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Development of microsatellite markers for *Hosta capitata* (Asparagaceae) and amplification in related taxa

Mi-Jung CHOI, Jung-Hyun LEE[1], Won-Bum CHO[1], Eun-Kyeong HAN[1] and Hyeok-Jae CHOI*

Department of Biology and Chemistry, Changwon National University, Changwon, 51140, Korea
[1]Department of Biology Education, Chonnam National University, Gwangju, 61186, Korea
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ABSTRACT: Microsatellite markers were developed as a tool for phylogeographic studies of *Hosta capitata*. We also assessed cross-amplification in species closely related to *Hosta capitata*. We produced 28 polymorphic microsatellite markers by mapping 300 bp paired-end reads obtained from Illumina MiSeq data of *H. capitata*. In *H. capitata*, the number of alleles per locus ranged from 1 to 13. Observed and expected heterozygosity ranged from 0.000 to 0.844 and 0.000 to 0.832, respectively. Additionally, 13 loci were successfully transferable to the related species of *H. minor* and *H. venusta*. These markers will provide a powerful genetic tool not only for elucidating the phylogeographic patterns of *H. capitata* populations but also for studying the genetic delimitation of *H. capitata* from its related species.

Keywords: *Hosta capitata*, microsatellite, cross-amplification, population genetics, phylogeography

The temperate climate zones in East Asia are one of the biodiversity hotspots with increased endemism and plant species richness in East Asian temperate forests (Qian and Ricklefs, 2000). Many phylogeographic surveys covering countries in Asia, such as China, Korea, and Japan, have demonstrated that the high plant diversity is largely derived from dynamic changes in land configurations during the Quaternary climate oscillations (e.g., Qiu et al., 2011; Lee et al., 2013). The processes and mechanisms underlying lineage diversification are major topics in evolutionary biology, and phylogeographic analysis is an essential tool to understand the speed and spatial scale of an organism's diversification (Crisp et al., 2011; Donoghue and Edwards, 2014). In addition, differences in behaviors, such as life cycles, seed dispersal modes, and pollinators, can explain complex patterns of speciation. Thus, understanding the phylogeographic history of a given species concerning ecological factors is critical for resolving systematic controversies (Sakaguchi et al., 2018).

The genus *Hosta* Tratt. is widely used in gardening owing to its ground cover, shiny leaf surfaces, leaf pattern, and brilliant purple to white flowers (Jones, 1989; Chung, 1990). With ca. 45 species, *Hosta* is naturally distributed in Korea, Japan, China, and some parts of Russia, mainly on the edge of the forest or on grasslands in alpine regions (Chen and David, 2000; Chung, 2018). This genus was traditionally under Liliaceae (Maekawa, 1940; Fujita, 1975; Chung, 1990; Chung et al., 1991; Schmid, 1991), but more recently it has been placed in Asparagaceae (The Angiosperm Phylogeny Group et al., 2016; Jo and Kim, 2017; Lee et al., 2018). In general, the infrageneric classification of *Hosta* includes 3 subgenera and 10 sections (Schimid, 1991).

Hosta capitata (Kodiz.) Nakai [sect. *Lamellatae* F. Maek., subg. *Bryocles* (Salisb.) F. Maek.] shows the disjunct distribution by occurring in the main mountain range (Baekdudaegan) of Korea and western parts of Japan (Fujita, 1975; Chung et al., 1991). This species is distinguished in sect. *Lamellatae* by its capitate raceme (Lee et al., 2018). However, this species still has a systematic ambiguous relationship especially with its closely related *H. minor* (Baker) Nakai, *H. venusta* F. Maek., and *H. nakaiana* F. Maek. within the section.

*Author for correspondence: hjchoi1975@changwon.ac.kr

Table 1. Locality and voucher information for the *Hosta* species examined in this study.

