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Isolation and characterization of 19 new microsatellite loci in *Colocasia esculenta* (Araceae)[1]

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- *Premise of the study:* Microsatellite primers were developed for taro (*Colocasia esculenta*) to investigate its population genetics and evolutionary history through germplasm improvement.
- *Methods and Results:* Nineteen microsatellite loci were identified in three populations. The number of alleles per locus ranged from two to seven, with a mean of 4.68. The observed and expected heterozygosities ranged from 0.231 to 0.820 and from 0.126 to 0.742, respectively.
- *Conclusions:* These new genetic markers will be useful for the study of taro germplasm management and population evolution in southwestern China.

Key words: *Colocasia esculenta*; microsatellite; polymorphism.

Taro (*Colocasia esculenta* (L.) Schott), a member of the monocotyledonous family Araceae, is an important root crop throughout the world (Jane et al., 1992; Mace et al., 2006; Hu et al., 2009). Taro is widely planted throughout most humid tropical and subtropical regions, including Southeast Asia, China, and Japan. It is generally accepted that taro originates in China, which is also its center of diversity (Kuruvilla and Singh, 1981; Hu et al., 2009). Although some microsatellite loci for taro are already published (Mace and Godwin, 2002; Quero-García et al., 2006; Hu et al., 2009), there are few reports on population evolution and genetic diversity of taro resources, especially in China. Additional loci will help us identify the origin and distribution of taro in China. In addition, microsatellite markers may be very useful for marker-assisted breeding of taro, one of the main food resources of southwestern China. Microsatellite markers may also be useful in distinguishing among taro species. In our study, 19 novel microsatellite markers have been developed from taro germplasm primarily sampled from Yunnan Province, southwestern China. Our study will aid efforts toward the conservation and utilization of this species as a food resource.

METHODS AND RESULTS

Genomic DNA was extracted from the leaves using a modified cetyltrimethyl ammonium bromide (CTAB) method (Zhou et al., 1999). We constructed an enriched partial genomic library for the repeat motif $(AG)_n$, and then isolated SSRs using the protocol reported by Hauswaldt and Glenn (2003). In brief, ~500 ng of DNA was completely digested with the restriction enzyme *Rsa*I and *Bst*UI (Fermentas, Burlington, Ontario, Canada) and then ligated to the SuperSNX linkers. For enrichment, the ligated products were hybridized with 5′-biotinylated probe $(AG)_{12}$ in a 100-μL hybridization solution (12× SSC and 0.2% SDS) at 95°C for 5 min, quickly ramped to 48°C, and maintained at this temperature for 2 h. Hybridized DNA was then mixed with Streptavidin-coated magnetic beads (Promega, Shanghai, China). Captured DNA was used as the template to amplify with SuperSNX-Forward primer (5′-GTTTAAGGCCTAGCTAGCAGAATC-3′), PCR products were purified with the QIAquick PCR Purification Kit (QIAGEN, Shanghai, China), and then these fragments with microsatellite loci were cloned using pMD18-T vectors (Takara Bio Inc., Dalian, China) according to the manufacturer's instructions and propagated in the *E. coli* DH5a strain. Positive clones were amplified using $(AG)_{10}$ and M13 primers. PCR products of 200–800 bp were selected for sequencing. Sequencing revealed that 19 clones contained microsatellite sequences, and we successfully designed primers for all of them. We then tested these primers in the 56 natural individuals in three populations collected in southwestern China: the Yunnan population (Kunming Botanical Garden, Yunnan Province, 21°54′47.5″N, 101°15′50.5″E, herbarium No. KUN-YP-Taro101), Sichuan population (Chendu, Sichuan Province, 30°68′28.05″N, 104°06′29.69″E, herbarium No. KUN-YP-Taro201), and Guangxi population (Nanlin, Guangxi Province, 22°84′15.04″N, 108°33′21.50″E, herbarium No. KUN-YP-Taro301). PCR was carried out in a volume of 25 μL consisting of 10–30 ng of genomic DNA, 1× PCR buffer, 0.4 μM for each forward and reverse primer, 1.5 mM $MgCl_2$, 300 μM for each dNTP, and 0.75 U *Taq* polymerase (QIAGEN). Thermocycling conditions were as follows: an initial denaturing step (94°C for 5 min), then 35 cycles (94°C for 40 s, annealing at primer-specific temperature (Table 1) for 45 s, and 72°C for 30 s, and a final elongation step (72°C for 5 min and then held at 15°C). After the amplification, PCR products were size-fractionated by electrophoresis on 6% denaturing polyacrylamide-sequencing gels running at 45 W for 60 min, then identified by silver staining. Analysis of polymorphism including number of alleles, observed and expected heterozygosities, tests for linkage disequilibrium (LD), and deviations from Hardy–Weinberg equilibrium (HWE) were calculated using Arlequin software (Excoffier et al., 2005) and GENEPOP version 4.0 (Raymond and Rousset, 1995).

