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PRIMER NOTE

Isolation and characterization of microsatellite markers from *Musa balbisiana*

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Abstract

This is the first report of targeted development of B genome microsatellite markers in *Musa*. A total of 44 sequences with microsatellites were isolated from an enriched library of *Musa balbisiana* cv. 'Tani' (BB genome). Of these, 25 were polymorphic when screened on 14 diverse diploid and triploid *Musa* accessions. The number of alleles detected by each marker ranged between one and seven. All 25 microsatellite markers generated amplification products in all species and genome complements. These new microsatellite markers fill an important gap for diversity assessment and linkage mapping studies in plantain (AAB) and cooking banana (ABB).

Keywords: B genome, microsatellite, *Musa*, polymorphism, polyploid

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Bananas and plantains (genus *Musa* L.) are amongst the most important global food commodities in terms of commercial value in South and Central America, and have nutritional importance for millions of poor farmers in sub-Saharan Africa and Asia (Gowen 1995). All the edible species in the family Musaceae are found within the genus *Musa.* They are generally triploids with various combinations of A and B genomes. The diploid (2n = 2*x* = 22) progenitors of these genomes are *Musa acuminata* Colla. (A genome) and *Musa balbisiana* Colla. (B genome). All cultivated accessions of *Musa* are vegetatively propagated through suckers or asexual micropropagation. Only wild bananas propagate through a sexual reproductive cycle via seed production. Simple sequence repeat (SSR) markers are routinely used for screening cultivated germplasm for diversity analysis and molecular breeding in many crops because of their high level of polymorphism, codominant nature, efficiency and cost-effectiveness. Microsatellite markers have been utilized for numerous applications in *Musa* (Creste *et al*. 2004 and Crouch *et al*. 1998b references therein). Yet all these studies have relied on A genome-derived SSR markers (Jarret *et al*. 1994; Kaemmer *et al*. 1997; Lagoda *et al*. 1998), despite the importance of the B genome in plantains and cooking bananas. Thus, we report on the targeted isolation and characterization of B genome SSR markers in *Musa*.

DNA was extracted from leaf tissue of *M. balbisiana* cv. 'Tani' as described in Crouch *et al*. (1998a) and purified on a CsCl gradient. Genomic DNA was restricted with *Cla*I (NEB) and fractionated by 1% agarose gel electrophoresis. Fragments of 300–600 bp were excised and ligated with T4 DNA ligase (NEB) into pGEM7zf(+) vector (Stratagene), heat-shock transformed into *Escherichia coli* JM109 competent cells, and inoculated onto Luria-Bertani (LB)/ampillicin/X-Gal/IPTG plates. White (positive) colonies were selected, streaked onto nylon membranes, placed on top of LB/ampillicin selective media, and grown at 37 °C overnight. Replica filters of the nylon membranes were prepared. Colonies on filters were lysed and DNA hybridized to membranes, these were screened with end-labelled (α^{32}PdATP) oligonucleotide probes: $(GT)_8$ $(AT)_8$ and $(CT)_8$. Hybridization and wash conditions were as described by Akkaya *et al*. (1992). A total of 101 positive clones

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Table 1 Optimized reagent components in the PCR for each marker

Reactions	Primer (pmol)	Template (ng)	$MgCl_2$ (mM)	dNTP (mM)	Enzyme (*u*)
1	0.1	2.5	1.5	0.1	0.1
2	0.1	10	2.5	0.15	0.2
3	0.2	10	2.0	0.2	0.3
4	0.2	5	2.5	0.1	0.2
5	0.3	5	1.5	0.2	0.3

were selected from approximately 2500 colonies. These clones were bidirectionally sequenced with SP6 and T7 primers (MWG Biotech). The B genome DNA sequences were screened against all *Musa* accessions in GenBank and unpublished A genome sequences using BLASTN alignment, resulting in insignificant homology (alignments of < 18 bp in length), which indicates that the new loci are different to markers derived from the A genome.

