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Genetic variation of *Sargassum horneri* populations detected by inter-simple sequence repeats

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ABSTRACT. The seaweed *Sargassum horneri* is an important brown alga in the marine environment, and it is an important raw material in the alginate industry. Unfortunately, the fixed resource that was originally reported is now reduced or disappeared, and increased floating populations have been reported in recent years. We sampled a floating population and 4 fixed cultivated populations of *S. horneri* along the coast of Zhejiang, China. Inter-simple sequence repeat (ISSR) markers were applied in this research to analyze the genetic variation between floating populations and fixed cultivated populations of *S. horneri*. In total, 220 loci were amplified with 23 ISSR primers. The percentage of polymorphic loci within each population ranged from 53.64 to 95.45%. The highest diversity was observed in population 3, which was the local species that was suspension cultured in the lab and then fixed cultivated in the Nanji Islands before sampling. The lowest diversity was obtained in the floating population 4. The genetic distances among

the 5 *S. horneri* populations ranged from 0.0819 to 0.2889, and the distance tendency confirmed the genetic diversity. The results suggest that the floating population had the lowest genetic diversity and could not be joined into the cluster branch of the fixed cultivated populations.

Key words: *Sargassum horneri*; Floating populations; Inter-simple sequence repeat (ISSR); Genetic variation

INTRODUCTION

Sargassum horneri, a large brown alga, belongs to Phaeophyceae, Fucales, Sargassaceae; it is widely distributed from the mid-littoral to sublittoral zones along the coast of the Pacific Ocean and the adjoining seas of Korea, Japan, and China (Hossain et al., 2003). *S. horneri* provides the habitat for a number of marine organisms so that they can avoid predators, inhabit, feed, and spawn (Zheng et al., 2008). *S. horneri* powder is the main feed ingredient of *Stichopus japonicus* culture, which expanded over 1 million acres in China (Zheng et al., 2008). Chemicals like alginate, laminaran, and fucoidan, which are extensively used in industry, can be extracted from *S. horneri* (Ermakova et al., 2011), and the algal polysaccharides can be used as drugs and drug intermediates because of their anticoagulant activity, antitumor activity, and antiviral activity (Witvrouw and De Clercq, 1997). On the other hand, sodium alginate can be used as an important food additive (O'Sullivan et al., 2010).

Macroalgae farming is an effective means of *in situ* remediation of the environmental eutrophication, which was an excellent way to protect the environment compared to the use of microalgae and phytoplankton (Yang and Fei, 2003). *S. horneri* was cultivated in the Nanji Islands, Zhejiang, China, in order to restore the mariculture field, which effectively alleviated the environmental pressure of the islands (Sun et al., 2009).

The natural resources of *S. horneri* were reported to be depleted, leading to sea desertification or "isoyake" areas, because of extensive collection for artificial seaweed breeding, coastal pollution, and herbivore grazing (Fujita, 2010; Nagai et al., 2011). Currently, *S. horneri* is one of the main candidates for seaweed bed reconstruction in Japan, the Republic of Korea, and China (Yamauchi, 1984; Choi et al., 2003; Sun et al., 2009). According to 40 years of observation (1969 to 2009) by Sun et al. (2010), the number of algae species at Mazuao, Nanji Islands, decreased with the gradual change in abundance. The original constructive species became the common species, and some constructive species such as *Chondria crassicaulis* Harv. and *Sargassum fusiforme* (Harv.) Setch became uncommon species; furthermore, *S. horneri* disappeared in the intertidal zone during the observation period; thus, the local fixed *S. horneri* cannot be found now. Komatsu et al. (2007) conducted research in the eastern East China Sea during May 2002 and March 2004, which revealed that the floating seaweeds were distributed along the front between the Kuroshio Current and coastal waters and were mainly composed of 1 seaweed species, *S. horneri*, from spring to early summer. The field survey revealed that *Sargassum* forests were mainly composed of *S. horneri* around Goqui Island, Shensi Prefecture, Zhejiang Province, China (Komatsu et al., 2007). Additionally, Abé et al. (2013) reported that the floating *S. horneri* probably originated in the southern East China Sea. In our research, the inter-simple sequence repeat (ISSR) technique was applied to detect the genetic variation of fixed cultivated populations and the floating population of *S. horneri* along the coast of Zhejiang, China.

MATERIAL AND METHODS

Sampling

In total, 5 populations of *S. horneri* were collected from 3 sites from April 2013 to June 2013 along the coast of Zhejiang Province, China (Table 1). The samples were collected randomly and cleaned by distilled seawater to remove the attachments; then, samples were placed in an icebox and taken to the lab as soon as possible. The samples were preserved at -20°C for DNA extraction.

Table 1. Information about 5 *Sargassum horneri* populations and the sampling location.

