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PRIMER NOTE

CHARACTERIZATION OF MICROSATELLITES IN *XANTHOSOMA SAGITTIFOLIUM* (ARACEAE) AND CROSS-AMPLIFICATION IN RELATED SPECIES[1]

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- *Premise of the study:* To investigate the genetic diversity of a root crop, *Xanthosoma sagittifolium*, and to facilitate germplasm conservation, microsatellite loci were developed and characterized by genotyping 39 accessions from different geographic origins.
- *Methods and Results:* Using a microsatellite-enriched library approach, 17 polymorphic microsatellite markers were identified and characterized. The number of alleles for each locus ranged from two to six. Observed and expected heterozygosities ranged from 0.00 to 0.97 and from 0.09 to 0.78, respectively. Additionally, cross-amplification of these microsatellite markers was tested successfully in other species of *Xanthosoma* and *Caladium*, with rates varying from 23.5% to 100%.
- *Conclusions:* These results indicate the effectiveness of microsatellite loci developed for the characterization of *X. sagittifolium* genetic diversity. They are crucial for the future investigation of population dynamics and clonal identification and, therefore, for prioritizing germplasm conservation. They should also enable research on other related species.

Key words: *Caladium lindenii*; genetic conservation; microsatellite markers; polymorphism; tuber crop; *Xanthosoma sagittifolium*.

Xanthosoma sagittifolium (L.) Schott (Araceae) is a monocotyledon aroid native to the tropical Americas, but its original place of domestication is still unknown. It is widely distributed throughout tropical regions in Africa, Southeast Asia, and Oceania. It is an allogamous species cultivated exclusively by vegetative propagation, preventing any possible genetic recombination. Its adaptive capacity is therefore almost nonexistent, and it is vulnerable to various pathogens such as dasheen mosaic virus (DMV) or *Pythium myriotylum* Drechsler (Lebot, 2009). Genetic resources are therefore of great value and need to be carefully identified, conserved, and protected. To date, the molecular genetic diversity of this plant has been investigated using only RAPD markers (Schnell et al., 1999; Offei et al., 2004) within a limited number of accessions and across restricted areas. The management of ex situ collections and the accurate identification of clones are often hampered by the lack of efficient markers. Present knowledge on wild crop relatives, population genetics, and spatial distribution of this species is insufficient and limited. It is therefore necessary to develop highly polymorphic codominant markers in *X. sagittifolium*.

Microsatellites or simple sequence repeats (SSRs) are highly polymorphic markers that are codominant and widespread in the genome. Such characteristics make them useful for a large range of applications in genetics. Consequently, they have been developed for a large number of plant species.

Here, we present the first set of polymorphic nuclear microsatellite markers suitable for germplasm diversity studies and further genetic conservation in *X. sagittifolium*. Additionally, the microsatellite primers that gave good results were tested for cross-amplification in related *Xanthosoma* and *Caladium* species.

METHODS AND RESULTS

Total genomic DNA was extracted from a silica gel–dried leaf sample following the protocol of Risterucci et al. (2000) and purified on NucleoBond PC20 columns (Macherey-Nagel, Düren, Germany). Genomic DNA of one accession of *X. sagittifolium* (living accession Xs10 at the Vanuatu Agricultural Research and Technical Centre [VARTC], Vanuatu) was restricted with *Rsa*I (Invitrogen, Carlsbad, California, USA) and then used to construct a $(GA)_n$ and $(GT)_n$ microsatellite-enriched library following the protocol of Billotte et al. (1999). The enriched microsatellite fragments were then cloned into pGEM-T Easy Vector (Promega Corporation, Madison, Wisconsin, USA) as indicated by the supplier and were used to transform *Escherichia coli* DH10B competent cells (Invitrogen). Overall, 288 white transformed clones then underwent PCR amplification in a 50-µL reaction mixture containing a bacterial colony, 0.16 mM dNTP, 0.2 µM for each M13 primer, 50 mM KCl, 10 mM Tris-HCl, 0.001% glycerol, 2 mM $MgCl_2$, and 1 unit *Taq* DNA polymerase. The PCR program was: initial denaturation at 95°C for 4 min; 30 cycles at 94°C for 30 s, 52°C for

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TABLE 1. Characteristics of 17 polymorphic microsatellite markers isolated from *Xanthosoma sagittifolium*.

