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Development of expressed sequence tag-derived microsatellite markers for *Saccharina* (*Laminaria*) *japonica*

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Abstract Expressed sequence tag-derived microsatellite markers (EST-SSR) were generated and characterized in *Laminaria japonica* using data mining from updated public EST databases and polymorphism testing. Fifty-eight of 578 ESTs (10.0%) containing various repeat motifs were used to design polymerase chain reaction (PCR) amplification primers. A total of 12 pairs of primer were generated and used in the PCR amplification. Alleles per locus ranged from two to ten (average of 5.7). The observed heterozygosities and expected heterozygosities were from 0.045 to 0.543 and from 0.056 to 0.814, respectively. All loci were in Hardy–Weinberg equilibrium and no linkage disequilibrium was detected. These robust, informative, and potentially transferable polymorphic markers appear suitable for population, genetic, parentage, and mapping studies of *L. japonica*.

Keywords Phaeophyta · Microsatellite · EST · Molecular marker

Introduction

Useful as food and as raw material for algin, mannitol, and iodine extraction, *Laminaria japonica* (Areschoug) Lane, Mayes, Druehl & Saunders (Lane et al. 2006) was introduced into China for cultivation in 1927 from Hokkaido, Japan (Tseng 1983). Innovation and technology developed in the 1950s has made Chinese *L. japonica* cultivation the largest mariculture industry in the world (Zemke-White and Ohno 1999). Many genetic studies have been conducted in efforts to improve *L. japonica* breeding in China, and molecular marker systems have been applied in germplasm identification (Wang et al. 2004), genetic diversity analysis (Yotsukura et al. 2001; Wang et al. 2005; Xia et al. 2005), parentage analysis (Billot et al. 1999), genetic mapping (Li et al. 2007), and prediction of heterosis of hybrids (Li et al. 2008). There are now 18 genomic simple sequence repeats (SSRs) for *L. japonica* (Shi et al. 2007).

While developing SSRs from genome traditionally involves time-consuming and labor-intensive construction, enrichment and sequencing of genomic libraries (Edwards et al. 1996), identifying SSRs from expressed sequences, is fast, efficient, and relatively low cost (Bouck and Vision 2007). EST-derived simple sequence repeat markers (EST-SSRs) associated with known function genes can be more useful than genomic SSR markers for comparative gene mapping. EST-SSRs facilitate physical mapping and tend to be widely transferable between species and even between genera (Liu et al. 1999).

With the hope of improving germplasm identification, genetic diversity analysis, parentage analysis, prediction of heterosis of hybrids, and genetic linkage mapping, the first set of EST-SSRs in *L. japonica* was developed. A total of 578 *L. japonica* EST sequences from GenBank were screened for di-, tri-, tetra-, penta-, and hexanucleotide microsatellite repeats using the SSRHUNTER program (http://www.bio-soft.net/dna/). Screening criteria include a minimum of five repeat units for dinucleotide and tetranucleotide and a minimum of four repeat units for tetra-,

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Table 1 Characterization of ten polymorphic EST-SSR primers in *L. japonica*

