Chinese Journal of Oceanology and Limnology
Vol. 30 No. 2, P. 225-230, 2012
DOI: http://dx.doi.org/10.1007/s00343-012-1098-7

High genetic diversity in gametophyte clones of *Undaria pinnatifida* from Vladivostok, Dalian and Qingdao revealed using microsatellite analysis*

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Received Apr. 10, 2011; accepted in principle May 19, 2011; accepted for publication May 23, 2011

Abstract Breeding practice for *Undaria pinnatifida* (Harvey) Suringar requires the screening of a large number of offspring from gametophyte crossings to obtain an elite variety for large-scale cultivation. To better understand the genetic relationships of different gametophyte cultures isolated from different sources, 20 microsatellite loci were screened and 53 gametophyte clone cultures analyzed for *U. pinnatifida* isolated from wild sporophytes in Vladivostok, Russia and from cultivated sporophytes from Dalian and Qingdao, China. One locus was abandoned because of poor amplification. At the sex-linked locus of Up-AC-2A8, 3 alleles were detected in 25 female gametophyte clones, with sizes ranging from 307 to 316 bp. At other loci, 3 to 7 alleles were detected with an average of 4.5 alleles per locus. The average number of alleles at each locus was 1.3 and 3.7 for Russian and Chinese gametophyte clones, respectively. The average gene diversity for Russian, Chinese, and for the combined total of gametophyte clones was 0.1, 0.4, and 0.5, respectively. Russian gametophyte clones had unique alleles at 7 out of the 19 loci. In cluster analysis, Russian and Chinese gametophyte clones were separated into two different groups according to genetic distance. Overall, high genetic diversity was detected in gametophyte clones isolated from the two countries. These gametophyte cultures were believed to be appropriate parental materials for conducting breeding programs in the future.

Keyword: *Undaria pinnatifida*; microsatellite analysis; sex-linked locus; genetic distance; variety breeding

1 INTRODUCTION

As one of the seven commercially important seaweed species, *Undaria pinnatifida* (Harvey) Suringar has been farmed in northern coastal waters of China for more than 20 years, reaching an annual production of ca. 300 000 tons wet weight. Unlike *Saccharina japonica* (Areschoug) Lane, Mayes, Druehl and Saunders, studies on varietal breeding of *U. pinnatifida* have lagged behind (Wu and Lin, 1987). Dozens of elite varieties of *S. japonica* have been bred so far, increasing the yield of this seaweed dramatically (Zhang et al., 2007; Li et al., 2007, 2008a). In comparison, there has been no officially accepted variety for *U. pinnatifida*. This seaweed is destined for the export market and thus quality standards are high. However, after a few generations of open sea cultivation, the commercial traits of the offspring degenerate gradually, which is probably due to inbreeding depression (Wang et al., 2007) and new parental plants need to be introduced to reverse this. In China, cultivation of both *U. pinnatifida* and *S. japonica* suffer from the problems of degeneration and contamination of varieties in sporeling production phases. The ultimate reason for all of these problems is the lack of an optimal operation system to breed and maintain the selected varieties. Recently in China, pairwise hybridization using separately

* Supported by the National Special Research Fund for Non-Profit Sector (Agriculture) (No. 200903030), the Agriculture Science Technology Achievement Transformation Fund (No. 2010GB24910699), and the Knowledge Innovation Programs of Chinese Academy of Sciences (No. KSCX2-EW-B-3)
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propagated vegetative gametophyte clones and artificially selecting elite hybrids has been approved as a reliable method for selecting desired varieties for large-scale cultivation in species in Laminariales (Pang and Wu, 1996; Li et al., 2007, 2008a). Sporophytic offspring derived from mono-crossing of gametophyte clones possess identical genotypes (Shan and Pang, 2009). In addition, the same crossings of gametophytes can be repeatedly used. In this way, heterosis can be fully utilized by crossing gametophyte clones that are genetically distant. For *S. japonica*, two elite varieties Dongfang Nos.2 and 3 (Li et al., 2007, 2008a) have been reported recently through mono-crossing between gametophyte clones.