Locus	Repeat motif	Clone size (bp)	Primer sequence (5′–3′)	GenBank accession no.	T_a (°C)	*A*	Allele size range (bp)	H_e	H_o	PIC
mXsCIR05	$(CA)_8(CACA)_3$	196	F: GCGCATTATTAACGAATATC R: GTCATCTATGGCTATCACCT	KF693219	50	2	212–216	0.0973	0.0000	0.099
mXsCIR07	$(TG)_7(AG)_{19}$	204	F: GGACTGGGAGTCTGAGTAG R: CCTTTCCCCTCACTATAAA	KF693220	50	2	224–241	0.2778	0.2308	0.225
mXsCIR10	$(AG)_{22}$	222	F: GATGTCTGTAGTGGCCTAGT R: AATTAAGTTGGGTGGTAGAT	KF693221	50	5	208–251	0.6586	0.5526	0.584
mXsCIR11	$(TG)_{10}(GA)_{16}$	204	F: AATTCTTAGCAGCATTGTTA R: CATTCGTATCAACTTCCTTT	KF693222	50	6	204–236	0.7883	0.9744	0.747
mXsCIR12	$(TC)_{17}(TTC)_7(TCCC)_3(TTCTTG)_3$	242	F: TACATTTCCATTGCCATC R: CAAATTAAAGAGGGAGACAG	KF693223	50	4	223–282	0.4316	0.4359	0.389
mXsCIR13	$(CA)_8(AG)_{16}$	209	F: GTTTCCTTTATTCGTTGATG R: GTAGTGGCTGAGAATTGAAA	KF693224	50	5	223–244	0.7699	0.7949	0.739
mXsCIR14	$(AG)_{20}$	196	F: TACCCTACATTTGGGATCT R: TTTTGGCTTTAGGTCTATTC	KF693225	50	2	190–216	0.1841	0.2051	0.178
mXsCIR16	$(AG)_{15}$	183	F: CTTATTGATGCCGAGAATAC R: TTCCTCACAATATGTTCTCAT	KF693226	50	3	202–205	0.6091	0.2308	0.531
mXsCIR19	$(AC)_8(AC)_{24}(AC)_8$	229	F: CAACTTGTGTATCCTACATCC R: GCGTGGTTTATGTGTATCTT	KF693227	50	4	184–204	0.6769	0.8947	0.619
mXsCIR20	$(CT)_{11}(TC)_{15}(TCTA)_3$	220	F: CCCTTATTGCTGTTTTCA R: CATATCTCTTCCTCTCACCA	KF693228	50	6	226–247	0.6483	0.9487	0.586
mXsCIR21	$(AG)_{30}$	194	F: CTTAACCTTGTCAGCCTCT R: GAGCGGTATAACAATTCATC	KF693229	50	4	194–226	0.6295	0.8421	0.577
mXsCIR22	$(AG)_{22}$	211	F: CGTGAGAAACACCTGAATTA R: AATTTGCTCTGTCATTGTG	KF693230	50	4	216–226	0.6972	0.9744	0.629
mXsCIR23	$(GA)_{23}$	166	F: TGTAGGTATGGACACATGG R: TTAAGACAAACCCTCAGC	KF693231	50	3	176–184	0.5513	0.7436	0.475
mXsCIR24	$(AG)_{23}$	204	F: AATTTGAAGTGAAACGATCA R: TTCCTGTCATCAGAATTGTA	KF693232	50	3	206–225	0.5362	0.4324	0.474
mXsCIR26	$(TC)_9(TC)_9$	266	F: TTCACCATTACTTGTCCACT R: TTAACATGGGAACGTATCTT	KF693233	50	3	245–285	0.5914	0.8974	0.511
mXsCIR27	$(AG)_{15}(GAA)_6$	198	F: TGCATGAATTGAAGAAAT R: AACAAAGAGTCTCACCACAT	KF693234	50	3	217–245	0.4467	0.5385	0.385
mXsCIR28	$(GA)_9$	201	F: ACAGAAGTTGACATGGAGAG R: AATGTTAAAGAGCAAAAGGA	KF709527	50	3	218–223	0.5017	0.7105	0.427

Note: *A* = number of observed alleles per locus; F = forward sequence; H_e = expected heterozygosity; H_o = observed heterozygosity; PIC = polymorphism information content; R = reverse sequence; T_a = optimized annealing temperature.

45 s, and 72°C for 1 min 30 s; and final elongation at 72°C for 8 min. PCR products were then electrophoresed on 1.2% agarose gels at 80 V.

