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Isolation, characterization, and cross-transferability of microsatellite markers from the whitebacked planthopper (*Sogatella furcifera*)

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ABSTRACT. The whitebacked planthopper *Sogatella furcifera* (Horváth) (Hemiptera: Delphacidae) is one of the most harmful pests of rice. In this study, 18 polymorphic microsatellite markers were developed from *S. furcifera* genomic libraries using the fast isolation by amplified fragment length polymorphism of sequences containing repeat protocols. Microsatellite polymorphism was investigated using 32 individuals from one natural population. These 18 simple sequence repeat markers showed a number of alleles that ranged from 3 to 15 and had observed and expected heterozygosities that ranged from 0.094 to 0.871 and from 0.148 to 0.924, respectively. The high cross-species transferability of these markers was evaluated in three other planthopper species: *Nilaparvata lugens*, *N. muiri* China, and *N. bakeri* (Muir). These microsatellite markers will provide powerful tools for population genetic and ecological studies of this pest and its related species in the future.

Key words: *Sogatella furcifera*; Microsatellites; Polymorphism; Genetic diversity

INTRODUCTION

Sogatella furcifera, which is the major insect pest of rice throughout Asia, is one of the most important pests for studying the interaction between rice plants and sucking insects. It causes major damage to the rice plants directly by sucking excess plant sap from the phloem and indirectly by transmitting rice plant diseases of economic importance such as *Erynia delphacis* (Entomophthorales) (Matsui et al., 1998). On the other hand, an effect on the rice also could be induced resistance to rice blast fungus *Magnaporthe grisea* and *Pyricularia grisea*, after being infested by *S. furcifera* (Kanno and Fujita, 2003; Kanno et al., 2005).

However, little is known about the economic importance, population genetic structure, and genetic diversity of this pest. Previously, the genetic basis for wing dimorphism was studied, and an internal transcribed spacer of the ribosomal RNA and *cox* sequences of the mitochondrion were used to distinguish regional populations (Matsumura, 1996; Fu et al., 2012; Matsumoto et al., 2013). In two other planthoppers, the smaller brown planthopper *Laodelphax striatellus*, and the brown planthopper, *Nilaparvata lugens*, many molecular markers have been used to study the population ecology and evolution, but none have been used to study *S. furcifera* (Jing et al., 2012a,b; Sun et al., 2012; Jairin et al., 2013). Microsatellites, also known as simple sequence repeats (SSRs), which are typically highly polymorphic, remain the most popular markers in population genetic studies (Guichoux et al., 2011). However, the lack of SSR primers that were developed specifically for *S. furcifera* bottlenecked progress in studies of its population genetics. In order to study the genetic diversity and structure of populations of *S. furcifera*, we developed and characterized 18 genomic SSR markers.

MATERIAL AND METHODS

S. furcifera samples were collected from rice fields in Wuhan, Hubei Province, China. We used 32 female adults from these natural populations to determine the level of polymorphism at these isolated microsatellite markers. Three other planthopper species, *N. lugens*, *N. muiri* China, and *N. bakeri* (Muir), were collected from rice and *Leersia hexandra* plants in fields in Wuyishan, Fujian Province, China, and were used to perform a cross-amplification test.