However, in selection practice, identification of target crossing combinations is usually based on a large number of crossings. For example, *Saccharina* variety Dongfang No.2 was selected from dozens of hybridization combinations (Li et al., 2007). Precise prediction of the hybrid performance or heterosis before performing hybridization experiments enhances the efficiency of the breeding process. Such investigations have been conducted in the guppy, maize and *Saccharina* species (Shikano and Taniguchi, 2002; Kiula et al., 2008; Li et al., 2008b). Shikano and Taniguchi (2002) revealed that genetic distance measured by molecular markers positively correlated with the degree of heterosis in guppies (*Poecilia reticulata*). Kiula et al. (2008) found a tight association between genetic distance and first filial performance in the intergroup in tropical maize. Similar results were obtained in species of *Saccharina*. Li et al. (2008b) found a significant relationship between the genetic distance of parental *Laminaria* (*Saccharina*) gametophyte clones measured using microsatellite markers and heterosis of agronomical traits of the first filial hybrids. All of these investigations have indicated that genetic distance between parental individuals, computed based on molecular markers such as microsatellites, AFLP and others, could be used to estimate the heterosis of their hybrids.

Microsatellites have been the marker of choice for a range of genetic studies because of their merits of codominance, high polymorphism and even genomic distribution (Liu and Cordes, 2004). Daguin et al. (2005) developed 20 microsatellite markers for *U. pinnatifida*. One microsatellite locus (Up-AC-2A8) was shown to be specifically linked to the female gametophyte (Shan and Pang, 2010). The objective of this study was to analyze the genetic diversity of representative gametophyte clones of *U. pinnatifida* isolated from Vladivostok, Dalian, and Qingdao. The results of this investigation can help to select optimal crossing combinations of gametophyte clones in future breeding practice.

2 MATERIAL AND METHOD

2.1 Sample information

Fifty-three gametophyte clones of *U. pinnatifida* were used for genetic analyses in this study (Table 1). Ten were isolated from five wild parental sporophytes growing in Vladivostok, Russia, five of them were from four cultivated parental sporophytes grown on longlines in a farm in Qingdao, China and the other 38 from 22 cultivated individuals grown on longlines in two farms in Dalian, China. Release of zoospores and isolation of gametophyte clones were conducted according to the method of Shan and Pang (2009). All the gametophyte clone cultures were preserved in sterilized Provasoli Enriched Seawater (PES) (Starr and Zeikus, 1987) at 20°C in dim florescent white light (2–3 μmol photon/m^2/s) under a 12:12 h light:dark regime. A code for gametophyte clone culture was given in the form: "Year of isolation – Parental sporophyte individual number – Gametophyte clone number ♀/♂".

Table 1 Sampling locations of the parental sporophytes of *Undaria pinnatifida* in this study

Code	Location	Code	Location
08-VL3-2♀	1	07-19-7♂	3
08-VL3-6♂	1	07-19-8♀	3
08-VL4-2♂	1	07-21-3♂	3
08-VL4-3♀	1	07-22-4♀	3
08-VL5-1♀	1	07-22-4♂	3
08-VL5-6♂	1	07-22-5♀	3
08-VL10-1♂	1	07-26-7♂	3
08-VL10-2♀	1	07-26-8♂	3
08-VL14-2♀	1	07-27-2♂	3
08-VL14-3♂	1	07-C-1♂	3
08-DL401-1♂	2	07-E-2♂	3
08-DL401-3♀	2	07-F-1♀	3
08-DL404-3♂	2	07-F-2♂	3
08-DL404-4♀	2	07-G-3♀	3
08-DL406-1♂	2	07-I-1♂	3
08-DL406-3♀	2	07-I-3♀	3
08-DL415-4♀	2	07-K-4♀	3
08-DL415-5♂	2	07-K-10♀	3
08-DL416-4♂	2	07-K-11♂	3
08-DL416-4♀	2	07-K-24♂	3
08-DL424-6♂	2	07-Z-15♀	3
08-DL424-6♀	2	06-C2-4♀	4
07-7-1♂	3	06-C2-11♂	4
07-7-3♀	3	06-J10-3♀	4
07-10-10♂	3	06-6-5♀	4
07-11-1♂	3	06-8-1♀	4
07-18-3♂	3		

Note: Location 1: Vladivostok, Russia, 43°12′N, 131°56′E; Location 2: Dalian, China, 38°43′N, 121°10′E; Location 3: Dalian, China, 38°49′N, 121°30′E; Location 4: Qingdao, China, 36°05′N, 120°19′E.

2.2 Genomic DNA extraction and microsatellite analyses

Genomic DNA extraction and microsatellite analyses were performed according to the procedures used in a previous study (Shan and Pang, 2009). In total, 20 microsatellite loci (Daguin et al., 2005) were used for PCR-amplification.

