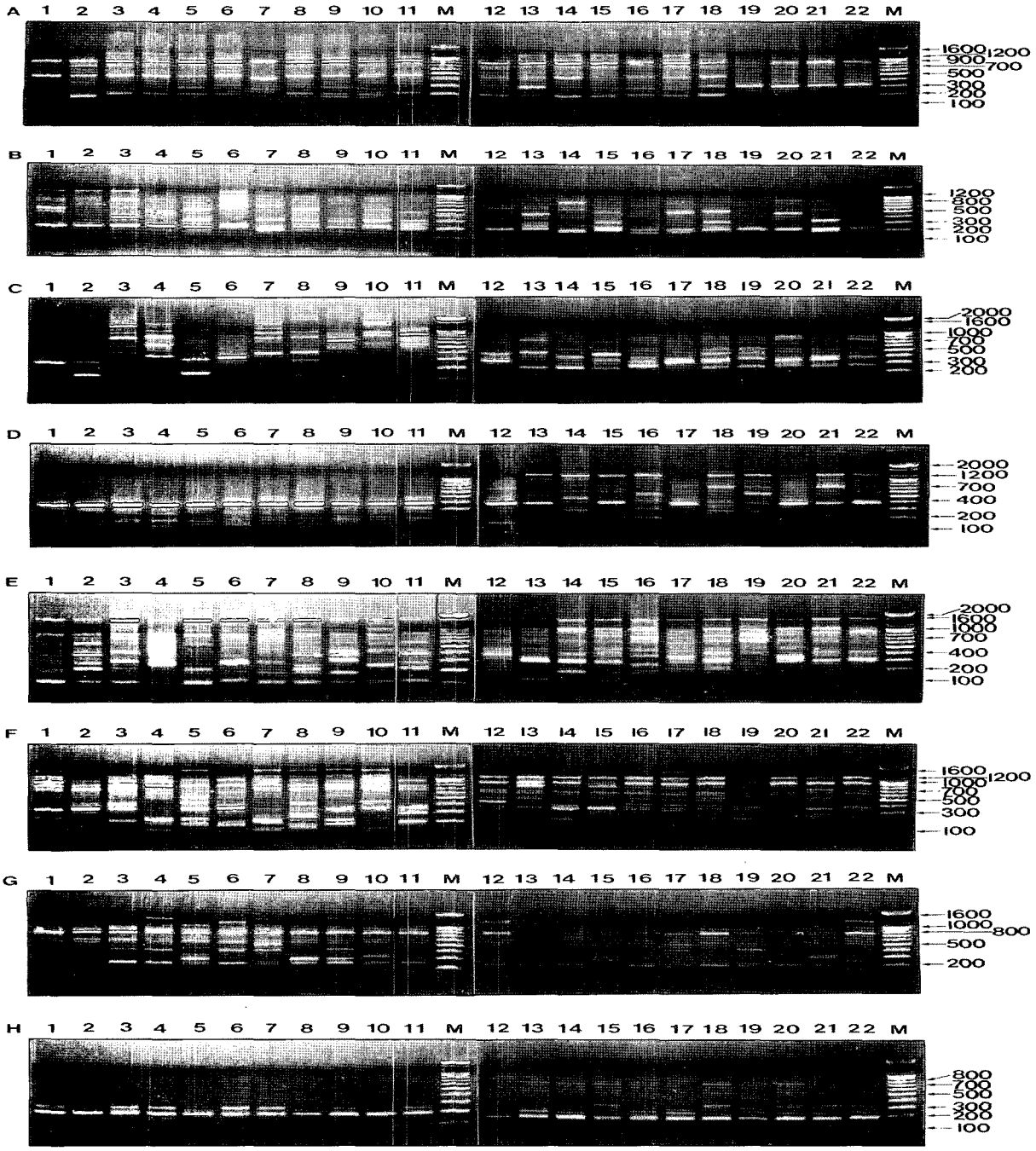

Fig. 1. RAPD-PCR-generated electrophoretic profiles of two *Porphyra* species. DNA isolated from *P. tenera* collected at Wando (lane 1-11) and *P. dentata* (lane 12-22) were amplified by random primer OPA-04 (A), OPA-06 (B), OPB-01 (C), OPB-08 (D), OPB-10 (E), OPB-11 (F), OPB-14 (G) and OPC-10 (H). Amplified products were electrophoresed on a 1.4% agarose gel and detected by staining with ethidium bromide. M, 100 bp Ladder DNA marker.

a total of 136 RAPD markers were produced and 88 DNA bands were polymorphic with an average 5.5 polymorphic bands per primer (Kwon et al., 2004).

