Title

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Journal

Applications in Plant Sciences, 2(2)

ISSN

2168-0450

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Publication Date

2014-02-01

DOI

10.3732/apps.1300079

Peer reviewed

Applications in Plant Sciences

Applications in Plant Sciences 2014 2(2): 1300079

PRIMER NOTE

TEN POLYMORPHIC MICROSATELLITE PRIMERS IN THE TROPICAL TREE CAIMITO, *CHRYSOPHYLLUM CAINITO* (SAPOTACEAE)[1]

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- *Premise of the study:* We developed microsatellite primers for the tropical tree *Chrysophyllum cainito* (Sapotaceae) to determine the native range of the species, investigate the origin of cultivated populations, and examine the partitioning of genetic diversity in wild and cultivated populations.
- *Methods and Results:* We developed 10 polymorphic primers from *C. cainito* genomic DNA libraries enriched for di-, tri-, and tetranucleotide repeat motifs. The loci amplified were polymorphic in samples collected from Jamaica and Panama. The number of alleles per locus ranged from two to 10 and three to 12, while observed heterozygosities ranged from 0.074 to 0.704 and 0.407 to 0.852 in Jamaica and Panama, respectively.
- *Conclusions:* The microsatellite primers will be useful in future population genetic studies as well as those aimed at understanding the geographic origin(s) of wild and cultivated populations.

Key words: caimito; *Chrysophyllum cainito*; microsatellite; Sapotaceae; tropical tree domestication.

Chrysophyllum cainito L. (Sapotaceae), commonly known as caimito or star apple, is a neotropical tree valued for its ornamental quality and for its edible fruits (Morton, 1987). The species is generally considered native to the Greater Antilles and naturalized in Central and South America (Pennington, 1990). Less commonly, the species is considered as native to Panama (e.g., Correa et al., 2004). In Panama, cultivated and wild *C. cainito* trees are found growing in close proximity and exhibit high levels of intraspecific variability for fruit traits such as fruit and seed size, sugar content, and levels of polyphenolics. These observed differences between cultivated and wild individuals suggest that the cultivated trees are semidomesticated (Parker et al., 2010).

Hypotheses as to the geographic origin of the species have been recently tested with DNA sequence data. Those data showed that wild populations in Panama are more diverse than wild populations from the Greater Antilles, suggesting a southern Mesoamerican origin for the species (Petersen et al., 2012).

Chrysophyllum cainito, as a semidomesticated species, is an excellent system to study anthropogenic impacts on the distribution of a neotropical fruit tree. In species in the early stages of domestication, wild forms may coexist with cultivated forms that have experienced varying degrees of management and modification through artificial selection. This situation provides an opportunity to study anthropogenic impacts on genetic and phenotypic variation within a single species. We developed 10 polymorphic microsatellite markers to further test hypotheses regarding the geographic origin of the species and the source of cultivated populations, and to evaluate the levels of genetic diversity and population structure in wild and cultivated trees.

METHODS AND RESULTS

We extracted 100 ng/μL of genomic DNA of leaf tissue from a single individual of *C. cainito* (Montgomery Botanical Center accession number 78601*C) using a DNeasy Plant Tissue Kit (QIAGEN, Valencia, California, USA). The extracted DNA was sent to the Savannah River Ecology Laboratory (University of Georgia, Athens, Georgia, USA), and the DNA was enriched for di-, tri-, and tetranucleotide repeats using three sets of oligonucleotide probe mixes (Cc2: AG, AAC, AAG, AAT, ACT, ATC; Cc3: AAAC, AAAG, AATC, AATG, ACAG, ACCT; and Cc4: AAAT, AACT, AAGT, ACAT). The enrichment process followed Glenn and Schable (2005) and included an initial digestion with the restriction enzyme *Rsa*I (New England Biolabs, Ipswich, Massachusetts, USA). Double-stranded SuperSNX linkers (Hamilton et al., 1999) were then ligated onto each end of the digested DNA fragments. The DNA was then denatured and hybridized to biotinylated microsatellite oligonucleotide probes and captured on magnetic beads. The nonhybridized DNA was washed away, and the hybridized DNA was eluted and amplified in a PCR using the SuperSNX forward primer. At the University of California, Davis, fragments of the three libraries of enriched DNA were cloned using TOPO-TA cloning kits (Invitrogen, Carlsbad, California, USA) (Glenn and Schable, 2005). Inserts from 91 clones were PCR-amplified using M13 forward and M13 reverse primers, and the PCR products were sequenced with those primers using ABI BigDye Terminator v3.1 Cycle Sequencing chemistry on an ABI 3730 Capillary