Genomic DNA was extracted from a single individual using the cetyltrimethylammonium bromide method (Tang et al., 2010). The fast isolation by amplified fragment length polymorphism of sequences containing repeat protocols was employed to construct the $(AC)_{13}$- and $(AG)_{13}$-enriched partial genomic libraries (Zane et al., 2002). Then, the fragments containing microsatellite repeats were ligated into the pUC18-T vector (TaKaRa, Daliang, China) and transformed into TOP10 *Escherichia coli* cells. Finally, positive recombinant clones with suitable insert length were identified and sequenced using an ABI 3730 DNA sequencer. One hundred and thirteen sequences were obtained and screened for the SSR motifs using the Simple Sequence Repeat Identification Tool (SSRIT) software available at http://www.gramene.org/db/markers/ssrtool. As a result, 70 sequences were confirmed to carry at least one microsatellite locus. Seventy-nine primer pairs were designed using BatchPrimer3 (You et al., 2008). A PTC-100 thermal cycler (MJ Research) was used for all polymerase chain reaction (PCR) amplifications, which were performed in 10-µL volumes according to the following conditions: 10 ng template DNA, 0.3 µM of each of the two primers, 0.2 mM deoxynucleotide triphosphates, 2.5 mM $MgCl_2$, 1X PCR buffer, and 1 U Taq DNA polymerase (Fermentas). The thermal profile for all loci consisted of an initial denaturation at 94°C for 5 min; 35 cycles

of 94°C for 15 s, 55°C for 15 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. Amplification fragments were separated by electrophoresis on 6% denaturing polyacrylamide gels that were run at a constant power of 60 W and then visualized by silver staining (Ji et al., 2007). Allele sizes were determined using pBR322 DNA/*Msp*I DNA size markers (Tiangen Biotech).

The number of alleles, observed heterozygosity, expected heterozygosity, tests for linkage disequilibrium, and deviations from Hardy-Weinberg equilibrium were calculated by the Arlequin 3.1 software (Excoffier et al., 2005). The cross-species amplification was classified as no PCR product; equivocal output - unclear, weak, or inconsistent PCR product; monomorphic locus in the tested set of isolates; and polymorphic locus in the tested set of isolates.

RESULTS AND DICUSSION

Forty-two of 79 primer pairs were successfully tested in natural populations of *S. furcifera*, and the amplification product sizes were within the expected size range; the other primer pairs showed multi-banding patterns on gels or no amplification. Eighteen markers were polymorphic in the natural population of *S. furcifera*. The number of alleles ranged from 3 to 15 per locus, with a mean number of 7.3 per locus. The observed and expected heterozygosities ranged from 0.094 to 0.871 (mean 0.572) and from 0.148 to 0.924 (mean 0.633), respectively (Table 1).

Table 1. Characteristics of 18 microsatellite markers of *Sogatella furcifera.*

Locus name	Repeat motif	Primer sequence (5'-3')	*S*	N_A	H_O	H_E	*D*	GenBank No.
WM1	$(TC)_2$	F: ATCTCCCTAATCAGGAATCTG R: GAATGGGAAGAGGAAAATAGA	134-164	10	0.667	0.712	NS	KF245615
WM2	$(CT)_2$	F: TAACACTTGTTGGTTTTCCAT R: CTAATGGAATGGTGTGTTTTG	130-164	15	0.871	0.924	NS	KF245616
WM4	$(TC)_2$	F: TGATCACAGCATTATCCTAGC R: TGAAGCACAAGAGAGAGAGAG	172-192	10	0.857	0.905	NS	KF245617
WM9	$(TG)_2$	F: GAAAAGAGAATAGGCATACCC R: TGTACATGGATCGTACATCTG	148-160	7	0.688	0.830	*	KF245618
WM11	$(GA)_2$	F: AGTCCTGAGTAACACTCAATCA R: CCATACAGTACCACTCCACTG	156-176	11	0.839	0.863	NS	KF245619
WM12	$(CA)_2$	F: CCAACAAAAACAACAAAGTTC R: TCGAGACGAGATAAACAAAAG	136-152	6	0.219	0.260	NS	KF245620
WM22	$(CT)_2$	F: ATTTTATCCCCTCATTCACTC R: TCTGTTAGCTATTATCCCTGCT	154-164	6	0.688	0.795	*	KF245621
WM25	$(AC)_2$	F: GCACACTTGCCTGAAGTAA R: GTCCTGAGTAACACTCAAACG	146-148	4	0.430	0.491	NS	KF245623
WM30	$(GT)_2$	F: CGATAACTGTGGATGGAATAA R: TTGAGCTTAGACAAGAGTTGTG	134-160	7	0.625	0.646	NS	KF245624
WM32	$(TCTCT)_5$	F: TCTTTGAACTTTACCAACTGC R: AACTTTTCGAATCTACCCATC	140-155	4	0.438	0.446	NS	KF245625
WM40	$(AG)_2$	F: CTTGTGAAACAAGCTACAAGC R: ATTCACACCCTCTTCCTCTAA	136-152	5	0.484	0.489	NS	KF245626
WM50	$(GT)_2$	F: ACAAGACATTGTTCAGACCAC R: CTCTGCTATGCTAACCCTTTC	186-208	12	0.839	0.896	NS	KF245627
WM57	$(TTC)_3$	F: TTTTTCTACTCCCCTCAACAT R: TAGAAAGAGAAAGAGGGGATG	154-169	4	0.406	0.563	NS	KF245628
WM59	$(GAGT)_4$	F: TGATTGAACGAGAGAGAAAGA R: ACACAGACAGGAAACAGGTAA	140-172	9	0.656	0.704	NS	KF245629
WM69	$(CCT)_3$	F: CACACCCATATAGCAAGAAAA R: TGGAGAAAAAGAAGAGTGACA	154-160	3	0.094	0.148	NS	KF245630
WM78	$(CA)_2$	F: CCCTTTCTCTCTTTCACTCTC R: TAAGGCGGCCTGACTTCG	136-166	10	0.742	0.767	NS	KF245631
WM79	$(TG)_2$	F: GGAAAACCGTTATTGTTTTGT R: CAGACTCGAAGAGATTTGTTT	164-178	6	0.531	0.630	NS	KF245632