In the present study a decamer primer generated 66.0 of average number of per primer in *P. tenera* population from Wando. A RAPD primer generated 6.0 loci on average observed per sample and ranged from 2.3 to 9.9 bands in this *Porphyra* species. In *P. tenera* species, the primer OPB-01 generated various sized bands, ranging from 100 bp to 2,200 bp, as summarized in Fig. 1.

In *P. tenera* species, the banding patterns of the shared loci, 220 bp, 480 bp and 900 bp bands, were generated by the decamer primer OPA-04, as shown in Fig. 1A. The banding patterns generated by decamer primer OPB-01 of individual

P. tenera varied widely, as shown in Fig. 1C. Moreover, the banding patterns also generated by decamer primer OPB-08 of individual *P. dentata* varied widely, as shown in Fig. 1D. The complexity of the banding pattern varied widely between primers and/or geographically locales. Generally, the size and the number of the bands generated unbiased depend on the nucleotide sequence of the primer used and on the source of the template DNA, resulting in a genome-specific DNA band (Welsh and McClelland, 1990).

In *P. tenera* species, the oligonucleotide decamer primer OPB-10 generated 44 shared loci, 100 bp, 600 bp, 800 bp and 1,600 bp, respectively, as shown in Fig. 1E (Table 1). The result indicates that the genome sizes of *P. tenera* from Wando are similar to seaweed (Park et al., 1998), black tiger shrimp (Tassanakajon et al., 1998), catfish (Yoon and Kim, 2001), penaeid shrimp (Yoon and Kim, 2003b), oyster (Kim et al., 2004), and bullhead (Yoon and Kim, 2004).

The number of unique loci to each species and number of shared loci by the two species were generated by RAPD-PCR using 8 random primers in *Porphyra* species from Wando, as shown in Table 2. Eighty-eight unique shared loci to each species, with an average of 11.0 per primer, were observed in *P. tenera* species. Eighty-eight unique shared loci, with an average of 11.0 per primer, were identified in the *P. dentata* from Wando. Especially, 55 numbers of shared loci by the two species, with an average of 6.9 per primer, were observed in the two *Porphyra* species. Especially, the decamer primer OPA-06 generated the shared loci by the two species, approximately 220 bp, between the two *Porphyra* species (Fig. 1B, Table 2). The oligonucleotide primer OPC-10 also generated the shared loci by the two species, approximately 200 bp, in *P. tenera* and *P. dentata*, as shown in Fig. 1H. By the way, the other primers, OPB-01 and OPB-14, did not generate the shared loci by two *Porphyra* species. The result demonstrates that *P. tenera* is genetically different from *P. dentata* species.

Table 2. The number of unique shared loci to each species and number of shared loci by the two species generated by RAPD-PCR using 8 random primers in *P. tenera* and *P. dentata* from Wando of Korea

Item	No. of unique shared loci to each species		No. of shared loci by the two species
Primer\Species	*P. tenera*	*P. dentata*	Two species
OPA-04	22	0	11
OPA-06	0	0	11
OPB-01	0	11	0
OPB-08	11	0	11
OPB-10	33	11	11
OPB-11	0	55	11
OPB-14	22	11	0
OPC-10	0	0	11
Total no.	88	88	55
Average no. per primer	11.0	11.0	6.9

Using numerous arbitrary primers, RAPD-PCR has been applied to identify specific/polymorphic markers particular to breed, line, species and geographical population as well as genetic similarity/polymorphism/diversity in organisms (Partis and Wells, 1996; Callejas and Ochando, 1998; Klinbunga et al., 2000a). In this study, 8 primers generated 22 polymorphic loci (22/528 loci, 4.17%) in *P. tenera* and 30 (30/443 loci, 6.77%) in *P. dentata* species, as illustrated in Table 1. The results demonstrate that a primer detects a great deal of polymorphic loci. This suggests the genetic variation in *P. dentata* is higher than in *P. tenera* species.