Taxon	Locality	No. of individuals sampled	Voucher No.
H. capitata	Gwangyang, Jeollanam-do (Baegunsan Mt.)	32	*CAP-15080601*
H. capitata	Taebaek, Gangwon-do (Deadeoksan Mt.)	24	*CAP-16072901*
H. capitata	Bonghwa, Gyeongsangbuk-do (Okdollbong)	24	*CAP-16052801*
H. minor	Gunwi, Gyeongsangbuk-do (Hwasan Mt.)	24	*MIN-15062801*
H. venusta	Seogwipo, Jeju-do (Mulyeongarioreum)	24	*VEN-15072801*

Voucher specimens were deposited in the plant taxonomy laboratory of Changwon National University.

In particular, *H. nakaiana* has been treated as a synonym of *H. cpitata* (Tamura and Fujita, 2016; Jo and Kim, 2017), but its phylogenetic identity is still controversial (Schimid, 1991; Sauve et al., 2005). In addition, despite population genetic analyses based on allozyme (Park and Chung, 1997) and isozyme (Chung, 1994) data for *H. capitata*, its phylogeography and evolutionary history have not been clearly addressed so far (Schmid, 1991). Accordingly, we isolated and characterized a set of polymorphic microsatellite markers from *H. capitata* to provide a basis for analyses of evolutionary patterns driven by historical factors in East Asia. We also tested cross-amplification in its related species, *H. minor* and *H. venusta* of sect. *Lamellatae*.

Materials and Methods

To acquire an NGS library for *H. capitata*, we used the DNeasy Plant Kit (Qiagen, Seoul, Korea) to extract genomic DNA from a fresh leaf of a single plant sample collected from Baegunsan Mt., Gwangyang-si, Jeollanam-do, Korea (Table 1). We evaluated the quality of the DNA using a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). The DNA passed the quality check (concentration 317.95 ng/μL; A260/280 = 1.83; A260/230 = 2.37) was sequenced on the Illumina MiSeq platform (LAS Inc., Seoul, Korea). To identify microsatellites, sequence reads were screened using SSR-pipeline version 0.951 (Miller et al., 2013). The parameter settings for the detection of di-, tri-, or tetranucleotide motifs were flanking regions larger than 100 bp and a minimum of 10, 6, and 4 repeats, respectively. In addition, AT and TA repeats, G + C contents under 30%, and G + C contents above 80%, as well as reads with additional repeat were removed. The resulting sequences were grouped by the repeat motives and mapped to the reference using Geneious R 10.1.3. For each assembled contig, sites with identities < 80% and qualities < 50% were removed. We selected fragments with unique patterns that had less than 20 reads, two separate alleles, little variation at the site to which the primer was attached, and no additional single nucleotide polymorphisms in the flanking region. The final selected reads were deduplicated through de novo assembly and used to develop microsatellite markers. Primer pairs were designed using Primer 3 (Rozen and Skaletsky, 2000), and three sets of M13 tag sequences were added (5′-CACGACGTTGTAAAACGAC-3′, 5′-TGTGGAAT TGTGAGCGG-3′, and 5′-CTATAGGGCACGCGTGGT-3′) to the forward primer with 6FAM, VIC, and NED fluorescent dyes, respectively. For multiplex PCR (nine primer sets), each marker was 100–300 bp. To select microsatellite markers with infraspecific polymorphism for *H. capitata*, 90 microsatellite markers were verified using 32 individuals collected from Baegunsan Mt. The PCR protocol consisted of initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 56°C for 1 min 30 s, and extension for 1 min at 72°C, and a final extension at 4°C for 10 min. The PCR products were analyzed using an ABI 3730XL sequencer with the GeneScan 500LIZ Size Standard (Thermo Fisher Scientific, Waltham, MAs, USA). Allele sizes and peaks for each sample were determined using Peak Scanner version 2.0 (Thermo Fisher Scientific). The number of alleles and the values for H_E (expected heterozygosity) and H_O (observed heterozygosity) were analyzed using GeneAlEx 6.5 (Peakall and Smouse, 2005). Deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were estimated using GENEPOP 4.0 (Rousset, 2008). In addition, Micro-checker version 2.2.3 (Van Oosterhout et al., 2004) was used to determine the null allele frequencies and scoring errors.