Out of 85% of clones giving positive amplification, 120 inserts were purified on NucleoSpin Gel and PCR Clean-up columns (Macherey Nagel), then sequenced using M13 primer and Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, California, USA), and run on ABI 3500XL Genetic Analyzers (Applied Biosystems). The vector and adapter flanking sequences were eliminated, SSR presence was detected, and PCR primers were designed from a subset of 30 appropriate sequences using the SAT Web application (Dereeper et al., 2007). The primers were commercially synthesized (Sigma-Aldrich, Dorset, United Kingdom) with forward primers having an M13-tail added to their 5′ end (5′-CACGACGTTGTAAAACGAC-3′). The added M13-tails were labeled with IRD700 or IRD800 fluorochromes. To test the efficiency of the markers, a sample of 39 genomic DNAs of *X. sagittifolium* from three different geographic origins (20 from Vanuatu, 18 from Burkina Faso, and one from India) was used. The accessions are kept as living material at the Vanuatu Agricultural Research and Technical Center (Vanuatu accessions), at the University of Ouagadougou (Burkina Faso accessions), and at Central Tuber Crops Research Institute (India accessions) (Appendix 1). PCR amplification was performed in a Techne TC-412 Thermal Cycler as described by Chaïr et al. (2010). Allele number and sizes were determined using AFLP Quantar Pro 1.0 software (KeyGene, Wageningen, The Netherlands). Expected and observed heterozygosities were estimated using GENETIX 4.04 software (Belkhir et al., 2002), and polymorphism information content (PIC) values were estimated using CERVUS 3.0.3 software (Kalinowski et al., 2007).

Seventeen out of the 30 loci were identified as polymorphic and generated consistent amplification products. The characteristics of these new markers are summarized in Table 1. The number of alleles observed for each locus ranged from two (mXsCIR05, mXsCIR07, and mXsCIR14) to six (mXsCIR11 and mXsCIR20), with an average of 3.65 alleles per locus. The observed (H_o) and expected (H_e) heterozygosities ranged from 0.00 (mXsCIR05) to 0.97 (mXsCIR11 and mXsCIR22) (average: 0.61) and 0.09 (mXsCIR05) to 0.78 (mXsCIR11) (average: 0.53), respectively. Twelve markers presented H_o values higher than the H_e values, imparting an excess of heterozygous individuals in the studied sample and suggesting high rates of asexual reproduction, mainly due to vegetative propagation. In the absence of random mating, the tests for Hardy–Weinberg equilibrium and linkage disequilibrium were not performed. The PIC values obtained ranged from 0.01 (mXsCIR05) to 0.07 (mXsCIR11) (average: 0.48), with values greater than 0.5 for nine loci.

Additionally, cross-amplifications were carried out to test marker transferability to *Caladium lindenii* (André) Madison, previously classified as *Xanthosoma*, and 16 *Xanthosoma* species (14 referenced and two unreferenced) namely: *X. atrovirens* K. Koch & C. D. Bouché, *X. blandum* Schott, *X. brasiliense* (Desf.) Engl., *X. ceronii* Croat & L. P. Hannon, *X. granvillei* Croat & S. A. Thomps., *X. harlingii* Croat & L. P. Hannon, *X. hylaeae* Engl. & K. Krause, *X. mexicanum* Liebm., *X. piquambiense* sp. nov. Croat, Scherber. & G. Ferry, *X. poeppigii* Schott, *X. pubescens* Poepp., *X. robustum* Schott, *X. violaceum* Schott, *X. viviparum* Madison, *X.* sp. Croat, and *X.* sp. nov. Croat (Appendix 2), using the experimental protocol described above (Table 2). One out of 17 primers (mXsCIR11) showed 100% cross-amplification. The remaining markers were restricted to certain species, with rates varying from 23.5% (mXsCIR27) to 94.1% (mXsCIR12), with an average of 58.1%. Interestingly, a high number of markers (rates varying from 88.2% to 94.1%) showed transferability to the species *X. brasiliense*, *X. robustum*, *X. violaceum*, *X. atrovirens*, and *X. blandum*, suggesting high phylogenetic proximity of these species with *X. sagittifolium*. The remaining species seemed to be moderately close to *X. sagittifolium* as suggested by a lower transferability of the markers, with a rate varying from 35.3% to 58.8%. DNA of *C. lindenii* was amplified by only two markers (mXsCIR11 and mXsCIR23), confirming its classification in the *Caladium* genus as demonstrated by Loh et al. (2000).