In the case of seaweed *Hizikia fusiformis*, thirty-one primers resulted in 715 PCR products of conserved and polymorphic bands (Park et al., 1998). Seventy primers produced 104 polymorphic bands, of them 89 were assigned to linkage groups in lentil (*Lens* sp.) (Eujayl et al., 1998). A genetic linkage map of *Lens* sp. was constructed with 89 RAPD markers, 79 AFLP markers and six RFLP markers. Thirty-eight primers were selected for analysis of total DNA RAPD and resulted in a total of 254 DNA fragments, of them 65% were polymorphic in sorghum (Jaiswal et al., 1998). The number of amplified bands generated by a primer varied from 19 to 27. 98.9% of these bands were polymorphic in *Digitalis* (Nebauer et al., 2000).

Here, 24 specific loci generated by the decamer primer OPA-06 exhibited the inter-individual-specific characteristics and DNA polymorphisms, as shown in Fig. 1B and Table 1. We have also identified 100 bp RAPD-PCR-amplified specific loci (lanes 13, 15 and 20) and 250 bp (lanes 16 and 18) in *P. tenera*, as shown in Fig. 1E. Especially, the 200 bp bands generated by the primer OPC-10 were identified commonly in two *Porphyra* species, which identified populations and/or species, as shown in Fig. 1H. The specific primer was found to be useful in the identification of individuals and/or population, resulting from the different DNA polymorphism among individuals/species/population (Yoon and Park, 2002; Yoon and Kim, 2003a). Although the main disadvantage of the RAPD method is its reproducibility, the method was considered suitable for the identification of a species. RAPD technique was used to characterize six iso-

lates of *Hizikia fusiformis* collected from six locations in Korea (Park et al., 1998). Representative isolate-specific and polymorphic banding patterns were observed among the six isolates with specific RAPD primer. Three out of 40 nanomer primers that amplified DNA bands specific to a grass species or to a durum cultivar were identified (Bommineni et al., 1997). The primer PR21 amplified DNA bands specific to five individual durum cultivars. The primers PR 22 amplified bands specific to a grass species. Similarly, the primer PR23 amplified bands specific to a grass species. The amplified bands ranging from 1,000 to 1,200 bp were specific to *Thinopyrum junceiforme*. The DNA band approximately 1.5 kb was specific to *Lophopyrum elongatum*. Generally, polymorphic loci generated by RAPD-PCR using arbitrary primers were suitable to detect genetic similarity/diversity/polymorphism among various organisms (Bommineni et al., 1997; Nicolosi et al., 2000).

In the present study, the bandsharing value based on the presence or absence of amplified bands was utilized to calculate the similarity indices, as illustrated in Table 3. The similarity matrix based on the average bandsharing value was 0.623±0.008 for *P. tenera* and 0.560±0.009 for *P. dentata*. Bandsharing value between two *Porphyra* species ranged from 0.305 to 0.564 with the average 0.40±0.004. The value difference between the two *Porphyra* species is statistically significant ($P<0.001$). Compared individuals separately, the bandsharing value of individuals for *P. tenera* was higher than that for *P. dentata*. Our bandsharing values between two *Porphyra* species are similar to the result of Park et al. (1998) who reported that the genetic similarity for the six isolates of seaweed *Hizikia fusiformis* ranged from 23% to 59%. Our bandsharing values are also similar to previously reported results of Kim et al, (1997a) in which similarity values obtained by RnRc primer analysis of nuclear DNA varied from 0.364 to 0.714 between *Porphyra tenera* (wild) and *Porphyra tenera* (Ariake). However, the bandsharing values are lower than the results of Kim et al, (1997a) reported that similarity values of *Porphyra* chloroplast DNA were high and ranged from 0.727 to 1.000.