[1]Manuscript received 30 September 2013; revision accepted 17 November 2013.

The authors thank the Montgomery Botanical Center and Fairchild Tropical Garden for providing leaf material and T. Commack, I. Lopez, H. Membache, and G. Proctor for fieldwork assistance. The authors thank the Smithsonian Tropical Research Institute for logistical support and the Autoridad Nacional del Ambiente (ANAM) and the Autoridad del Canal de Panamá (ACP) for granting us permission to conduct research in the Republic of Panama. The authors thank M. Jasieniuk and M. Okada for technical assistance. J.J.P. acknowledges financial support from the University of California, Davis, and Henry A. Jastro and Peter J. Shields Research Scholarships.

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doi:10.3732/apps.1300079

TABLE 1. Characteristics of 10 polymorphic microsatellite loci developed for *Chrysophyllum cainito.*

Locus	Primer sequences (5′–3′)	Repeat motif	Allele size (bp)	T_a (°C)	Fluorophore[a]	GenBank accession no.
CaiC1	F: GCGTAGAGTTTCGCCTATCG R: TGTAGGGGATTGGAGTCGAG	$(GA)_{12}(GT)_{11}$	249	50	HEX	KF688727
CaiC2	F: CAGCCATCAACCAAAAGTCA R: GGAGGAAATTTGGAGGGAAT	$(CT)_{15}(CA)_{17}$	212	56	HEX	KF688728
CaiD8	F: GGAAGGGAAAGATGGTGTGA R: CCCGTGAGATACAGGGAAGA	$(AG)_{14}$	283	50	NED	KF688730
CaiD9	F: TCCATATTCCGTAAACCATGC R: TGATGGCTATGCATTCTTTGTT	$(AG)_{14}$	156	50	NED	KF688731
CaiE5	F: TCCCAAACAAGCCAATTCTC R: CCGCCTGAATTGAAGAAAAC	$(AG)_{11}$	174	60	FAM	KF688732
CaiE7	F: CTCCACTTGGAAGGTGCTTG R: TTACCAGATGCCTGGGAAAC	$(CT)_8(AC)_8$	151	56	FAM	KF688729
CaiF4	F: ACAAGCAAACCAAGCCAAAC R: TGCAACGTCAGAACAACCAT	$(AG)_{14}$	159	50	FAM	KF688726
CaiG4	F: TAAATGGGGCTTTGTTGAGG R: TCTGGAACACAATACAAACTTGAG	$(AG)_{20}$	223	50	FAM	KF688733
CaiG6	F: TCTTGGGGGTCTTCTTGATG R: TTGAATCCAGTTTACAAACCAA	$(AG)_{20}$	214	50	FAM	KF688734
CaiG7	F: AGATGCCCATCTCGTGAGTC R: GGACACTTTGATTTCCCCAGT	$(AG)_{18}(AAG)_2$	201	50	HEX	KF688735

Note: T_a = annealing temperature.
[a]Fluorophore used to label the forward primer.

Electrophoresis Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Forward and reverse sequences were assembled and edited in Sequencher 3.1.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Thirty-three of the 91 sequenced inserts contained a microsatellite motif with a minimum of 10 subunit repeats. We designed primers using the default program settings in Primer3 (Rozen and Skaletsky, 2000) for 22 assembled contigs (forward and reverse sequences) that were between 100 and 400 bp in length and had sufficient flanking regions (at least 20 bp) on either side of the microsatellite repeat.

