Research Article

J. Algal Biomass Utln. 2013, 4 (4): 1–6

ISSN: 2229- 6905

Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © 2009 PHYCOSPECTRUM - ISSN: 2229 – 6905

Genetic Diversity between *Cystoseria crinita* (Desf.) Bory Populations Detecting By Random Amplified Polymophic DNA Polymerase Chain Reaction (RAPD-PCR)

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Abstract

Cystoseira sp. is one of the most widely distributed genera of the Fucales order and provides an essential habitat for many epiphytes, invertebrates, and fish. *Cystoseira sp.* is found mostly in temperate regions of the Northern Hemisphere, such as the Mediterranean, Indian, and Pacific Oceans. *Cystoseira sp.* is characterized by highly differentiated basal and apical regions and presence of catenate pneumatocysts air-vesicles. Random Amplified Polymorphic DNA (RAPD-PCR) method is commonly used in toxicity, mutation detection and identification studies in macro and microalgae. Among PCR based studies it is widely recognised that RAPD-PCR is a rapid and reliable method for intra and inter-specific differentiation of most plant species. In this study RAPD method was used to determine genetic diversity between *Cystoseria crinita* (Desf.) Bory populations. 6 different populations of *Cystoseria crinita* (Desf.) Bory used as a material in the study. 11 different 10 bp RAPD-primers used and 8 of them gave acceptable bands. Some species identification keys for *Cystoseira* species are not generally give satisfying results. The *Cystoseira* species looks the same in some cases. Besides the morphological features are change according to seasons, enviromental conditions, life cycles and some enviromental effects. For these reasons molecular methods may be used to support morphological identification.

Key Words : *Cystoseira crinita*, RAPD-PCR

Introduction:

Cystoseria crinita (Desf.) Bory (Fig 2) preferentially thrive in the shallow waters of sheltered coves and bays of Mediterranean Sea like other *Cystoseira* species. They generally exist in gravelly-sandy intertidal shores. The thallus is in bushy form in 20-30 cm length. It is an iridescence species. *C.crinita* species reported from East Mediterranean, Aegean and Libyan shores (Hamel., 1939, Nizamuddin et al., 1979) and in Turkey coasts by Güner 1981, 1984, 1987, 1999, Zeybek 1976, Cirik 1978 , Oztürk,1984, Sukatar 1992.

Fig 1 *Cystoseira crinita* (Desf.) Bory

ISSN: 2229- 6905

Furthermore, over the past decade, several molecular techniques have been developed to provide information on diversity, genotoxicology, genetic relationships etc. One of the methods that used for these aims is RAPD-PCR (Random Amplified Polymorphic DNA-Polymerase Chain Reaction). The RAPD procedures were first developed in 1990 [Welsh and McClelland, 1990; Williams *et al.,* 1990] using PCR to amplify anonymous segments of nuclear DNA with identical primers which are 10 bp length. Genetic variation and divergence within and between the taxa of interest are assessed by the presence or absence of each product. RAPD polymorphisms can occur due to base substitutions at the primer binding site sor to indels in the regions between the sites. The RAPD primers are commercially available and do not require prior knowledge of the target DNA sequence. RAPD markers are have been used for species identification, analysis of population structure, analysis of genetic impact of environmental stress and analysis of genetic diversity.

RAPD-PCR method is commonly used in toxicity, mutation detection and identification studies in macro and microalgae. Bartolozzi et al., (1998) used RAPD-PCR analysis to show the genetic differences between Californian almond cultuvars. In a similar study Martins et al., (2001) find high levels of polymorphysims between almond populations by RAPD-PCR. Martinez et al., (1999) showed the genetic differences between haploid male and famele and diploid individuals of *Gracilaria gracilis* by this method.

Based on the studies about species identification and genetic mapping RAPD and RFLP analysis show related results. RAPD requires very small quantities of DNA and there is no need extra procedures like sequencing, cloning or hybridizating. For these reasons it is cheap and rapid technique for genomic characterization studies. Among PCR based studies it is widely recognised that RAPD-PCR is a rapid and reliable method for intra and inter-specific differentiation of most plant species. But there is not much studies about *Cystoseira* species. Because of these reasons we try to exhibit the genetic diversity between *Cystoseria crinita* (Desf.) Bory populations by RAPD analysis. Besides, increasing of the salinity, temperature and polution in Mediterranean sea affects the *Cystoseira* growth and spreading. Decreasing of *Cystoseira* populations takes the attention on to this genus.

Materials and Method

Plant Material

Samples were collected from the rocky shores of Kaş / Antalya, Urla / İzmir, Seferihisar / İzmir, Kuşadası / İzmir and Fethiye / Muğla (Figure 2) on the same season. The sea temperature was nearly the same at the collecting stations (18-21°C). After the samples cleaned with distileted water and kept in ice during trasportation to the labratory the epiphytes cleaned under the stereoscop. Then kept at -20°C until DNA isolation.

DNA isolation: DNA extracted according to modified CTAB method (Doyle & Doyle, 1987, Tuney 2005).