Population	Resource origin	Collection site	Sample time
1	North cultivar (Dalian)	Nanji Islands; 27°27' N, 121°05' E	April 13, 2013
2	Local cultivar (Nanji)	Nanji Islands; 27°27' N, 121°05' E	April 13, 2013
3	Local species suspension cultured in lab and cultivated in the sea (Nanji)	Nanji Islands; 27°27' N, 121°05' E	April 13, 2013
4	Floating species (Unknown)	Dongtou; 27°50' N, 121°09' E	May 18, 2013
5	Wild species (Dongji)	Dongji Islands; 30°11' N, 122°41' E	April 30, 2013

DNA extraction

About 5 leaflets were plucked from the alga plantlet when it was thawed completely, and they were washed thoroughly with disinfected seawater, sterilized in 0.7% KI solution for 10 min, and dried on filter paper. After being finely ground with liquid nitrogen, the seaweed powder was transferred to a sterilized 1.5-mL centrifuge tube for DNA extraction. The total genomic DNA was extracted following the cetyltrimethylammonium bromide method (Porebski et al., 1997). The concentrations of the extracted DNA were measured by a NANODROP1000 (Thermo Scientific, USA), and these results correspond with ISSR standards. The DNA quality was tested by 1% agarose gel electrophoresis.

ISSR amplification

Twenty-eight ISSR primers were synthesized by Sangon (China), and 23 of these primers were finally selected according to the polymorphism, quality, and stability of the amplification (Table 2). The ISSR reaction system was 20 μL and consisted of 15 ng template DNA solution, 10 μM primers, 10X polymerase chain reaction (PCR) buffer, 0.2 mM dNTP mix, 0.2 mM Mg^{2+}, and 1.0 U Taq DNA polymerase (TaKaRa Biotech, China).

Table 2. Inter-simple sequence repeat (ISSR) primers.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
807	$(AG)_8T$	834	$(AG)_8YT$	864	$A(TGA)_3TG$
808	$(AG)_8C$	835	$(AG)_8YC$	866	$(CTC)_6$
809	$(AG)_8G$	841	$(GA)_8YC$	873	$(GACA)_4$
810	$(GA)_8T$	844	$(CT)_8RC$	880	$GGA(GAG)_2AGGAG$
811	$(GA)_8C$	848	$(CA)_8RG$	889	$BHBG(AG)_6A$
812	$(GA)_8A$	849	$(GT)_8YA$	890	$(GGAGA)_3$
823	$(TC)_8C$	851	$(GT)_8YG$	891	$HVH(TG)_7$
828	$(TG)_8A$	855	$(AC)_8YT$		

*Y = C/T; R = A/G.

PCR amplifications were conducted with a thermal cycler (Eppendorf, Germany) with the following conditions: initial denaturation at 94°C for 5 min; 40 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 45 s, and extension at 72°C for 2 min; and a final extension at 72°C for 10 min. PCR products were separated on 1.5% agarose gels in 1X Tris, acetic acid, ethylenediaminetetraacetic acid buffer. DNA marker DL 2000 (TaKaRa Biotech, China) was used as a size marker. PCR products were recorded with a digital imager (Bio-Rad, USA) after staining with ethidium bromide.

Data analysis

The ISSR electrophoretic amplification bands were scored for their presence (1) or absence (0), which excluded smeared and weak bands. The results were analyzed by the PopGene 32 software based on the data matrix to obtain the following: 1) the percentage of polymorphic loci (P%); 2) N_A, observed number of alleles; 3) N_E, effective number of alleles; 4) H, Nei's (1973) gene diversity; and 5) I, Shannon's information index. The Nei's (1978) unbiased genetic distance (D) between each population was determined, and a dendrogram based on it was constructed by unweighted pair group method with arithmetic mean (UPGMA).

RESULTS

All of the 220 loci ranged from 100 to 2000 bp in size in the 5 populations, and there was an average of 9.1 loci per primer. The P% within each population ranged from 53.64 to 95.45% (Table 3). The highest parameters were detected in population 3 from Nanji Island (P% = 95.45%, N_A = 1.95 ± 0.21, N_E = 1.69 ± 0.31, H = 0.38 ± 0.14, I = 0.56 ± 0.19), which was the local species that was cultured in the lab. The lowest P%, N_A, and I were detected in the floating samples of population 4 collected from the Dongtou area (P% = 53.64%, N_A = 1.54 ± 0.5, I = 0.31 ± 0.31), while the lowest N_E and H was observed in the population 2 Nanji local cultivar (N_E = 1.31 ± 0.30, H = 0.20 ± 0.16).

Table 3. Genetic diversity of 5 *Sargassum horneri* populations by ISSR analysis.

Population	Sample size	P%	N_A	N_E	H	I
1	16	76.36%	1.76 ± 0.43	1.41 ± 0.36	0.24 ± 0.19	0.37 ± 0.26
2	17	75.45%	1.75 ± 0.43	1.31 ± 0.30	0.20 ± 0.16	0.32 ± 0.23
3	12	95.45%	1.95 ± 0.21	1.69 ± 0.31	0.38 ± 0.14	0.56 ± 0.19
4	17	53.64%	1.54 ± 0.5	1.38 ± 0.41	0.21 ± 0.22	0.31 ± 0.31
5	20	85.45%	1.85 ± 0.35	1.34 ± 0.29	0.22 ± 0.16	0.35 ± 0.22
Total	82	100.00%	2.00	1.52 ± 0.23	0.33 ± 0.10	0.50 ± 0.13

P% = percentage of polymorphic loci; N_A = observed number of alleles; N_E = effective number of alleles; H = Nei's genetic diversity; I = Shannon's information index.