The bandsharing values are also similar to previously reported results in which the average bandsharing value obtained by five random primers was 0.40±0.05 in the wild crucian carp population and 0.69±0.08 in the cultured population (Yoon and Park, 2002). The average bandsharing value of our study is also similar to those of the between the species common carp and Israeli carp (0.57±0.03) (Yoon, 2001), in bullhead population (0.504±0.115) (Yoon and Kim, 2004). On the contrary, the average bandsharing value of our study is higher than that in turkey lines (0.202~0.230) (Ye et al., 1998). The average bandsharing value of our study is also lower than that in Spanish barbel species (0.71~0.81) (Callejas and Ochando, 1998). Even if other molecular method, the sequence homology was 96% similar between the brown and green, 95.1% between the brown and pink, and 94.3% between the green and pink strains from Lake Biwa, Japan (Kane et al., 1997).

The average genetic difference was 0.379±0.009 within *P. tenera* and 0.440±0.009 within *P. dentata* species. As compared individuals separately, the average genetic difference was higher in *P. dentate* than in *P. tenera*. The difference between the two *Porphyra* species is statistically significant ($P<0.001$). Accordingly, as mentioned above, RAPD-PCR analysis showed that *P. tenera* was distinctly separated from *P. dentata*.

Based on the similarity matrix generated by bandsharing values and genetic distances, hierarchical clustering analysis was performed to obtain the dendrogram, as shown in Fig. 2. The dendrogram obtained by the eight primers, indicates four genetic clusters. The longer genetic distance displaying significant molecular differences was between the individual DENTATA no. 19 and TENERA no. 04 between two *Porphyra* populations (0.353). Especially, two *P. dentata* between the individual DENTATA no. 21 and DENTATA no. 19 showed the longest genetic distance (0.627) in comparison with other individuals used, as illustrated in Fig. 2. The genetic similarity for the six isolates of seaweed *Hizikia fusiformis* ranged from 23% to 59% (Park et al., 1998). Especially, isolates collected from Kijang and Chungmu showed that they are most distantly related to the others based on genetic similarity (23%).

Our cluster analysis showed the similar pattern illustrated by Yoon and Kim (2004) in which the single linkage cluster analysis indicating four genetic groupings and dendrogram revealed close relationships between individual identities within two geographical populations of Korean catfish (*S. asotus*) and bullhead (*P. fulvidraco*). In cyanobacterial picoplankton strains, the sequences of the three strains from Lake Biwa, Japan were closely related to those of marine cyanobacterial picoplankton strains and *Prochlorococcus marinus* found in the Pacific and Atlantic Ocean (Kane et al., 1997). In plant, RAPD data analysis of genetic distance and parsimony methods, family clustering, and the analysis of molecular variance were applied to study genetic relationships of a

Table 3. Similarity matrix including bandsharing values and genetic differences calculated using Nei and Li's index of the similarity of *P. tenera* and *P. dentata* from Wando