2.3 Data analyses

Data were recorded using visual scoring of bands at the expected size locations. Null alleles were identified as gametophyte individuals that repeatedly failed to amplify at some loci but amplified for other loci (Muhlin and Brawley, 2009). Number of alleles (N_a) across all loci, gene diversity (H, Nei, 1973) and Nei's genetic distance (Nei, 1978) among all gametophyte clones were computed using POPGENE version 1.31 (Yeh et al., 1999). Based on the genetic distance matrix a dendrogram was constructed to show the relationships among all of the gametophyte clones using TFPGA software (Miller, 1997). During bootstrapping, 1 000 permutations were performed to evaluate the robustness of the groupings.

3 RESULT

PCR products failed to be amplified repeatedly in a large number of samples at microsatellite locus Up-AC-1B2, thus this locus was abandoned. Null alleles were identified for locus Up-AC-2E8 in Russian gametophyte clones. At the female-linked locus Up-AC-2A8, three alleles were generated from 25 female gametophyte clones. The five gametophyte clones from Russia possessed the largest allele, with a sequence length of 316 bp. One gametophyte clone 08-DL416-4♀ from China had the smallest allele with a sequence of 307 bp and the other female gametophyte clones possessed an allele of 313 bp (Fig.1).

At the other 18 microsatellite loci, 3 to 7 alleles were found at each locus, with Up-AC-1C1 having the most alleles. The average number of alleles found at each locus was 1.3 and 3.7 for Russian and Chinese gametophyte clones, respectively, with an overall average of 4.5 when all gametophyte samples were combined. For the 43 gametophyte clones from China all 18 microsatellite loci were polymorphic, whereas 12 microsatellite loci were monomorphic for the 10 gametophyte clones from Russia. Russian gametophyte clones had unique microsatellite alleles at locus Up-AC-1B5, Up-AC-1H4, Up-AC-1H5, Up-AC-2B2, Up-AC-4G2, and Up-AC-4G9. The average gene diversity of Russian gametophytes was 0.1, ranging from 0 to 0.42, and that of Chinese gametophytes was 0.4, ranging from 0.13 to 0.68. The average gene diversity for all gametophytes combined was 0.5 (Table 2).

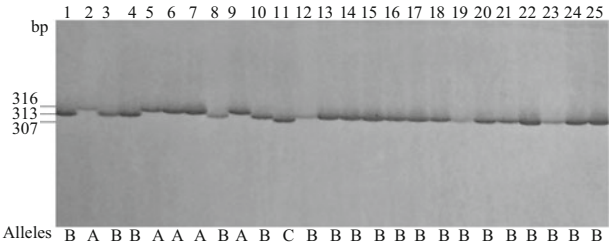

Fig.1 Electrophoresis profile of PCR-amplification bands of 25 female gametophyte clones of *Undaria pinnatifida* at the sex-linked microsatellite locus Up-AC-2A8

The three alleles that were amplified are indicated as A, B and C with sequence length of 316, 313 and 307 bp, respectively. Lanes 1-25 indicate 08-DL416-4♀, 08-VL4-3♀, 08-DL424-6♀, 08-DL404-4♀, 08-VL10-2♀, 08-VL14-2♀, 08-VL5-1♀, 08-DL401-3♀, 08-VL3-2♀, 08-DL415-4♀, 08-DL406-3♀, 07-F-1♀, 07-G-3♀, 07-I-3♀, 07-7-3♀, 07-19-8♀, 07-22-4♀, 07-22-5♀, 07-Z-15♀, 06-J10-3♀, 07-K-4♀, 06-C2-4♀, 06-6-5♀, 07-K-10♀, 06-8-1♀, respectively.

Cluster analysis based on the genetic distance separated all the gametophyte clones into two groups which corresponded to Russia and China respectively (Fig.2). 08-VL10-1 ♂ and 08-VL10-2 ♀ and

Table 2 Number of alleles and gene diversity of the gametophyte clones of *Undaria pinnatifida* at eighteen microsatellite loci