Eighteen of the 19 primer pairs yielded polymorphic amplification products in three populations, with one monomorphic in the Yunnan population, one monomorphic in the Sichuan population, and one monomorphic in the Guangxi population. The number of alleles (N_a) and observed (H_o) and expected heterozygosities (H_e) were obtained for each locus and population using GenAlEx version 6.1 (Peakall and Smouse, 2006). The number of alleles varied from two to seven per polymorphic locus (Table 2), while observed and expected heterozygosities ranged from 0.000 to 0.768 and 0.000 to 0.897, respectively. Tests for departure from

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TABLE 1. Characteristics of 19 microsatellite primers showing the forward (F) and reverse (R) primer sequences, repeat motif, allele size (bp), optimal annealing temperature (T_a), and GenBank accession number.

Primers	Primer sequences (5′–3′)	Repeat	Size (bp)	T_a (°C)	GenBank Accession No.
Taro01	F: CTGACTCTTGTAAGGTCGCTC	$(AG)_8$	1004	56	FJ895330
	R: CAAAAGCAGGTCTGGATG				
Taro02	F: ACTAATTCCGCAATACTTCACTA	$(G)_{12}$ $(GA)_{10}$	708	53	FJ895331
	R: ATCGCCGGGTGTGAGCTGATT				
Taro03	F: CGTGAGGGCGGTTTTGTCAGG	$(CT)_{12}$	616	60	FJ895332
	R: ACGAGCGAGCAGCTCACCGC				
Taro04	F: ACTTTATGTAATAGTGAACATT	$(TC)_{18}$ $(TAA)_6$	947	57	FJ895333
	R: CGAAGCAGCGCCACCGGC				
Taro05	F: ACGTTTGGACACCCATGTG	$(GA)_{10}$	742	58	FJ895334
	R: CCGGCCAAGATTTGATCTT				
Taro06	F: GCGCGGCCAAAGATCAAATCT	$(GA)_{24}$	389	52	FJ895335
	R: CACATTTATTATTAATGCAAAC				
Taro07	F: CGGCCCCACGATCTGACGC	$(CT)_{10}$	705	59	FJ895336
	R: CACCCTTTTGTTGAAGGTCGTT				
Taro08	F: CGTTCGGATTAGGAAACCAC	$(CT)_{20}$	1038	58	FJ895337
	R: GCTCCCCCTGCTACTTTCTTCC				
Taro09	F: CGATAGACAGAGAGAGAGA	$(AGGG)_4$	641	62	FJ895338
	R: ACCAGGTCTCCGACACATG				
Taro10	F: TTGGGGGGGAAGATCATGTG	$(CT)_4$	854	60	FJ895339
	R: ACTATGAGGATTTTAAGGGTA				
Taro11	F: CGGCCAAGAAGGAGAGCCA	$(GA)_8$	540	58	FJ895340
	R: ACAAGCTTATTTATAGTGGCTA				
Taro12	F: CGCTTTGCCTTTCGGTGTTGAGA	$(GA)_8$	726	56	FJ895341
	R: ACTTGGTGTGCAGCAAGACTT				
Taro13	F: GTTAATGGGATATAAACGGCA	$(CT)_8$	582	58	FJ895342
	R: CGCCAAAGTCTATTGAGTGTT				
Taro14	F: ACAAAATATGTTCTCTGTGATAT	$(GA)_{22}$	743	55	FJ895343
	R: ACCTAGTCTACTATCGAGCCA				
Taro15	F: ACACATGTGTAAACTGATTTA	$(TC)_8$	640	56	FJ895344
	R: CCGCATACAGAGAGAGAG				
Taro16	F: ATTACTGCAGCTATCAGATCC	$(AG)_8$	450	56	FJ895345
	R: AGGCTAGACTGACGTCGAT				
Taro17	F: TTAGAGCTAGCTAGCGAGCT	$(TC)_{10}$	550	55	FJ895346
	R: CGATCGATCGAGCTATTGAC				
Taro18	F: CTAGCTAGCGATTGAGACCTC	$(CT)_9$	610	56	FJ895347
	R: CTAGAAGGCTGAGCTTGACG				
Taro19	F: TTCGACGTACCGATCGAGACCG	$(AGG)_4$	630	54	FJ895348
	R: TTACCGAGACTGACGAAGCTAG				