		Genetic differences of *P. tenera* from Wando												Genetic differences of *P. dentata* from Wando									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Bandsharing values of *P. tenera* from Wando	1	-	0.268	0.340	0.446	0.430	0.419	0.405	0.434	0.408	0.449	0.468	0.595	0.619	0.581	0.599	0.563	0.532	0.641	0.654	0.563	0.645	0.595
	2	0.732	-	0.340	0.443	0.325	0.475	0.481	0.349	0.307	0.400	0.368	0.579	0.601	0.532	0.644	0.594	0.507	0.602	0.658	0.663	0.584	0.572
	3	0.660	0.660	-	0.288	0.441	0.357	0.354	0.295	0.306	0.325	0.350	0.550	0.546	0.533	0.528	0.573	0.523	0.532	0.618	0.643	0.605	0.562
	4	0.554	0.557	0.712	-	0.437	0.376	0.411	0.329	0.327	0.397	0.439	0.583	0.618	0.610	0.595	0.608	0.570	0.575	0.635	0.634	0.644	0.627
	5	0.570	0.675	0.559	0.563	-	0.465	0.485	0.236	0.325	0.356	0.418	0.516	0.596	0.637	0.611	0.637	0.563	0.524	0.587	0.636	0.614	0.570
	6	0.581	0.625	0.643	0.624	0.535	-	0.434	0.449	0.395	0.469	0.423	0.623	0.594	0.614	0.611	0.604	0.470	0.646	0.585	0.615	0.614	0.551
	7	0.595	0.519	0.646	0.589	0.515	0.566		0.373	0.331	0.432	0.360	0.641	0.636	0.592	0.627	0.628	0.594	0.629	0.649	0.654	0.633	0.587
	8	0.566	0.651	0.705	0.671	0.764	0.551	0.627	-	0.301	0.274	0.335	0.436	0.599	0.595	0.547	0.644	0.572	0.491	0.532	0.610	0.609	0.545
	9	0.592	0.693	0.694	0.673	0.675	0.605	0.669	0.699		0.310	0.270	0.584	0.576	0.564	0.610	0.600	0.530	0.573	0.592	0.646	0.625	0.623
	10	0.551	0.600	0.675	0.603	0.644	0.531	0.568	0.726	0.690	-	0.400	0.484	0.613	0.620	0.615	0.615	0.607	0.518	0.615	0.681	0.649	0.619
	11	0.532	0.632	0.650	0.561	0.582	0.577	0.640	0.665	0.730	0.600		0.582	0.579	0.543	0.583	0.592	0.525	0.567	0.603	0.695	0.571	0.556
Bandsharing values of *P. dentata* from Wando	12	0.405	0.421	0.450	0.417	0.484	0.377	0.359	0.564	0.416	0.516	0.418	-	0.536	0.461	0.497	0.519	0.395	0.422	0.390	0.528	0.412	0.379
	13	0.381	0.399	0.454	0.382	0.404	0.406	0.364	0.401	0.424	0.387	0.421	0.464		0.476	0.352	0.344	0.511	0.369	0.532	0.265	0.546	0.492
	14	0.419	0.468	0.467	0.390	0.363	0.396	0.408	0.405	0.436	0.380	0.457	0.539	0.524	-	0.443	0.456	0.468	0.345	0.504	0.380	0.484	0.434
	15	0.401	0.356	0.472	0.405	0.389	0.389	0.373	0.453	0.390	0.385	0.417	0.503	0.648	0.557		0.454	0.491	0.339	0.581	0.364	0.384	0.478
	16	0.437	0.406	0.427	0.392	0.363	0.396	0.372	0.356	0.400	0.385	0.408	0.481	0.656	0.544	0.546	-	0.485	0.431	0.491	0.368	0.450	0.473
	17	0.468	0.493	0.477	0.430	0.437	0.530	0.406	0.428	0.470	0.393	0.475	0.605	0.489	0.532	0.509	0.515		0.481	0.523	0.442	0.427	0.498
	18	0.359	0.398	0.468	0.425	0.476	0.354	0.371	0.509	0.427	0.482	0.433	0.578	0.631	0.655	0.661	0.569	0.519	-	0.435	0.365	0.413	0.412
	19	0.346	0.342	0.382	0.365	0.413	0.415	0.351	0.468	0.408	0.385	0.397	0.610	0.468	0.496	0.419	0.509	0.477	0.565		0.518	0.465	0.413
	20	0.437	0.337	0.357	0.366	0.364	0.385	0.346	0.390	0.344	0.319	0.305	0.472	0.735	0.620	0.636	0.632	0.558	0.635	0.482	-	0.392	0.351
	21	0.355	0.416	0.395	0.356	0.386	0.386	0.367	0.391	0.375	0.351	0.429	0.588	0.454	0.516	0.616	0.550	0.573	0.587	0.535	0.608		0.348
	22	0.405	0.428	0.438	0.373	0.430	0.449	0.413	0.455	0.377	0.381	0.444	0.621	0.508	0.566	0.522	0.527	0.502	0.588	0.587	0.649	0.652	-