Locus	Russia		China		Total	
	N_a	H	N_a	H	N_a	H
Up-AC-1B5	2	0.32	5	0.29	6	0.51
Up-AC-1C1	1	0	7	0.62	7	0.54
Up-AC-1C9	1	0	3	0.35	3	0.29
Up-AC-1G2	1	0	5	0.65	5	0.58
Up-AC-1H4	2	0.32	4	0.49	5	0.64
Up-AC-1H5	1	0	4	0.35	5	0.54
Up-AC-2A2	2	0.32	5	0.62	5	0.66
Up-AC-2B2	2	0.32	4	0.29	6	0.51
Up-AC-2B4	1	0	3	0.21	3	0.44
Up-AC-2C1	2	0.42	5	0.61	5	0.69
Up-AC-2E8	-	-	5	0.58	5	0.58
Up-AC-3D1	1	0	3	0.21	3	0.42
Up-AC-3H12	1	0	3	0.21	3	0.17
Up-AC-4C12	1	0	4	0.63	4	0.70
Up-AC-4E9	1	0	5	0.68	5	0.74
Up-AC-4G2	1	0	2	0.13	3	0.39
Up-AC-4G9	1	0	4	0.21	5	0.45
Up-AC-4H6	1	0	3	0.13	3	0.38
Average	1.3	0.1	3.7	0.4	4.5	0.5

Note: N_a: number of alleles; H: Nei's (1973) gene diversity; null alleles are indicated by the symbol "-".

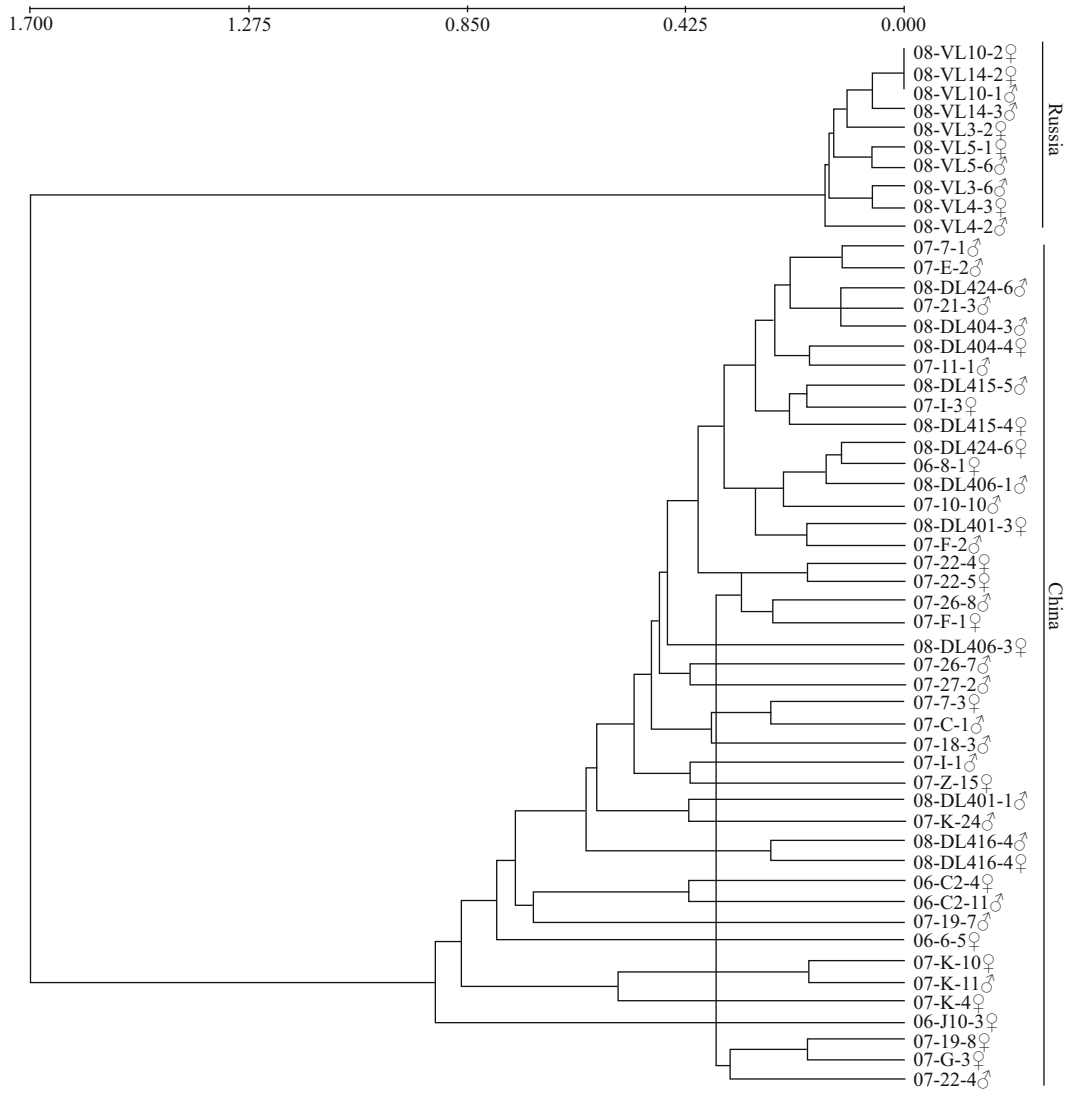

Fig.2 Dendrogram of 53 gametophyte clones of *Undaria pinnatifida* based on Nei's genetic distance

The scale bar at the top represents the corresponding genetic distance.

