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Development and characterization of 10 microsatellite markers from *Wisteria floribunda* (Fabaceae)

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Abstract

Wisteria floribunda is a deciduous liana species widely distributed in Japan. It is essential to evaluate the clonal structure of this species because high clonal ability enables lianas to make dense colonies after canopy gaps occur. Therefore, we developed 10 microsatellite markers for *W. floribunda* from genomic shotgun sequences available in the publicly available database to study clonal structure and life history strategy of this species. The number of alleles per locus ranged from 7 to 16, with an average of 10.2. The observed and expected heterozygosity ranged from 0.483 to 0.926 and from 0.642 to 0.916, with averages of 0.769 and 0.800, respectively. These findings will contribute to the understanding of ecological roles and management of this species.

Keywords: *liana, Fabaceae, microsatellite, simple sequence repeat, genomic*

Introduction

Wisteria floribunda is a deciduous, liana (woody vine) species, which is commonly distributed in Japan, and is often predominant in cool temperate forests (Satake et al. 1989; Mori et al. 2016). This species is dependent on large-scale past disturbances (Mori et al. 2016), and has high clonal ability because of its stolon that spreads along the ground surface (Sakai et al. 2002). Because high clonal ability enables lianas to make dense colonies after canopy gaps occur (Yorke et al. 2013), it is essential to evaluate the clonal structure with sufficient number of individuals in large areas (> 1 ha) of their natural habitat including growth under various conditions (e.g., canopy gap after disturbance). However, the clonal ability of this species was revealed by excavation experiments with only three large *W. floribunda* individuals (Sakai et al. 2002). Genetic tools, such as microsatellite markers, are necessary for such conditions. Furthermore, lianas are often problematic in forest management and conservation; for instance, they cause negative impact on host trees (Ladwig and Meiners 2009; Ingwell et al. 2010), showing invasiveness outside of their original habitat (reviewed in Webster et al. 2006; Leicht-Young and Pavlovic 2015) and formation of hybrids with native species (Trusty et al. 2008; Zaya et al. 2015). In the present study, we developed 10 microsatellite markers for *W. floribunda* from genomic shotgun sequences available in the publicly available database. These markers would help clarify the genetic structure of *W. floribunda* and would aid in the understanding of their life history strategies, management, and conservation of temperate forests.

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Methods

Sequence data and primer design

Whole genome sequencing data for *Wisteria floribunda* were downloaded from the Sequence Read Archive (accession number SRR1265941) at NCBI (http://www.ncbi.nlm.nih.gov/sra). The total amount of sequence data was 1.9 gigabase pairs (Gbp). Reads were processed with Trim Galore! version 0.3. (Krueger 2015) and assembled de novo with CLC Genomics Workbench ver. 8.0.2 (CLC Bio; https://www.qiagenbioinformatics.com/) using default parameters to construct the contigs. All the contig sequences were used as input for the CMIB (CD-HIT-EST, MISA, ipcress and BlastCLUST) pipeline (Ueno et al. 2012) to obtain PCR primers for amplifying unique microsatellite sequences with the number of repeat units ≥6, 5, 4, 3, and 3 for di-, tri-, tetra-, penta-, and hexa-simple sequence repeats (SSRs), respectively. The contigs for which primer pairs were successfully designed were BLASTed against the NCBI nt database with an e-value of 1e-3. We selected 44 primer pairs for SSRs with the number of repeat units ≥12 and BLAST hits, and primers with tail sequences for forward primers (Blacket et al. 2012) and reverse primers (Brownstein et al. 1996) were synthesized by Eurofins Genomics (Tokyo, Japan) and Hokkaido System Science (Hokkaido, Japan).

Sampling, PCR and fragment analysis

Leaf samples from 29 individuals along the roadside were collected near Ogawa Forest Reserve (OFR) located in the north of Ibaraki prefecture, Japan. All the samples were collected at least 100-m apart to avoid collecting clonally reproduced stems.

DNA was extracted from leaf tissue (10 mg) from 29 samples using extraction buffers from DNeasy kit (Qiagen). The initial polymerase chain reaction (PCR) was performed for two *W. floribunda* individuals in a 10-μL volume containing 1X Multiplex PCR master mix (Qiagen), primer mix, and 5–10 ng of template DNA. The primer mix contained both forward and reverse primers and one of the fluorescently-labeled TAIL primers (Blacket et al. 2012). PCR was performed using the following thermal profiles: 15 min at 95°C, followed by 35 cycles of 30 s at 94°C, 90 s at primer-specific annealing temperature (Table 1), 60 s at 72°C, and then a final extension step at 60°C for 30 min. The products were analyzed using a 3130 Genetic Analyzer (Applied Biosystems) with GeneScan 600 LIZ size standard (Thermofisher). After electropherograms from each marker were critically checked for clear peak pattern using Genotyper ver. 3.7 (Applied Biosystems), we selected 16 primer pairs and constructed primer combinations for multiplex PCR using Multiplex Manager (Holleley and Geerts 2009). PCR amplification and genotyping for the rest of the samples were performed in the same way as described above.