RAPD-PCR:

Amplification of genomic DNA was performed in 50 µl reaction mixture containing 18.5 Mili Q water, 1.75 µl of 10 x Taq polymerase buffer, 1.2 µl of $MgCl_2$, 1.5 µl of dNTPs, 0.6 µl of primer and 0.6 µl of Taq DNA polymerase before adding DNA.

ISSN: 2229- 6905

Fig 2 Collection site (yellow arrows)

RAPD-PCR was performed by 10 different RAPD primers (Table 1) after checking the DNAs by agarose gel electrophoresis. PCR bands were checked again by agarose gel electrophoresis after PCR analysis. The PCR analysis performed three times as control. Amplification done in ABI Prisim 9700 Thermal Cycler with 5 cycles; denaturation at 94°C for 1 minute, annealing at 40 ^{o}C for 30 seconds and extension at 72 ^{o}C for 1 minute, 40 cycles; denaturation at 94°C for 45 seconds, annealing at 60 ^{o}C for 1 minute and extension at 72 ^{o}C for 45 seconds, final extension at 72 ^{o}C for 11 minutes. The amplified product kept in 4°C until gel electrophoresis.

Table 1: RAPD-PCR primers and sequences

Primer	Primer Sequence
OPA9	5’-GGG TAA CGC C-3’
OPB14	5’-TCC GCT CTG G-3’
OPB17	5’-AGG GAA CGA G-3’
OPB5	5’-TGC GCC CTT C-3’
OPB6	5’-TGC TCT GCC C-3’
OPB7	5’-GGT GAC GCA G-3’
OPB8	5’-GTC CAC ACG G-3’
OPB10	5’-CTG CTG GGA C-3’
OPB11	5’-GTA GAC CCG T -3’
OPB12	5’-CCT TGA CGC A-3’
OPD13	5’-TCG TCA CCC C-3’

ISSN: 2229- 6905

Agarose Gel Electrophoresis:

9 µl of each PCR product was electrophoresed on agarose gel (%1.2) in TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.0) at 80 V for 1 hour. The gel was stained with ethidium bromide and photographed.

Results and Discussion

RAPD analysis has been successfully to investigate the genetic diversity and structure of populations of rare or endangered species of plants (Fischer et.al., 2000; Chen et al., 2004). RAPD analysis is unaffected by sample deterioration, therefore, it can be used to detect genetic polymorphysims far more successfully (Perez et al., 1998). The main changes observed in the RAPD profiles have been resulted both in appearance or disappearance of different bands and variation of their intensity. 3 of 11 different RAPD-PCR primers didn’t give any bands while 8 of them gave 44 bands. The primers and their band numbers shown in Table 2. The most band obtained by primers OPB 6 (Figure 3) and OPB 9, respectively. Also the most polymorphic band patterns observed by the same primers.

Figure 3: RAPD-PCR band patterns by OPB6 primer; M (Marker), N (Negative control), 1 (Fethiye), 2 (Kaş), 3 (Urla), 4 (Kuşadası), 5 (Foça), 6 (Seferihisar)

According to the statistical results of RAPD-PCR analysis; 57% differences observed between *C.crinita* samples collected from Fethiye and Urla. This value is 11% between Fethiye and Seferihisar samples. The band differences found among Kuşadası and Fethiye is 29%. The less differences found between Foça and Seferihisar is 14 %.

The collected samples from Urla shown 16 % difference with the samples from Seferihisar while 33% and 25 % differences with samples from Foça and Kaş, respectively.

In general the biggest differences observed between the samples from Fethiye and Urla while the biggest similarity found Fethiye and Seferihisar.

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Table 2: Primers, band numbers and polmorphysim percentages

Primer No	Primer	Band Numbers		
		Total band (a)	Polimorphic band (b)	b/a x 100
1	OPB6	15	3	20
2	OPB5	4	4	100
3	OPB1	2	2	100
4	OPB17	2	2	100
5	OPB8	5	3	60
6	OPB10	9	5	55.5
7	OPB7	3	3	100
8	OPD13	4	-	0
Total		44	22	535.5
Average		5.5	2.75	66.9

On the bases of the RAPD-PCR results we observed that different populations of *Cystoseira crinita* species show different band patterns. In the first case we assume that these differences are because of the environmental differences for example adaptation for environmental changes, however the nitrite, nitrate, phosphate and temperature analysis nearly the same for all stations. The other environmental condition such as existence of herbivore and opportunist organisms, epiphytes and invaders could form different conditions for *C. crinita* species. The species grown in these different kind of circumstances it seems normal that they show differences in their DNA sequences. Besides it is known that *Cystoseira* have high hybridisation potential within species. For these reasons some differences in morphological and molecular levels are expected in different populations of same species. Costal morphology, buttom nature, nutrient concentration, urbanization

ISSN: 2229- 6905

level of the coast and other factors like inorganic chemical pollution, increased turbidity levels, overgrazing and climate changes have been suggested as other possible causes. In this experiment the differences between *C. crinita* populations collected from Kaş, Fethiye, Kuşadası, Seferihisar, Urla and Foça shores searched by RAPD-PCR method. The reasons of the differences between the populations may be the high hybridisation ability of Cystoseira species and some enviromental (physical and chemical) differences between the stations.

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ISSN: 2229- 6905