S = size range (bp); N_A = number of alleles; H_O = observed heterozygosity; H_E = expected heterozygosity; *D* = deviation from Hardy-Weinberg equilibrium; NS = not significant. *Significant deviations from Hardy-Weinberg expectations ($P < 0.05$).

The degree of polymorphism of WM2 was the highest in the population. No significant deviation from Hardy-Weinberg equilibrium was detected excepted for the loci WM9 and WM22. Most of the polymorphic loci were found to be independent in this natural population, whereas there was linkage disequilibrium between WM59 and WM1, WM11, or WM50 and between WM11 and WM79.

The cross-species transferability was high for these newly developed microsatellite markers of *S. furcifera*; this was demonstrated by amplifying the SSR markers in the three other planthopper species, *N. lugens*, *N. muiri* China, and *N. bakeri* (Muir). Among the 18 polymorphic markers, only three markers (WM57, and WM69) could not be detected in the three additional species. Seven of the 15 remaining markers were polymorphic in the three related species, and 9 markers successfully amplified in at least one species (Table 2). The number of markers that could successfully amplify in each species was similar.

Table 2. Cross-species amplification of the 18 microsatellite markers.

Locus	Species		
	Nilaparvata lugens	*Nilaparvata muiri* China	*Nilaparvata bakeri* (Muir)
WM1	P	P	P
WM2	M	M	±
WM4	P	P	±
WM9	-	M	-
WM11	P	P	P
WM12	-	-	M
WM22	±	±	P
WM25	P	P	P
WM27	-	-	-
WM30	-	P	M
WM32	P	P	P
WM40	P	M	M
WM50	P	P	P
WM57	-	-	-
WM59	P	P	P
WM69	-	-	-
WM78	M	-	-
WM79	P	P	P

(-) = no PCR product; (±) = equivocal output - unclear, weak, or inconsistent PCR product; M = monomorphic locus in the tested set of isolates; P = polymorphic locus in the tested set of isolates.

This is the first time that genomic SSR markers were developed in *S. furcifera*. The cross-transferability revealed that these markers could be used to study *S. furcifera* and related species. Therefore, these microsatellite loci will be useful for future genetic diversity studies and population genetic structure analysis of *S. furcifera*.

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