Primer sequences were designed using GENEFISHER version 1.1 (http://bibiserv.techfak.uni-bielefeld.de/genefisher/) and NETPRIMER software (http://www.premierbiosoft.com/netprimer/). Initially, 44 primer pairs were screened on *M. balbisiana* cv. 'Tani' to optimize polymerase chain reaction (PCR) conditions and concentrations of primer, template DNA, Mg^{++}, dNTPs and Tag polymerase using a modified (5 × 5) grid approach (Cobb & Clarkson 1994). As the annealing temperatures of the markers ranged from 59 to 62 °C, two categories of touchdown temperature cycles were used, consisting of initial denaturation of 3 min at 95 °C for both profiles, followed by five cycles reduced by 1 °C per cycle (either 60–55 °C or 55–45 °C), culminating with 30 cycles of denaturation for 20 s at 94 °C, annealing for 20 s at either 56 or 48 °C respectively, extension for 30 s at 72 °C , and a final extension at 72 °C for 10 min. The PCR were performed in a volume of 5 μL using PCR component concentrations (Table 1) in a GeneAmp 9700 thermocycler (Applied Biosystems). The amplified products were resolved on 6–10% nondenaturing polyacrylamide gels, and the bands were visualized through silver staining.

Twenty-two microsatellites showed perfect dinucleotide repeats, the majority of which were GA repeats, there were also two 'imperfect' repeats consisting of a mixture of di-, tetranucleotide repeats, or both and one marker (Mb 1–126), which showed a compound repeat (Table 2). Following PCR optimization using *M. balbisiana* cv. 'Tani', 25 markers produced amplification products, summary details of these markers are shown in Tables 1 and 2. These 25 markers were used for screening Eumusa cultivars (one sample per accessions) to assess the level of polymorphism and cross-species specificity: Calcutta 4 (AA), Safet Velchi (AB), Kunnan (AB), Tani (BB), Mbwazirume (AAA), Yangambi km5 (AAA), Pisang baker (AAA), Prata Ana (AAB), Orishele (AAB), Figue Pomme Geante (AAB), Kluai Tiparot (ABB), Pelipita (ABB), Saba (ABB), and Monthan (ABB). All microsatellites markers detected polymorphisms amongst the 14 accessions and the number of alleles detected varied from two to seven, with allele sizes ranging from 119 to 600 bp. As the allelic relationships for allotriploid accessions are uncertain, calculation of the polymorphic information content and of gene diversity index was not considered appropriate.

All markers described here appeared to detect homoeologous loci, as they all produced amplification products in AA and AAA cultivars. These results support similar observations with A genome-derived microsatellite markers that were frequently observed to detect homoeologous loci in B genome accessions (Crouch, unpublished data). Ortiz and Vuylsteke (1994) observed trisomic segregation between AAB plantain × AA (Calcutta 4) for some traits which indirectly suggest the occurrence of A and B homoeologous pairing in *Musa*.

This is the first report of the isolation and characterization of microsatellite markers from the B genome in *Musa*. Despite the apparent close relationship between A and B genomes, the availability of B genome-derived SSR markers is a fundamentally important resource for systematically ensuring good coverage of the allopolyploid genomes of plantain and cooking banana. Thus, these new markers will be an important resource for diversity estimates and taxonomic analyses of Eumusa germplasm (including gene flow analysis and clonal diversity) and for genetic mapping and molecular breeding of agronomically important traits.

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Table 2 Characteristics of *Musa balbisiana* SSR markers. The name, repeat motif, primer sequences, predicted allele size, number of alleles (N_a), allele size range, PCR touchdown profile, PCR component concentrations (linked to Table 1) and GenBank Accession nos are detailed for 25 markers