Number	Accession no.	Motif	Primer sequence (5′–3′)	T_a (°C)	N_a (size range, bp)	H_o	H_e	P
1	CX943148	(ta)5	F: TGAACTGACGAGGTAGAGC R: TTGTATTAGAGGCAAGAGC	49	7 (300–359)	0.543	0.694	0.456
2	CX943137	(cg)5	F: CGAAGATGTCGCAGAACG R: CGCAAAGGCTACGGAAAA	56	2 (449–460)	0.306	0.689	0.234
3	CX943083	(agac)5	F: GCGTGGGAGGCTTTCTTC R: CGTGGGATTGCTGATACTG	56	5 (370–420)	0.045	0.505	0.346
4	CX943071	(tgc)11	F: GGTAGCCACTTGAAGATAACG R: TCTGAGGGAAGAGGAGGG	54	2 (494–500)	0.359	0.306	0.086
5	CX943061	(gcct)18	F: CGGCTTCATCTCCCACAG R: TGACGGAATAGACCCAAA	53	7 (450–496)	0.524	0.706	0.933
6	CX942986	(gt)5	F: ATCATACAAAGCAGACCG R: AAAATCAAGGCAAGGACC	50	10 (290–400)	0.129	0.814	0.452
7	CX942986	(cag)5	F: AACCGCTCTTTCCACCCA R: TCAAGGCAAGGACCGTAG	56	4 (210–256)	0.195	0.182	0.683
8	CX942983	(ac)5	F: GCAGGCTCGTGTCGTGTA R: CGCAGCATCAAGAAGGTA	53	9 (246–320)	0.165	0.169	0.556
9	CX942859	(tgc)7	F: CAATAATCGCAGAAAGGG R: CCGTCCGAACAACCAACT	54	5 (448–499)	0.057	0.056	0.524

The observed heterozygosity (H_o), expected heterozygosity (H_e), and probability of Hardy–Weinberg equilibrium (P) were calculated for 96 *Laminaria japonica* individuals

T_a annealing temperature, N_a number of alleles

penta-, and hexanucleotide. Fifty-eight SSR loci were identified and used for microsatellite marker optimization. Twelve primers were designed using the primer premier 5.0 program (http://www.premierbiosoft.com/), which were designed to define product sizes ranging from 200 to 500 bp in length.

Ninety-six *L. japonica* individuals were collected from an aquafarm located in Rongcheng City, China (N36.53, E118.44) and preserved in our lab, IOCAS. Genomic DNA was extracted from kelp sporophytes using a plant genomic DNA kit (Tiangen Biotech Co., Ltd, Beijing, China) according to manufacturer's instructions. Polymerase chain reactions (PCRs) were carried out in a total volume of 20 μL containing 0.5 U *Taq* DNA polymerase (MBI), 1× PCR buffer, 0.2 mM dNTP mix, 0.8 μM of each primer pairs, 2.0 mM $MgCl_2$, and about 50–100 ng template DNA. PCR conditions were as follows: one 4-min denaturation cycle at 94°C, followed by thirty-five 1-min cycles at 94°C, 1 min at annealing temperatures (see Table 1), 1 min at 72°C, and 10 min at 72°C. Amplification products were resolved via 6% denaturing polyacrylamide gel (0.4 mm× 30 cm×40 cm) and visualized by silver staining. The expected and observed heterozygosities were calculated using the TFPGA program (Miller 1997). Hardy–Weinberg equilibrium and linkage disequilibrium were tested by Genepop 3.4 software (Raymond and Rousset 1995).

Results and discussion

Eleven of the 12 designed primers amplified the expected products. One primer pair yielded no products and five primer pairs yielded more products than expected, suggesting the presence of intron. For the primers with successful amplification yields, nine pairs of primer revealed polymorphism among the 96 individuals of *L. japonica* tested. The number of alleles per locus ranged from two to ten and averaged 5.6. EST-SSRs commonly have less polymorphism and fewer alleles than genomic SSRs because EST-SSRs derive from expressed conservative genes. However, the 5.7 average of alleles for EST-SSRs loci in our study is slightly higher than the 4.7 average of alleles for genomic SSRs loci (Shi et al. 2007). This abnormality may be due to the fact that our samples had higher original polymorphism, but more research is needed to clarify the abnormality. The observed and expected heterozygosities ranged from 0.045 to 0.543 and from 0.056 to 0.814, respectively (Table 1). All loci are in Hardy–Weinberg equilibrium and no linkage disequilibrium was detected between loci ($P>0.01$ in each case).

EST-SSR markers produced in this experiment may find application in germplasm identification, genetic diversity analysis, parentage analysis, prediction of heterosis of hybrids, and genetic linkage mapping in *L. japonica.*

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