08-VL14-2 ♀ could not be distinguished based on genetic distance revealed by the 18 microsatellite loci because they had uniform genotypes at all loci. The gametophytes which were derived from the same parental sporophyte plant were not necessarily grouped together. For example, gametophytes 08-VL14-2 ♀ and 08-VL14-3 ♂, 08-VL3-2♀ and 08-VL3-6♂ were genetically distant within the cluster. This was likely to relate to the heterozygous nature of their parental sporophyte plants.

4 DISCUSSION

The most important result of the present study was the detection of high genetic difference between Russian and Chinese gametophyte clones. Russian individuals possessed unique alleles at 7 of the 19 microsatellite loci analyzed including the sex-linked locus. In the cluster analysis, Russian and Chinese gametophyte clones separated into two conspicuously different groups with high genetic distance, similar to previous observations in *S. japonica* using AFLP markers (Shan et al., 2011). There is a large geographical distance between Vladivostok and Dalian/Qingdao and the Korean peninsula, which is located in the middle of these two populations, blocks transport via currents between Russian and Chinese waters. Thus it is reasonable to consider that geographical isolation has made gene exchange between Russian and Chinese populations of *U. pinnatifida* rare. In addition, the surface seawater temperature between the two regions is different. The temperature ranges of Vladivostok, Dalian and

Qingdao are 0°C–24°C (Skriptsova et al., 2004), 2°C–25°C and 4°C–26°C respectively. As a result the genetic variations between these two populations may reflect adaptations to their different environments. The average number of alleles at each locus was 4.5, slightly higher than that detected by Daguin et al. (2005), who recorded an average number of 4.2 alleles from three populations from Japan, New Zealand, and France. In particular, three alleles were detected at the sex-linked locus Up-AC-2A8 in Russian and Chinese gametophytes, more than the two alleles detected by Daguin et al. (2005) in three populations of sporophytes. It is thus confirmed that the sex-linked microsatellite marker found in female gametophytes is locus specific rather than sequence or allele specific.

The results of this study also revealed high genetic diversity in Chinese gametophyte clones. The Chinese gametophyte clones used in the present study were all derived from cultivated sporophyte individuals. The average number of alleles and gene diversity was 3.7 and 0.4 at each locus, respectively, which was higher than that found by Daguin et al. (2005) from a population from Nagasaki, Japan. In the farming grounds of China, parental plants need to be introduced from time to time to solve the problem of degeneration in cultivated populations and to thus keep the resource economically viable. As a result, the cultivated populations of *U. pinnatifida* in China are expected to be complex having different genetic backgrounds. These expectations were in accordance with the high genetic variations detected in the gametophytes from the three farming sites in China. In the cluster analysis, the sister gametophyte clones, i.e. the gametophyte clones derived from the same paternal sporophyte were not distinctly grouped. On one hand, this result revealed the heterozygous status of the parental plants for some microsatellite loci. On the other hand, it indicated that 18 microsatellite loci were not sufficient to assign all the gametophyte clones accurately in accordance with their pedigrees. The fact that three gametophyte clones did show an identical genotype in the 18 microsatellites and could not be distinguished in cluster analysis supports this. Therefore, the application of more microsatellite loci in combination with multi-locus markers such as AFLP would better decipher the genetic relationships of the gametophyte clones used in this study.

This is the first analysis of the genetic relationship between parental individuals with the purpose of breeding new varieties of *U. pinnatifida*. The high genetic diversity of the gametophyte clones used in this investigation, should allow heterosis to be obtained through hybridization between gametophytes with high genetic distance, particularly Russian vs. Chinese crossing combinations. The first filial hybrids derived from different crossing combinations of the gametophyte clones are currently growing on longlines in the farming ground and will be evaluated in the future. The field test data and the genetic distance data obtained in this future study and the present study could be correlated and can guide future breeding programs.

5 CONCLUSION

High genetic diversity was detected in gametophyte clones isolated from Vladivostok, Russia, and Dalian and Qingdao, China. These gametophyte cultures are believed to be appropriate parental materials for conducting breeding programs in the future.

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