Cluster analysis

Based on the ISSR markers, the D values among the 5 *Sargassum horneri* populations ranged from 0.0819 to 0.2889 (Table 4). The lowest value was between populations 2 and 5, and the highest value was between populations 1 and 4. The genetic identity values ranged from 0.749 to 0.921. In addition, the lowest value was between populations 1 and 4, and the

highest value was between populations 2 and 5. Therefore, the genetic identity and genetic distance exhibited the same tendency.

The UPGMA dendrograms (Figure 1) based on ISSR data showed that populations 2 and 5 clustered together first, then with populations 1 and 3, and finally with population 4.

Table 4. Nei's unbiased measures of genetic identity and genetic distance.

Population	1	2	3	4	5
1	-	0.8860	0.8072	0.7491	0.9037
2	0.1211	-	0.8065	0.7671	0.9213
3	0.2141	0.2151	-	0.7814	0.8314
4	0.2889	0.2652	0.2466	-	0.7528
5	0.1013	0.0819	0.1847	0.2840	-

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

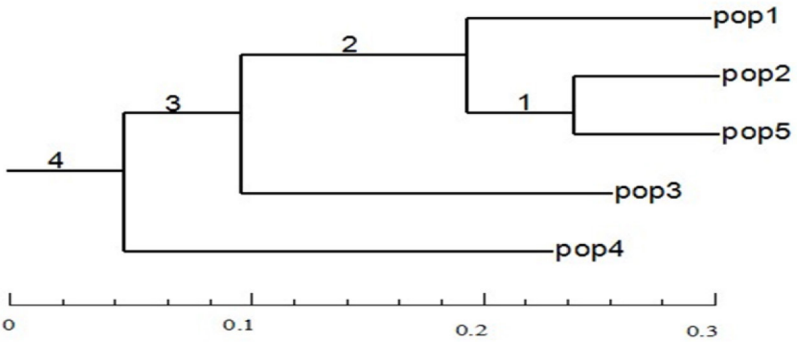

Figure 1. Unweighted pair-group method with arithmetic mean dendrogram of the *Sargassum horneri* populations.

DISCUSSION

Generally, natural selection factors, geographical distance, and ecological factors, such as current systems, temperature, and salinity, in specific geographical locations influence the genetic diversity of *Sargassum* (Sun and Lin, 2003). Zhao et al. (2007, 2008) reported that a comparatively low genetic variation, a P% of about 40-50%, among *Sargassum thunbergii* populations and *Sargassum muticum* populations was revealed by ISSR and random amplification of polymorphic DNA (RAPD). Similar results were obtained in studies on *Sargassum* species in Oman Sea by Noormohammadi et al. (2011). Yu et al. (2013) reported that high genetic variability and differentiation existed among *S. horneri* populations: the inter-population P% was 99.4 and 100.0% by ISSR and RAPD, respectively. In the same study, the highest P% within a population was detected in the YT and NJ populations (50%). Our results showed a higher intra-population P% of 95.45% in population 3 than in Yu et al.'s study. Population 3 was the collection of fixed wild seaweeds from Nanji Islands; this population was once a suspension culture in the lab for several months and then was cultivated along the Nanji coast before sampling. The north cultivar of population 1 that originated from Dalian (north of the Yellow Sea), the local cultivar of population 2 from Nanji, and the wild samples of population 5 from the Zhoushan Islands shared a similar genetic diversity, which indicated that the diversity

of these populations had little relation to the origin areas. The floating population 4 showed the lowest diversity, which coincided with the result that the genetic diversity of floating populations is lower than that of fixed populations (Zhao, 2012). Theoretically, populations 2 and 3 originated from the same area; therefore, they should have similar genetic variation, but our results did not support this assumption.

Yu et al. (2013) reported that the genetic differentiation of the *S. horneri* populations along China's coast agreed with the geographic isolation, but some populations are genetically closer than others, even when these populations are widely separated geographically. The cluster results of our study also showed the closed genetic distance between populations 1, 2, and 5, which originated broadly from the north to south. Meanwhile, populations 1 and 3, which had the same origin, were not joined in 1 branch, which differed from the results of Zhao et al. (2008). The only difference between these populations was that population 3 was once suspension cultured in the lab. Whether this behavior changes the genetic structure of the seaweed is not clear. An interesting observation was that the floating population 4 was separated from the other populations. The genetic distance relationship and the cluster dendrogram showed that population 4 was different from the other populations. These results did not support the hypothesis that the floating *S. horneri* possibly originated in the southern part of the East China Sea (Pang et al., 2009; Abé et al., 2013).

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