TABLE 2. Cross-amplification of 17 SSR loci from *Xanthosoma sagittifolium* in other species of *Xanthosoma* and *Caladium*.[a]

Locus	*C. lindenii* (0-u-4195)	*X. violaceum* (2004)	*X. atrovirens* (013188)	*X. blandum* (080119)	*X. brasiliense* (070064)	*X. ceronii* (070377)	*X. granvillei* (031506)	*X. harlingii* (020201)	*X. hylaeae* (011463)	*X. mexicanum* (060617)	*X. piquambiense* sp. nov. (120412)	*X. poeppigii* (070769)	*X. pubescens* (031520)	*X. robustum* (060616)	*X. viviparum* (031522)	Transferability of SSR marker loci (%)
mXsCIR05	—	+	+	+	+	+	—	+	—	+	—	+	—	+	+	66.7
mXsCIR07	—	+	+	+	+	+	+	—	—	—	+	+	—	+	—	60.0
mXsCIR10	—	+	+	+	+	—	—	+	—	—	—	—	—	+	—	40.0
mXsCIR11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100
mXsCIR12	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	93.3
mXsCIR13	—	+	+	+	+	—	—	+	—	—	—	—	—	+	—	33.3
mXsCIR14	—	+	+	+	+	+	+	+	+	—	+	+	—	+	+	80.0
mXsCIR16	—	+	+	+	+	—	—	+	—	—	—	—	—	+	—	33.3
mXsCIR19	—	+	+	+	+	+	+	+	+	—	+	+	+	+	+	86.7
mXsCIR20	—	+	+	+	+	—	—	+	—	+	+	—	—	+	—	46.7
mXsCIR21	—	+	+	+	+	—	—	—	—	—	—	—	—	+	—	33.3
mXsCIR22	—	+	+	+	+	—	—	—	—	—	—	—	—	+	—	33.3
mXsCIR23	+	+	+	+	+	+	+	+	+	+	—	+	+	+	—	86.7
mXsCIR24	—	—	—	—	—	+	+	—	—	—	—	—	—	+	—	26.7
mXsCIR26	—	+	+	+	+	+	+	—	—	+	—	+	+	+	+	73.3
mXsCIR27	—	—	—	—	+	+	+	+	+	—	—	—	—	—	—	20.0
mXsCIR28	—	+	+	+	+	+	+	+	+	+	—	+	+	+	+	86.7

Note: + = successful amplification; — = failed amplification.
[a]Voucher numbers are given in parentheses.

CONCLUSIONS

We present a first attempt to develop SSR molecular markers for *X. sagittifolium*. The 17 polymorphic markers identified here represent powerful tools for investigating genetic diversity, population genetic structure, and conservation biology. In addition, they are useful for clone and provenance identification—a necessary prerequisite for germplasm maintenance and the development of core collections. This research will considerably improve our knowledge of wild and cultivated cocoyam

relationships. It will also serve as a base for the development of conservation strategies for this neglected crop. In addition, this set of markers proved to have broad transferability with other related species of the same genus. The information gained in the current study is therefore essential for future research on these species.

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Appendix 1. *Xanthosoma sagittifolium* specimens used in this study. All specimens were conserved as living accessions at the Vanuatu Agricultural Research and Technical Centre (VARTC), Vanuatu; at the University of Ouagadougou, Burkina Faso; and at the Central Tuber Crops Research Institute (CTCRI), India.