Results and Discussion

Using the 90 newly designed primer pairs, we developed 28 polymorphic microsatellite loci from *H. capitata* (Table 2). The number of alleles per locus ranged from 1 to 13 (average, 4.7). H_E and H_O values ranged from 0.000 to 0.832

Table 2. Characterization of the 28 microsatellite loci developed from *Hosta capitata*.

Locus	Primer sequence (5'–3')	Repeat motif	Size range (bp)	GenBank accession No.
Hca004	F: GGAACTGGGCTAAGTGTAAA R: TACTGACACGCACAAAATTC	$(TC)_{10}$	124–138	MT084644
Hca005	F: GCTGGATCAACTGTAATGGA R: CAAAGCCAGGTTAGTGTACT	$(GA)_{11}$	162–186	MT084620
Hca007	F: ATGATATGCAGTTGGGATGT R: ACACACTTACATGCACACA	$(GT)_{10}$	129–133	MT084645
Hca010	F: CGAGCATCACACTAAATCAC R: AGGAGCATAATAGAACCCCT	$(TC)_{10}$	125–161	MT084621
Hca012	F: CCTGCTGAGATACACACAAT R: TGAGAGTTCTAGGGATTGGT	$(GA)_{12}$	224–244	MT084622
Hca014	F: CATTAAGAGAGGGTTGGCAT R: GGGCACTGAAGAACATAGAA	$(GAT)_{6}$	180–198	MT084623
Hca017	F: TACTGAGATCCCTTGTTCGT R: GTCAGCTCACAGGAATAGAG	$(CT)_{13}$	188–208	MT084624
Hca031	F: TGTAATCCTCTCCATCTCCA R: CAATGCACAAATCAGAGACA	$(TC)_{14}$	133–149	MT084625
Hca033	F: ATCTCGGCGTAAATCTGATC R: ATGCTTTACCTCACCATCTG	$(AGA)_{12}$	232–256	MT084626
Hca037	F: AGCTAACATTTGACTTTTGCT R: AGATTCCTGGCAAATTTCCA	$(GTG)_{7}$	151–160	MT084646
Hca038	F: AGACTGGAGAACTGCCTAA R: GATGCGAGGGTCATTCTATT	$(CT)_{13}$	192–206	MT084627
Hca040	F: TGTTCTTTGCTTGCTTCTTC R: GGTATTTCCTTTGAGAGAAACT	$(AG)_{11}$	160–168	MT084628
Hca041	F: AGACCAAATAAAAGCCTGCA R: CAGAGTTGGTTTTAAAGCGT	$(AG)_{17}$	198–220	MT084629
Hca045	F: ACTCCATTGTAACCTTCTCTC R: AGAGGTTTGGATATGGGAGA	$(TG)_{20}$	262–284	MT084630
Hca048	F: CTGCAACACATGGCAAAATA R: ATCACTCCATAGCTGATCCA	$(AG)_{17}$	248–278	MT084631
Hca050	F: AAATCTTTCAGCCGATGGAT R: AGTAATTATCAACACGGCAA	$(TC)_{15}$	203–219	MT084632
Hca051	F: TGTTAAAGGGGATTAAGTGAGA R: GGTCTCCTCCAACAAGATTT	$(GA)_{21}$	244–272	MT084633
Hca055	F: AGAGTAAGAGACACACGGAA R: GGTTTGGGCCATCTCTATC	$(GA)_{11}$	132–142	MT084634
Hca057	F: TCTAAAGATAAAATGGGCCTCT R: TTTTCTGCATTTACCTAACACC	$(AG)_{17}$	203–236	MT084635
Hca062	F: TCGATACGGCTTAGACAAAG R: GGAAAATTGTGGTCTTCGTC	$(CT)_{12}$	203–217	MT084636
Hca065	F: TGGAAGCAAGTGGTGAAATA R: ACTTGTGTTGCCTATTCAAAC	$(GT)_{17}$	190–218	MT084637
Hca066	F: AAACCGGCATGACCAATTTT R: TGGGTCTAGTTACTCATTCCA	$(GT)_{11}$	253–260	MT084647
Hca069	F: TGTTGTAGATACAGAGCACG R: TAGATGCATGAGAAGGAAGC	$(GA)_{13}$	214–246	MT084638
Hca070	F: CAGAACTCATCACTGGACTC R: GGAACTCTGCTCTTCAATCA	$(CT)_{11}$	122–138	MT084639
Hca071	F: AAGGAGTACAAACAGCAACA R: GTCCTACAGAACTCGTCCT	$(AGA)_{8}$	163–181	MT084640
Hca072	F: CTCAAACCAGCTGATGCATA R: TTTGAAGCTTCTTGACCCAT	$(GA)_{11}$	200–224	MT084641
Hca085	F: GCGCCCTCAGTTATATTCAA R: GGAGATCAGAGGAGTCAGTA	$(AC)_{14}$	126–140	MT084642
Hca086	F: CTGATTTTAACGTCCATGGAC R: TTTGGCATTTTGGAGATCCT	$(AC)_{10}$	196–202	MT084643