Characterization of SSR markers

For each locus, genetic diversity indices such as the number of alleles (N_a), the effective number of alleles (N_e), observed heterozygosity (H_o) and expected heterozygosity (H_e) were calculated using GenAlEx version 6.5 (Peakall and Smouse 2012).

Results and Discussion

Of the 44 primer pairs, 10 were successfully amplified in the multiplex PCR, and exhibited polymorphism (Table 1). For the 10 polymorphic loci, the number of alleles per locus ranged from seven to 16, with an average of 10.2. The observed and expected heterozygosity ranged from 0.483 to 0.926 and from 0.642 to 0.916, with averages of 0.769 and 0.800, respectively. There were no significant deviations from HWE ($P > 0.05$) for all the loci. Significant linkage disequilibrium ($P< 0.05$) was detected between two pairs of loci: wf02–wf04 and wf06–wf09, after Bonferroni correction using FSTAT 2.9.3 (Goudet 1995). The 10 microsatellite markers described here will facilitate the investigation of genetic diversity and genetic structure within and among the populations of *Wisteria floribunda*. These findings will contribute to the understanding of ecological roles and management of this species.

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Table 1
Characteristics of 10 microsatellite loci for *Wisteria floribunda* (N = 29)

Locus ID	Forward primer sequence	Reverse primer sequence	SSR motif sequence	T_a (°C)	PCR product size range	Na	Ne	Ho	He	DDBJ Accession number
wf01	GCCTCCCTCGCGCCAGGGAGG-TACAGAAACACCACTG	GTTTCTTACTAAGGTAGTCAGGCT-CAGGCC	(TG)14	65	198 - 230	11	6.371	0.793	0.843	LC179881, LC179882
wf02	GCCTTGCCAGCCCGCAGG-CATGCCGGTTAATCAATGGATC	GTTTCTTGCACACTCTCATTCCTT-TCCTTTC	(CT)11	65	198 - 230	10	5.144	0.724	0.806	LC179883, LC179884
wf03	CAGGACCAGGCTACCGTG-AAACGCTCTGCCTCATCA-CAAGG	GTTTCTTGGGTTGGAAACTACAG-AATTGTGGCC	(TG)13	60	198 - 226	9	4.833	0.483	0.793	LC179885, LC179886
wf04	GCCTTGCCAGCCCGCGGCAGG-CATACCAATAGACCAATGC	GTTTCTTTGTGTCGAGGCCCGGA-CATTG	(CA)15	60	166 - 198	13	6.23	0.828	0.839	LC179887, LC179888
wf05	CAGGACCAGGCTACCGTGTGG-GAGAGTGATTGGAGTAAG-AAGG	GTTTCACTCCTCCTCTCCTTCTCCTT-CGC	(AG)11	60	284 - 300	7	5.496	0.769	0.818	LC179889, LC179890
wf06	GCCTTGCCAGCCCGCTAC-CAACTACCGAGCCATGCAGC	GTTTCTTAAGAGCTTCCCGAGT-CATGGAGC	(GA)11	60	110 - 130	7	5.962	0.857	0.832	LC179891, LC179892
wf07	GCCTCCCTCGCGCCACCG-AATAATTTCAGCCACTCGTGAC	GTTTCTTACGGGAATTGTTTGTCAA-GAGGCTG	(TC)11	60	158 - 202	10	3.162	0.724	0.684	LC179893, LC179894
wf08	GCCTTGCCAGCCCGCAGATG-GAGGCTTGCCATGGAACC	GTTTCTTCAAGTTCAGGCGCAGGG-AAGTG	(GAT)11	60	188 - 220	8	2.793	0.731	0.642	LC179895, LC179896
wf09	CGGAGAGCCGAGAGGTGTCTG-CGGTTAAGGTTTGTTCAGCC	GTTTCTTCTGCTGTATTCCAAGG-CAGCCTC	(TC)11	60	248 - 296	16	11.854	0.852	0.916	LC179897, LC179898
wf10	CAGGACCAGGCTACCGTGT-CAACCACGTGCATGAGAGAGCC	GTTTCTTAGCTCACAGACA-CAGGTTGGGTTC	(TC)11	60	120 - 143	10	5.695	0.926	0.824	LC179899, LC179900

Ta: annealing temperature of primer pair, Na: Number of alleles per locus, Ne: Number of effective alleles per locus, Ho: observed heterozygosity, He: expected heterozygosity

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