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Genetic analysis of the gametophytes of *Undaria pinnatifida* (Phaeophyceae) with ISSR method

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Abstract

Inter-simple sequence repeat (ISSR) analysis was used to assess eleven pairs of *Undaria pinnatifida* (Harv.) Suringar male and female gametophytes. After screening fifty primers, 18 ISSR primers were selected for final analysis. A total of 104 loci were obtained, of which 77 were polymorphic among the gametophytes studied. Genetic relationships were analyzed with simple matching (S), Jaccard's (J) and Dice's (D) distance coefficients. Little genetic variations were found among the selected *Undaria* gametophytes, for instance, the genetic distances ranging from 0.010 to 0.125 with Dice coefficients. UPGMA dendrograms showed that 11 pairs of *Undaria* gametophytes were distributed into five groups. Most *Undaria* strains cultivated in China exhibited closely genetic relationships with the strains from Japan. However, gametophytes from Qingdao appeared as distinct clades from other *Undaria* strains with all three distance coefficients used. Mantel test showed that the three distance measurements generated congruent clustering patterns on the same data. Our results demonstrated the feasibility of applying ISSR markers for genetic analysis of *Undaria* gametophytes.
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Keywords: *Undaria*; Gametophytes; Inter-simple sequence repeat; Genetic analysis; Mantel test; Dendrogram

1. Introduction

Undaria pinnatifida (Harv.) Suringar has been cultivated as food and industrial use in eastern Asian countries such as in Japan, Korea and China. Nowadays *Undaria* is one of the three main cultivated seaweeds (*Laminaria*, *Porphyra* and *Undaria*) in China (Wu et al., 2004; Tseng, 2004).

It is well known that the seaweed cultivation is based on the knowledge of their life histories (Wikfors and Ohno, 2001). *Undaria* genus is one of the Laminaria kelps characterized by a heteromorphic life cycle, with an alternative between highly differentiated diploid sporophytes and microscopic haploid gametophytes. At maturity, the sporophytes release spores that germinate and grow into microscopic gametophytes, and the gametophytes become fertile to release sperms and eggs that join to form embryonic sporophytes (van den Hoek et al., 1995). Since the 1980s, the commercial cultivation of *Undaria* has begun in China. Zoospores method, with spores collected onto the seeding substrates for sporeling culture, was applied to *Undaria* cultivation. Zoospores are collected from numerous *Undaria* sporophylls of one strain or usually several strains dumped together and gametophytes and young sporelings are cultured in greenhouse at ambient temperature before cultivated in the sea (Tseng, 2001).

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This will quickly make the *Undaria* strain lose its economic characteristics because of randomly hybridizing. Fang et al. (1978) found that isolated gametophytes can be propagated vegetatively under controlled conditions as genetically "seeds" for breeding, and a new method of culturing sporelings by use of male and female gametophytes was developed (Pang and Wu, 1996; Wu et al., 2004). Using this method, it is convenient to keep the selected *Undaria* parent gametophytes in stock culture which can be propagated quickly in the need of sporeling production, with the desired characteristics of the sporophytes conserved well. Various *Undaria* gametophytes with specific characteristics are obtained, preserved and applied in breeding and large-scale crossing (Pang et al., 1997). But little is known about their genetic background at DNA level, it is necessary to perform germplasm analysis of various gametophytes using DNA marker.

To date, many DNA-based molecular markers, such as SNP, AFLP, SSR, ISSR, RAPD and RFLP have been applied in aquaculture genetics (Liu and Cordes, 2004). In seaweed they have been used for population genetics (Batley and Hayes, 2003; Schaeffer et al., 2002; Vis, 1999), biogeography (Wattier et al., 1997), parentage determination (Billot et al., 1999), heterosis (Patwary and van der Meer, 1994) and germplasm identification (Wang et al., 2004). Generally, the choice of DNA marker should depend on the aims, technical considerations, availability of laboratory facilities and costs. Compared with other DNA marker systems, the ISSR techniques was an intermediate in technical difficulties, reproducibility and cost (Pradeep Reddy et al., 2002). Similar to the RAPD technique that can be performed without knowledge of the sequence information for genomic DNA, the ISSR method with longer microsatellite primers anchored at the 3′- or 5′-end by two to four degenerate nucleotide is highly reproducible and allows detection of interspecific and intraspecific DNA polymorphisms. ISSR analysis has been applied to terrestrial plants for studying genetic diversity (Fernández et al., 2002), germplasm identification (Fang and Roose, 1997), population genetics (Qian et al., 2001), linkage map construction (Sankar and Moore, 2001), and gene tagging (Collard et al., 2003). Vis (1999) investigated algal genetic variability among individual gametophytes of a single population of *Batrachospermum boryanum* using this method. Sun et al. (2003) used ISSR analysis to examine genetic variations among different species of *Gracilaria*. Recently, this technique has been successfully used for identifying the toxic phytoplankton species (Bornet et al., 2005).

In a previous study, the ISSR technique has been used for the genetic analysis of selected *Laminaria* gametophytes from China (Wang et al., 2005). Here in this study, this method was used to assess the genetic background of some preserved *U. pinnatifida* (Harv.) Sur. gametophytes. To assist the analysis, the efficiencies of three distance coefficients (simple matching, Dice's and Jaccard's) in estimating genetic relationships among them were also examined with respect to the number of ISSR marker loci used.

2. Materials and methods

2.1. Materials

Eleven pairs of *Undaria* gametophytes (male and female) were analyzed in this study along with a pair of *Laminaria* gametophytes as an outgroup (Table 1). All materials were from the Germplasm Stock, IOCAS (Institute of Oceanology, Chinese Academy of Sciences), most of which were widely used in *Undaria* cultivation in China. All of the *Undaria* gametophytes were from the same species—*U. pinnatifida*. Prior to DNA extraction, gametophytes were cultured in sterilized seawater according to the modified method of Pang and Wu (1996).

2.2. DNA extraction and ISSR analysis

Genomic DNA was extracted from cultured gametophytes according to a modified cetyltrimethylammonium bromide (CTAB) method (Murray and Thompson, 1980; Wang et al., 2004). Fifty ISSR primers were synthesized by Sangon Co. Ltd. (Shanghai, China). The 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 each of dATP, dGTP, dCTP, and dTTP (Promega, Shanghai), 1.5 mM Mg^{2+}, 1 × polymerase buffer (Promega), and 1.0 U *Taq* DNA polymerase (Promega). PCR amplification reaction was conducted using a Masteral Thermal Cycler (Effendorf, Germany). The 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 on 1.5% agarose gel. All tests were repeated twice.

2.3. ISSR data analysis

Reproducible DNA fragments or loci were selected for data analysis: 0 indicated absence of the locus and 1

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