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Inter-simple sequence repeat (ISSR) analysis of genetic variation of *Chondrus crispus* populations from North Atlantic

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Abstract

ISSR analysis was used to investigate genetic variations of 184 haploid and diploid samples from nine North Atlantic *Chondrus crispus* Stackhouse populations and one outgroup Yellow Sea *Chondrus ocellatus* Holmes population. Twenty-two of 50 primers were selected and 163 loci were scored for genetic diversity analysis. Genetic diversity varied among populations, percentage of polymorphic bands (PPB) ranged from 27.0 to 55.8%, *H* (Nei's genetic diversity) ranged from 0.11 to 0.20 and *I* (Shannon's information index) ranged from 0.16 to 0.30. Estimators PPB, *H* and *I* had similar values in intra-population genetic diversity, regardless of calculation methods. Analysis of molecular variance (AMOVA) apportioned inter-population and intra-population variations for *C. crispus*, showing more genetic variance (56.5%) occurred in intra-population, and 43.5% variation among nine populations. The Mantel test suggested that genetic differentiation between nine *C. crispus* populations was closely related with geographic distances ($R = 0.78$, $P = 0.002$). Results suggest that, on larger distance scale (ca. >1000 km), ISSR analysis is useful for determining genetic differentiations of *C. crispus* populations including morphologically inseparable haploid and diploid individuals.

Keywords: Rhodophyta; *Chondrus crispus* Stackhouse; Population; Genetic variation; Geographic distance; ISSR; AMOVA; Mantel test

1. Introduction

Non-random distribution of genetic variation usually refers to the genetic structure of a population. Geographic distance is generally regarded as an important influence on both genetic structure and gene flow because distance confines the movements of gametes, propagules and individuals that change the spatial distributions of genes. The relation of genetic variation and distance scales has been studied for populations of many organisms, including some species of seaweed (Avise, 1994; Wattier and Maggs, 2001).

A number of DNA markers have been used in studies of the genetic structure of populations. SSR (simple sequence repeat), has been applied in studies of seaweed species such as *Laminaria digitata* (Hudson) Lamouroux (Billot et al., 2003), *Fucus serratus* Linnaeus (Coyer et al., 2003), *Cladophoropsis membranacea* Brøgesen (Van der Strate et al., 2003), and *Gracilaria gracilis* (Engel et al., 2004). RAPD and AFLP have been commonly used in studies of genetic structures of seaweed population (Coyer et al., 1997; Engelen et al., 2001; Faugeron et al., 2001, 2004; Bouza et al., 2006; Donaldson et al., 2000; Kusumo and Druehl, 2000). Another marker similar to RAPD is ISSR, which can be used without knowing the sequence information for genomic DNA (Zietkiewicz et al., 1994). ISSR has mild technical difficulty, good reproducibility and reasonable cost, permitting its use for genetic studies of population in higher plants (Wei et al., 2005), and seaweed (Li et al., 2005; Wang et al., 2005, 2006).

Chondrus crispus Stackhouse (Gigartinaceae, Rhodophyta) is distributed along shores of countries bordering the North Atlantic and is often harvested for carrageenan extraction (McHugh, 2003). Many studies on *C. crispus* relate to its distribution and life cycles in different seasons and places (Craigie and Pringle, 1978; Lazo et al., 1989; Lindgren and

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Åberg, 1996). Genetic studies of *C. crispus* began with Cheney and Mathieson (1979) examining isozyme patterns of eight individuals from New Hampshire in the USA and from the Maritime Provinces of Canada. Unfortunately, the isozyme technique used by Cheney and Mathieson was applied to a limited number of genetic markers and results could be negatively affected. Therefore, later studies applied sequences and multilocus techniques to *Chondrus* populations; 5.8 S rRNA, ITS sequences and plastid DNA RFLP banding patterns have been used to compare *C. crispus* from both sides of the North Atlantic (Chopin et al., 1996). Another drawback of early *C. crispus* studies was that a limited number of samples were examined. Therefore, 10 *C. crispus* populations, each having ten isolates of unknown ploidy state, were investigated using AFLP (Donaldson et al., 2000). However, AFLP discerned excessive genetic variation in *C. crispus* populations and the population structure was poorly reconstructed.

To improve on earlier studies, our research applied ISSR to haploid and diploid individuals of nine *C. crispus* populations selected from both sides of the North Atlantic. Our research aimed to analyze genetic structure of *C. crispus* and to relate genetic variations to the distances separating populations.