We tested the amplification and levels of polymorphism of products from 22 primer pairs using a panel of five individuals including the Montgomery Botanical Center accession (78601*C) and Fairchild Tropical Garden accession (981444A), as well as three individuals collected from Jamaica and Panama, which are indicated by asterisks in Appendix 1. The DNA of each individual was extracted from fresh or silica-dried leaf tissue using a DNeasy Plant Tissue Kit (QIAGEN). PCR reactions were performed in 25-μL reaction volumes containing 2.5 μL10× buffer, 1.9 μL $MgCl_2$, 0.25 μL bovine serum albumin (BSA), 0.25 μL AmpliTaq (Applied Biosystems), 0.5 μL each of the forward and reverse primers (10 μM) and dNTPs (10 mM), 17.65 μL H_2O, and 1 μL genomic DNA (20 ng/μL). PCR amplification was carried out on an Applied Biosystems 2720 thermal cycler using the following cycling parameters: a 94°C initial denaturing for 4 min followed by 35 cycles of 94°C for 30 s, 50–60°C for 30 s, and 72°C for 30 s, with a final extension of 72°C for 7 min. We ran out the PCR products on a 2% agarose gel stained with ethidium bromide and then visualized the products under UV light. For the 13 loci that produced reliable amplification, we excised the PCR products from the agarose gels and used QIAquick Gel Extraction Kits (QIAGEN) to purify amplified fragments. To confirm that the primers were amplifying the target microsatellite motif, the purified PCR products were sequenced directly in both directions using the amplification primers. In all cases, we confirmed that the primer pairs were amplifying the target microsatellite motif.

We fluorescently labeled forward primers for 13 loci using FAM, NED, and HEX and analyzed those PCR products on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). We separated and scored alleles using GeneMapper version 3.7 (Applied Biosystems) using GeneScan 400HD ROX as an internal size standard (Applied Biosystems). Ten primer pairs yielded polymorphic amplification products that we were able to score consistently (Table 1). We further tested these primers for ease of scoring and levels of polymorphism on a total of 54 individuals from *C. cainito* collected from 12 and 11 localities from Jamaica and Panama, respectively (Appendix 1). We performed conventional genetic analyses of the Jamaica and Panama collections in GenAlEx version 6.3 (Peakall and Smouse, 2006) and tested for the presence of null alleles using

TABLE 2. Locus-specific measures of genetic diversity for *Chrysophyllum cainito*, collected in Jamaica and Panama.

	Jamaica					Panama				
Locus	*N*	*A*	H_o	H_e	HWE[a]	*N*	*A*	H_o	H_e	HWE[a]
CaiC1	27	7	0.704	0.715	0.966	27	6	0.519	0.597	0.496
CaiC2	27	5	0.556	0.735	0.029*‡	27	9	0.815	0.767	0.890
CaiD8	27	6	0.259	0.269	0.280	27	7	0.704	0.639	0.846
CaiD9	27	5	0.259	0.236	1.000	25	12	0.560	0.816	0.000*‡
CaiE5	27	2	0.074	0.071	0.842	27	4	0.519	0.459	0.897
CaiE7	27	10	0.667	0.751	0.508	27	8	0.852	0.782	0.058
CaiF4	27	5	0.259	0.267	0.061	26	7	0.692	0.643	0.874
CaiG4	26	3	0.385	0.578	0.035*‡	26	7	0.692	0.658	0.843
CaiG6	27	4	0.556	0.562	0.790	27	3	0.407	0.411	0.941
CaiG7	26	5	0.615	0.556	0.831	25	7	0.720	0.742	0.613

Note: *A* = number of observed alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; HWE = Hardy–Weinberg equilibrium; *N* = sample size.
[a]*P* values for Hardy–Weinberg equilibrium tests; * indicates a significant departure from HWE ($P > 0.05$), and ‡ indicates possible null alleles detected by MICRO-CHECKER.