Marker name	SSR motif	Primer 5′–3′	Predicted product size (bp)	N_a	Allele size range (bp)	Touch down	PCR	GenBank Accession no.
Mb1–5	$(GA)_{12}$	F: CGGAAGTGGCAGGGTAGAGA R: CCCAACAACTTATGGCGGAGA	343	6	360–405	55–45	5	CL449381
Mb1–12	$(TTCC)_6$ $(GA)_{16}$	F: CCTCTTCTCTCCTTCACTTTCTCA R: AGGATGGCGGAGATCTGGTCA	272	2	258–300	60–55	1	CL449384
Mb1–18	$(GA)_{14}$	F: CTACAACAATAATCCAGGGCAA R: GGTCATCACGGCGTTCTCCA	359	4	450–550	60–55	1	CL449434
Mb1–30	$(GA)_{12}$	F: GTGTGTGTGTGTGTGTGTGTGA R: GAATTCGTGCTTTCTGTTTGGTGA	213	5	260–310	60–55	4	CL449388
Mb1–50	$(TC)_{11}$	F: GTCGAACTCCTTCCACTTCCA R: TATGTAGGCTGTGTGTGTGTCTGA	281	2	420–600	55–45	5	CL449393
Mb1–52	$(GA)_{14}$	F: CCAGCGATACCCTTCATGACCA R: CTGATTAGGATTTGAAAGGGGCAA	399	4	420–440	60–55	1	CL449394
Mb1–53	$(GA)_{19}$	F: GTTCACATGAAGACCGGGCAA R: CTCTCGATGGGTTTCCCAAGGA	400	4	405–440	60–55	1	CL449437
Mb1–63	$(GA)_{20}$	F: AATGGCTGCCTGCCATGCA R: GAATCAAAGAGGCGAGAAGACGA	191	6	119–210	55–45	3	CL449396
Mb1–69	$(TC)_{15}$	F: CTGCCTCTCCTTCTCCTTGGAA R: TCGGTGATGGCTCTGACTCA	386	7	450–500	55–45	4	CL449442
Mb1–100	$(TC)_{12}$	F: TCGGCTGGCTAATAGAGGAA R: TCTCGAGGGATGGTGAAAGA	201	6	190–235	60–55	2	CL449445
Mb1–113	$(GA)_{18}$	F: AGGTGCCACACAGTTCAGACA R: CAACCCAAACCTGTTCGACCAA	399	7	425–437	60–55	1	CL449398
Mb1–119	$(GA)_{24}$	F: ACTTGGGACGTCGAAGCA R: TGTGCTGGAATTCCCCTCTGA	384	5	130–160	55–45	4	CL449400
Mb1–126	$(GA)_{12}(GT)_7$ $(GC)_7(CCTC)_5$	F: CACGGTTTTTCTTGCCCCGAA R: TTCCACGTGCAAGCCTGCA	167	3	190–200	60–55	1	CL449450
Mb1–134	$(GA)_{21}$	F: ATGCCCAAGAAGGGAAGGGAA R: TAATGCCGGAGGATCAGTGTGA	398	7	440–500	60–55	2	CL449407
Mb1–139	$(GA)_{21}$	F: AGGGAGGGATCAGAAGGAGCA R: CGGCTTCCTCTGAAGGTTCCA	190	6	250–285	55–45	4	CL449409
Mb1–146	$(TC)_8$	F: CCGTTGGATTTCTCCCCCACA R: GAAGAACTGGGCTTACCCAGGA	204	4	220–235	60–55	1	CL449414
Mb1–141	$(GA)_{12}$	F: ATTCGAGGCATCGTCGTCCA R: GAAGGTTCCCAGAATCGATAGTGA	398	4	420–600	55–45	4	CL449411
Mb1–147	$(GA)_{11}$	F: AGATTCCGTTTCCGTTGCTA R: AGTTTATTCGGTGGACGTTAACGA	326	5	335–370	60–55	1	CL449415
Mb1–148	$(GA)_{14}$	F: GATCTGTGTATTTGGTCGACGGTA R: TTCGTGACGCAAGCCTGA	157	4	175–215	55–45	2	CL449416
Mb1–149	$(GA)_{17}$	F: CCGAAACGAAGGTTACAACAA R: GCGCCACCTGTATCACTGT	190	3	180–200	60–55	1	CL449417
Mb1–156	$(GA)_{13}$	F: AGCAGACCCATCGAGATACG R: TTCTCTTTGCCTCGTTGCTT	229	3	350–400	55–45	4	CL449422
Mb1–174	$(GA)_{16}$	F: AGTGAGTGTCCCCAAACGTGA R: TAAAATGTGCAAATGGGCGTGGA	400	4	435–455	60–55	1	CL449429
Mb1–175	$(TG)_8(GA)_7$	F: TCACATACCGAACAGAGAGAGTCA R: CCGACCGTGAACTTCTTTTCCA	400	5	430–465	60–55	3	CL449430
Mb1–179	$(GA)_{21}$	F: ACTAACTGAGGAGAATCAAGGAGA R: AAACCGCCTCTACATCCGGAA	287	3	350–400	55–45	4	CL449432
Mb1–49–2	$(CT)_{11}$	F: GCGGTCAAGGATCCGACCAA R: GGCTTCCTCTGAAGGTTCCAGA	375	3	430–450	55–45	4	CL449392

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