2. Materials and methods

2.1. Sample collection

Hundred and eighty-four *Chondrus* individuals were collected from 10 populations in the North Atlantic ocean and the Yellow Sea (four populations from France, two from Britain, and one each from Canada, Portugal, Germany and China), with 14–21 individuals from each population (Table 1). The *Chondrus ocellatus* Holmes population QD from the Yellow Sea was assigned as outgroup to the nine other *C. crispus* populations. Tetraphytes (tetrasporangia presence) and female gametophytes (cytocarps presence) were visually determined. Individuals that could not be assigned as haploid or diploid were collected locally in Qingdao or sent to our lab by air-mail. In our lab, isolates were rehydrated in deionized water and gently rubbed to remove epiphytes. Treated thalli from each population were used for DNA extraction and ISSR analysis.

2.2. DNA extraction and ISSR analysis

About 300 mg of algal material was cut into pieces (ca. 1/4 cm^2/piece) and put into a sterilized, pre-cooled mortar. The algal tissue was ground in liquid nitrogen using a pestle and the resulting powder was transferred into 1.5 mL Eppendorf tubes. Genomic DNA was extracted using a Universal Genomic DNA Extraction Kit (TaKaRa Biotechnology, Dalian, China). Concentrations of extracted DNA were tested by comparison with known quantities of λDNA/Hind III markers (Sangon, Shanghai, China) using 0.8% agarose gel. Yield was approximately 0.3 μg mg^{-1}.

ISSR reactions were performed in a 20 μL reaction volume containing 1 μL template DNA (approximately 20 ng), 0.2 μM primers, 0.2 mM dATP, 0.2 mM dGTP, 0.2 mM dCTP, and 0.2 mM dTTP (Promega, Shanghai), 1.5 mM Mg^{2+}, 1× polymerase buffer (Promega), and 1.0 U *Taq* DNA polymerase (Promega). PCR was conducted using a Master Thermal Cycler (Eppendorf, Germany). Cycling parameters were 94 °C for 5 min and 40 cycles of: denaturation at 94 °C for 30 s, annealing at the proper temperature for 45 s, and extension at 72 °C for 2 min. Amplification products were detected using 1.5% agarose gel. All tests were repeated.

2.3. Data analysis

Assuming two alleles per locus, ISSR profiles were scored for each individual as discrete characters based on the presence or absence of amplified bands. Only reproducible DNA bands or loci were selected for additional data analysis. Three comparable estimators in POPGENE 1.31 (Yeh et al., 1999), including the percentage of polymorphic band (PPB), Nei's genetic diversity (H) and Shannon indices of diversity (I), were used to calculate genetic diversity for each population. Assuming Hardy–Weinberg equilibrium, Nei's unbiased genetic distances separating populations (Nei, 1978) were determined using POPGENE 1.31 software.

AMOVA (Excoffier and Schneider, 2005) was used to examine the parameter F_{st}, indicating variances partitioned into intra-population and inter-population. Unlike Nei's analysis using POPGENE, AMOVA is not based on the assumption of Hardy–Weinberg equilibrium. Rather, AMOVA assumes that

Table 1
Details of examined *Chondrus* populations

Species	Population codes	Locality	Latitude and longitude	Sample size	Collection date	Collector
Chondrus crispus Stackhouse	GP	Green Point, Lepreau, New Brunswick, Canada	45°06′N, 66°18′W	20	19 November 2003	G.W. Saunders
Chondrus crispus Stackhouse	BB	Buarcos Bay, Portugal	40°09′N, 8°51′ W	22	5 June 2004	L. Pereira
Chondrus crispus Stackhouse	HE	Helgoland, North Sea, Germany	54°10′N, 7°54′E	14	8 July 2003	L.A. Franklin
Chondrus crispus Stackhouse	SV	St Vaast La Hougue, France	49°34′N, 1°17′W	17	9 February 2004	A.T. Critchley
Chondrus crispus Stackhouse	PS	Portsall, France	48°35′N, 4°40′W	18	4 June 2004	Solene Conan
Chondrus crispus Stackhouse	DL	Dellec, France	48°21′N, 4°34′W	21	5 June 2004	Solene Conan
Chondrus crispus Stackhouse	FB	Fort Bloque, France	47°42′N, 3°26′W	16	3 June 2004	Solene Conan
Chondrus crispus Stackhouse	WB	Wembury Beach, Devon, England	50°22′N, 4°08′W	21	5 December 2003	M. Brown
Chondrus crispus Stackhouse	BR	Black Rock, Dale Pembrokeshire, Wales	51°40′N, 4°55′W	20	5 December 2003	S.L. Morrell
Chondrus ocellatus Holmes	QD	Ba Da Guan, Qingdao, China	36°03′W, 120°20′ E	15	26 March 2002	Z.M. Hu