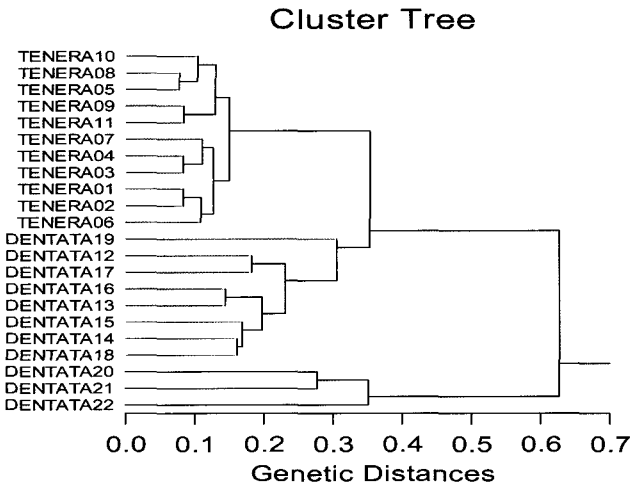

Fig. 2. Hierarchical dendrogram of genetic distances obtained from two *Porphyra* species. The relatedness among different individuals of *P. tenera* of Wando (TENERA 01 - TENERA 11) and *P. dentata* of Wando (DENTATA 12 - DENTATA 22) generated according to the bandsharing values and similarity matrix illustrated in Table 3.

few of species within a genus. They reported that the species relationships revealed by RAPD-PCR approach should be consistent with the previously obtained data using morphological affinities (Nebauer et al., 2000). In the dendrogram, the 42 individuals grouped into seven main clusters, as would be expected from their morphological characters.

Results of our cluster analysis were similar in pattern as those described by Orozco-Castillo et al. (1994). They reported that RAPD analysis reflected morphological differences between the sub-groups as well as the morphological origin of the coffee plants. The phenogram using RAPD data obtained by all primers revealed the close relationships between accessions identities in Native American maize accessions (Moeller and Schaal, 1999). Adams (2000) identified the systematics of genus *Juniperus* (15 species) with data obtained by leaf essential oils and RAPDs. He reported that *Juniperus saltuaria* and *J. przewalskii* are not very similar in their oils but link at the highest similarity (0.92) of any taxa by their DNA. He reported that these one-seeded *Juniperus* divided into three groups. The neighbor-joining tree based on these similarities had three major groups corresponding to the subgenera of *Dendroseris* (Esselman et al., 2000). Mean RAPD locus similarities for individuals of different species ranged from 0.243 to 0.575, with an average of 0.405 for all pairwise comparisons. The genetic distance obtained by 102 polymorphic bands in maize population was 0.08 for the most-similar individuals and 0.28 for the more-variable ones with the average 0.17 (Guo et al., 2001).

In our study, RAPD-PCR analysis revealed a significant genetic distance between two *Porphyra* species pairs ($P<0.001$). The existence of population discrimination and DNA polymorphism between two *Porphyra* species was detected by RAPD-PCR. This shows that the method can be an adequate tool to compare DNA in individuals, species and populations. Furthermore, the basic knowledge of DNA polymorphisms and molecular markers of *Porphyra* sp. may contribute significantly to the seedling selection and the selective seaweed-breeding program.

The genetic identification of black tiger shrimp (*Penaeus monodon*), penaeid shrimp (*Penaeus chinensis*), bullhead (*Pseudobagrus fulvidraco*) and oyster (*Crassostrea* sp.) populations is a necessary step for invertebrate/teleost breeding programs (Tassanakajon et al., 1998; Yoon and Kim, 2003a; Kim et al., 2004; Yoon and Kim, 2004). The classification of geographical populations/species of *Porphyra* needs to be based on the morphological variation in lanceolate and conchocelis in shape, frond size, frond type, and frond color. As mentioned above, the potential of RAPD to identify diagnostic markers for cultivar, breed, stock, species and population identification in plants/seaweeds (Kim et al., 1997a; Moeller and Schaal, 1999; Adams, 2000; Esselman et al., 2000; Kim and Choi, 2003; Kwon et al., 2004) has also been demonstrated.

Nevertheless, further analysis with more individuals, primers and species will be required to establish fully the specificity of loci to particular taxa and subsequent inter-specific gene flow in the genus *Porphyra*. Further sampling sites and isolates will be necessary to determine precisely the area where the phylogeographic break occurs. Additional statistical analyses such as AMOVA, Hardy-Weinberg equilibrium, bootstrapping and principal components based on the RAPD-PCR data will be necessary to obtain more profound and further assessment of genetic relationships among species. In future, we need to develop a genetic linkage map for *Porphyra* based on morphological traits, RAPD, RFLP, AFLP and microsatellite markers.

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