MICRO-CHECKER (van Oosterhout et al., 2004). The overall observed number of alleles per locus ranged from two to 10 (mean = 5.2) for the Jamaica samples and from three to 12 (mean = 7) for the Panama samples. The slight difference in the observed and mean number of alleles between the Jamaica and Panama samples may reflect higher genetic diversity of *C. cainito* in Panama compared to Jamaica. We would expect a higher number of alleles in the area of origin as well as in the center of domestication. Observed heterozygosities ranged from 0.074 to 0.704 and 0.407 to 0.852 for the Jamaica and Panama samples, respectively (Table 2). Significant departures from Hardy–Weinberg equilibrium (HWE) were observed at microsatellite loci CaiG4 and CaiC2 in the Jamaica samples and at locus CaiD9 in the Panama samples. MICRO-CHECKER reported that null alleles might be present at these same locus/sample locality combinations (Table 2). No locus was observed as having null alleles or significant deviations from HWE in both Jamaica and Panama.

CONCLUSIONS

These newly developed primers amplified highly polymorphic microsatellite loci in *C. cainito* individuals. Observed differences in genetic diversity between Jamaica and Panama, both of which have been reported to include the native range of the species, indicate that these highly polymorphic loci will be useful in establishing the origins and genetic differentiation of wild and cultivated *C. cainito* populations. In addition, the loci would be useful for studies that evaluate gene flow and the evolution of mating system in this semidomesticated neotropical fruit tree, as well as studies that include closely related congeneric *Chrysophyllum* species.

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Appendix 1. Voucher specimens used in this study. All specimens of *Chrysophyllum cainito* are deposited at the University of California, Davis, Center for Plant Diversity (DAV).

Country	Collection no.[a]	Collection locality	*N*	Latitude	Longitude
Jamaica	JP135	Albert Town, Trelawny Parish	1	18°17.340 N	77°32.594 W
	JP95*	Cave Valley, St. Ann Parish	2	18°12.869 N	77°22.695 W
	JP108	Ipswich/Red Gate, St. Elizabeth Parish	4	18°10.588 N	77°49.963 W
	JP101	Johnson, St. James Parish	2	18°15.708 N	77°49.755 W
	JP128	Kinloss–Clark Town Road, Trelawny Parish	2	18°24.157 N	77°33.716 W
	JP112	Lacovia to Slipe, St. Elizabeth Parish	1	18°04.377 N	77°46.530 W
	JP119	Marshal's Pen, Manchester Parish	1	18°03.608 N	77°31.822 W
	JP124	Mountainside, St. Elizabeth Parish	4	17°59.415 N	77°44.760 W
	JP100	Newton, St. Elizabeth Parish	1	18°07.543 N	77°44.879 W
	JP98	Scott's Pass, Clarendon Parish	2	18°00.588 N	77°23.000 W
	JP115	Slipe, St. Elizabeth Parish	3	18°03.533 N	77°47.133 W
	JP129	Windsor Estate, Trelawny Parish	2	18°22.125 N	77°38.786 W
Panama	JP222*	Arraijan-Burunga, Panama	4	08°58.190 N	79°40.286 W
	JP152	Balboa, Panama	2	08°57.272 N	79°33.344 W
	JP178	Camino de Cruces, Panama	4	09°06.658 N	79°41.512 W
	JP165	Chilibre, Panama	4	09°11.107 N	79°36.621 W
	JP189	Clayton, Panama	2	09°00.441 N	79°34.056 W
	JP141	Ella Purua, Panama	3	09°07.810 N	79°41.749 W
	JP216	Gamboa, Panama	1	09°07.890 N	79°42.690 W
	JP157	Madden, Panama	2	09°06.906 N	79°36.945 W
	JP205	Pipeline Road, Panama	4	09°09.066 N	79°43.946 W
	JP194*	San Antonio, Panama	2	09°07.758 N	79°41.733 W
	JP201	Venta de Cruces, Panama	1	09°07.707 N	79°41.081 W

Note: *N* = number of individuals sampled in a given locality.

[a] First author collection numbers. Individuals that were used in the initial primer screen are indicated with an asterisk.