and from 0.000 to 0.844, respectively. After Bonferroni correction, three markers (Hca069, Hca70, and Hca72) showed a significant deviation from HWE ($p < 0.0018$), but the deviation was not consistent across the three populations of *H. capitata* (Table 3). We tested the 28 loci for cross-amplification in its two closely related species of *H. minor* and *H. venusta* (Table 4). From the results, 13 loci were successfully amplified and were polymorphic in both *H. minor* and *H. venusta*, with 2–10 alleles per locus. The newly developed microsatellite markers will provide a powerful genetic tool for elucidating the phylogeographic patterns of *Hosta capitata* populations in Korea and Japan. Furthermore, these results will be beneficial for studying the genetic delimitation of *H. capitata* and its related species in sect. *Lamellatae*.

Table 3. Genetic properties of 28 microsatellite loci in separate populations of *Hosta capitata*.

Locus	*H. capitata*								
	Baegunsan Mt. ($n = 32$)			Daedeoksan Mt. ($n = 24$)			Okdollbong ($n = 24$)		
	A	H_E	H_O	A	H_E	H_O[a]	A	H_E	H_O[a]
Hca004	5	0.286	0.258	1	0.000	0.000	1	0.000	0.000
Hca005	8	0.743	0.645	3	0.569	0.583	3	0.436	0.261*
Hca007	3	0.606	0.594	1	0.000	0.000	2	0.478	0.292
Hca010	9	0.439	0.438	6	0.734	0.750	5	0.688	0.583
Hca012	7	0.644	0.531	7	0.737	0.667	3	0.390	0.304
Hca014	2	0.390	0.406	3	0.378	0.375	3	0.484	0.375
Hca017	7	0.823	0.781	4	0.442	0.458	2	0.041	0.042
Hca031	7	0.710	0.688	3	0.508	0.375	4	0.194	0.167
Hca033	8	0.799	0.806	3	0.536	0.458	3	0.546	0.500
Hca037	2	0.324	0.406	1	0.000	0.000	1	0.000	0.000
Hca038	4	0.630	0.690	3	0.508	0.542	3	0.155	0.167
Hca040	6	0.686	0.719	3	0.588	0.583	3	0.503	0.250**
Hca041	11	0.817	0.719	4	0.416	0.417	2	0.469	0.333
Hca045	8	0.827	0.781	5	0.358	0.250	4	0.120	0.125
Hca048	13	0.807	0.781	3	0.555	0.542	5	0.326	0.357
Hca050	6	0.758	0.750	4	0.610	0.583	7	0.472	0.458
Hca051	9	0.832	0.844	4	0.536	0.583	10	0.831	0.667
Hca055	6	0.623	0.469	4	0.602	0.417*	5	0.617	0.417**
Hca057	9	0.791	0.688	2	0.457	0.292	5	0.537	0.435**
Hca062	7	0.771	0.688	6	0.668	0.542**	3	0.392	0.417
Hca065	13	0.813	0.688	5	0.666	0.609	4	0.463	0.417
Hca066	7	0.541	0.433	4	0.665	0.500**	3	0.457	0.417
Hca069	10	0.820	0.645	2	0.043	0.043	8	0.347	0.130**
Hca070	3	0.563	0.500	6	0.362	0.292**	3	0.223	0.250
Hca071	5	0.566	0.531	2	0.492	0.458	4	0.120	0.125
Hca072	7	0.721	0.700	3	0.385	0.125**	3	0.368	0.136**
Hca085	6	0.657	0.531	5	0.624	0.583	5	0.458	0.500
Hca086	4	0.539	0.594	2	0.444	0.417	3	0.119	0.125