Collection no.	Locality (GPS coordinates)
MZN 28	Batié, Burkina Faso (09°59.496′N, 002°55.978′W)
BDG 11a	Bérégadougou, Burkina Faso (10°43.393′N, 004°44.780′W)
BDG 11b	Bérégadougou, Burkina Faso (10°43.393′N, 004°44.780′W)
MDD 1	Middebdo, Burkina Faso (09°59.258′N, 003°08.482′W)
TNS 1	Tansié, Burkina Faso (10°51.717′N, 003°14.135′W)
CAD 1a	Cascade Bo, Burkina Faso (10°43.213′N, 004°44.745′W)
CAD 1b	Cascade Bo, Burkina Faso (10°43.213′N, 004°44.745′W)
CAD 1	Cascade Bo, Burkina Faso (10°43.213′N, 004°44.745′W)
CAD 2	Cascade, Burkina Faso (10°43.261′N, 004°44.650′W)
CAD 3a	Séréfédougou, Burkina Faso (10°43.401′N, 004°42.089′W)
CAD 3b	Séréfédougou, Burkina Faso (10°43.401′N, 004°42.089′W)
DTA 1	Diatara, Burkina Faso (09°52.813′N, 002°55.980′W)
CAD 4a	Cascade Bo, Burkina Faso (10°43.305′N, 004°44.801′W)
CAD 4b	Cascade Bo, Burkina Faso (10°43.305′N, 004°44.801′W)
GUR 1	Guiéri, Burkina Faso (10°58.384′N, 005°02.149′W)
CAD 5a	Cascade Bo, Burkina Faso (10°44.717′N, 004°43.226′W)
CAD 5b	Cascade Bo, Burkina Faso (10°44.717′N, 004°43.226′W)
CAD 6	Banfora, Burkina Faso (10°36′N, 004°45′W)
Xs1	Vanuatu (unknown)
Xs2	Vanuatu (unknown)
Xs3	Vanuatu (unknown)
Xs4	Vanuatu (unknown)
Xs5	Vanuatu (unknown)
Xs6	Vanuatu (unknown)
Xs7	Vanuatu (unknown)
Xs8	Vanuatu (unknown)
Xs9	Vanuatu (unknown)
Xs10	Vanuatu (unknown)
Xs Hyb 1	Vanuatu (unknown)
Xs Hyb 102	Vanuatu (unknown)
Xs Hyb 157	Vanuatu (unknown)
Xs Hyb 180	Vanuatu (unknown)
Xs Hyb 184	Vanuatu (unknown)
Xs Hyb 210	Vanuatu (unknown)
Xs Hyb 227	Vanuatu (unknown)
CTCRI_IND_19	India (unknown)

Appendix 2. *Xanthosoma* and *Caladium* voucher specimens used in this study. All specimens were deposited at the botanical gardens, France.

Voucher no.	Species	Provider[a]	Collection locality	GPS coordinates
0-u-4195	*Caladium lindenii* (André) Madison	Chevreloup Botanical Garden	unknown	unknown
2004	*X. violaceum* Schott	Montpellier Botanical Garden	unknown	unknown
013188	*X. atrovirens* K. Koch & C. D. Bouché	Lyon Botanical Garden	unknown	unknown
080119	*X. blandum* Schott	Lyon Botanical Garden	Rémire-Montjoly, Cayenne, French Guyana	4°54′35.45″N, 52°16′34.62″W
070064	*X. brasiliense* (Desf.) Engl.	Lyon Botanical Garden	unknown	unknown
070377	*X. ceronii* Croat & L. P. Hannon	Lyon Botanical Garden	Sucumbios	00°13′06″N, 77°29′22″W
031506	*X. granvillei* Croat & S. A. Thomps.	Lyon Botanical Garden	Nouragues Research Center, French Guyana	4°04′18″N, 52°43′57″W
020201	*X. harlingii* Croat & L. P. Hannon	Lyon Botanical Garden	Mera, Haz. San Jose, Pastaza, Ecuador	1°27′26.15″S, 78°06′28.52″W
011463	*X. hylaeae* Engl. & K. Krause	Lyon Botanical Garden	Road from Tena to Puyo, Ecuador	unknown
060617	*X. mexicanum* Liebm.	Lyon Botanical Garden	Pena del Angel, Baja Verapaz, Guatemala	15°05′00.74″N, 90°24′46.69″W
120412	*X. piquambiense* sp. nov. Croat, Scherber. & G. Ferry	Lyon Botanical Garden	Lita, Esmeraldas, Ecuador	0°52′28.66″N, 78°28′04.07″W
070769	*X. poeppigii* Schott	Lyon Botanical Garden	San Juan Bosco, Morona-Santiago, Ecuador	3°06′08.69″S, 78°31′12.39″W
031520	*X. pubescens* Poepp.	Lyon Botanical Garden	Perou	11°07′49.03″S, 75°21′37.31″W
060616	*X. robustum* Schott	Lyon Botanical Garden	Alotenango, Sacatepequez, Guatemala	14°29′14.15″N, 90°48′35.74″W
120401	*X.* sp. Croat	Lyon Botanical Garden	Colombia	unknown
120452	*X.* sp. nov. Croat	Lyon Botanical Garden	Medellin, Caldas, Vereda Santa Clara, Antioquia, Colombia	5°44′40.14″N, 74°40′38.51″W
031522	*X. viviparum* Madison	Lyon Botanical Garden	Puyo, Pastaza, Ecuador	1°29′32.41″S, 78°00′09.04″W

[a]Voucher specimens are deposited at the provider institutions.