TABLE 2. Locus-specific measures of genetic diversity of three populations of *Colocasia esculenta*: N_a = number of alleles, H_o = observed heterozygosity, H_e = expected heterozygosity, P = P values of population-level Hardy–Weinberg exact tests (conducted using GENEPOP). P values with an asterisk indicate significant departure from Hardy–Weinberg equilibrium ($P < 0.05$).

	Yunnan population (N = 16)				Sichuan population (N = 14)				Guanxi population (N = 15)			
Locus	N_a	H_o	H_e	P	N_a	H_o	H_e	P	N_a	H_o	H_e	P
Taro01	2	0.232	0.456	0.0034*	2	0.670	0.786	0.0023*	3	0.343	0.564	0.0014*
Taro02	3	0.560	0.453	0.2334	2	0.124	0.437	0.0672	3	0.346	0.458	0.0763
Taro03	4	0.478	0.560	0.0028*	4	0.323	0.398	0.0035*	4	0.456	0.679	0.0532
Taro04	4	0.078	0.452	0.0000*	4	0.000	0.000	—	3	0.193	0.675	0.0000*
Taro05	1	0.000	0.000	—	3	0.123	0.786	0.0000*	1	0.000	0.000	—
Taro06	4	0.635	0.807	0.0055*	3	0.235	0.437	0.0672	3	0.323	0.789	0.0034*
Taro07	2	0.038	0.125	0.0063*	2	0.024	0.237	0.0053*	2	0.127	0.679	0.0035*
Taro08	3	0.445	0.670	0.0853	3	0.235	0.657	0.1021	2	0.023	0.356	0.0977
Taro09	2	0.239	0.457	0.0893	2	0.129	0.236	0.0046*	2	0.238	0.367	0.0342*
Taro10	3	0.347	0.767	0.1021	3	0.237	0.657	0.1163	3	0.238	0.460	0.0982
Taro11	5	0.234	0.376	0.0035*	4	0.655	0.787	0.0034*	5	0.289	0.788	0.0000*
Taro12	4	0.443	0.678	0.0843	4	0.340	0.678	0.0455*	3	0.346	0.758	0.0021*
Taro13	5	0.768	0.845	0.0000*	6	0.345	0.783	0.0000*	5	0.376	0.765	0.0007*
Taro14	7	0.456	0.543	0.0000*	5	0.234	0.124	0.0000*	5	0.127	0.675	0.0006*
Tar015	3	0.723	0.897	0.0989	3	0.712	0.879	0.1022	3	0.560	0.768	0.0894
Taro16	3	0.236	0.654	0.0024*	4	0.348	0.775	0.0028*	3	0.120	0.675	0.0023*
Taro17	4	0.344	0.567	0.0047*	4	0.740	0.678	0.2408	3	0.233	0.459	0.0045*
Taro18	5	0.456	0.785	0.0078*	5	0.456	0.828	0.0023*	6	0.235	0.679	0.0021*
Taro19	4	0.344	0.454	0.0034*	4	0.220	0.782	0.0020*	3	0.129	0.770	0.0018*

Hardy–Weinberg equilibrium and from linkage equilibrium were performed in GENEPOP version 4.0 (Raymond and Rousset, 1995). Among the 19 microsatellite markers, 38 loci showed significant deviation from Hardy–Weinberg equilibrium ($P < 0.005$) (Table 2), including 13 in the Yunnan population, 12 loci in the Sichuan population, and 13 loci in the Guangxi population, respectively. There was significant LD between most polymorphic loci following Bonferroni correction at 0.001 for multiple tests. These deviations may have resulted primarily from either the asexual reproduction of the taro or from human cultivation factors, because taro has been cultivated by humans for centuries and its distribution tends to follow human movement patterns.

CONCLUSIONS

We present 19 nuclear microsatellite markers developed specifically from taro (*Colocasia esculenta*). These newly developed nuclear microsatellite markers will be a useful tool for studying the population genetics and evolutionary history of taro in southwestern China. In addition, the set of novel makers are also helpful for the improvement of taro germplasm and identifying commercial germplasm.

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