n, number of individuals sampled; A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity.
[a]Significant deviation from Hardy-Weinberg equilibrium (*$p < 0.05$, **$p < 0.01$).

Table 4. Results of cross-amplication and genetic properties of 28 microsatellite loci developed from *Hosta capitata* in *H. minor* and *H. venusta*.

Locus	*H. minor* (n = 24)				*H. venusta* (n = 24)			
	A	H_E	H_O[a]	*Sr* (bp)	A	H_E	H_O[a]	*Sr* (bp)
Hca004	2	0.187	0.125	124–126	4	0.293	0.208**	124–130
Hca005	-	-	-	-	-	-	-	-
Hca007	-	-	-	-	-	-	-	-
Hca010	10	0.753	0.708	125–165	6	0.684	0.227**	123–155
Hca012	-	-	-	-	-	-	-	-
Hca014	3	0.234	0.087**	183–195	5	0.596	0.409**	183–201
Hca017	6	0.550	0.625**	188–204	7	0.691	0.625**	190–204
Hca031	5	0.622	0.250	123–137	3	0.317	0.375	123–135
Hca033	-	-	-	-	-	-	-	-
Hca037	2	0.499	0.708	160–163	2	0.486	0.583	160–163
Hca038	-	-	-	-	-	-	-	-
Hca040	3	0.269	0.087**	156–162	4	0.588	0.000	154–160
Hca041	3	0.317	0.333	202–206	4	0.631	0.542*	202–216
Hca045	-	-	-	-	-	-	-	-
Hca048	7	0.757	0.958**	254–268	8	0.804	0.304**	244–278
Hca050	-	-	-	-	-	-	-	-
Hca051	-	-	-	-	-	-	-	-
Hca055	4	0.563	0.250**	132–138	5	0.726	0.417**	132–140
Hca057	-	-	-	-	-	-	-	-
Hca062	-	-	-	-	-	-	-	-
Hca065	-	-	-	-	-	-	-	-
Hca066	3	0.314	0.000**	255–271	4	0.734	0.529*	253–271
Hca069	-	-	-	-	-	-	-	-
Hca070	2	0.496	0.217**	122–124	4	0.544	0.542**	122–142
Hca071	-	-	-	-	-	-	-	-
Hca072	5	0.619	0.091**	198–224	3	0.322	0.261	198–228
Hca085	-	-	-	-	-	-	-	-
Hca086	-	-	-	-	-	-	-	-

n, number of individuals sampled; A, number of alleles; H_E, expected heterozygosity; H_O, observed heterozygosity; *Sr*, size range; -, unsuccessful amplification.

[a]Significant deviations from Hardy-Weinberg equilibrium (*$p < 0.05$ and **$p < 0.01$).

ORCID: Mi-Jung CHOI https://orcid.org/0000-0002-4409-9765; Jung-Hyun LEE https://orcid.org/0000-0003-0066-6032; Won-Bum CHO https://orcid.org/0000-0002-0098-3559; Eun-Kyeong HAN https://orcid.org/0000-0002-8530-9925; Hyeok-Jae CHOI https://orcid.org/0000-0003-3644-6795

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Conflict of Interest

The authors declare that there is no conflict of interest.

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