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PRIMER NOTE

Novel microsatellite markers for *Bactris gasipaes* (Palmae)

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Abstract

Ten new polymorphic microsatellites were isolated and characterized in *Bactris gasipaes* using a microsatellite enrichment protocol and selective hybridization with oligonucleotide probes. The loci are highly polymorphic, with a mean of 14.6 alleles per locus and a mean expected heterozygosity of 0.83 among 62 individuals of the Pampa Hermosa landrace. These microsatellites will be useful for population genetic analysis and germplasm characterization for heart-of-palm breeding.

Keywords: *Bactris gasipaes*, landrace, microsatellites, peach palm

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Peach palm (*Bactris gasipaes* Kunth) was domesticated in the American humid tropics for its fruit and is important today for its heart-of-palm. Numerous distinct landraces possess morphological, chemical and yield characteristics for use in genetic improvement programmes (Mora Urpí *et al*. 1997). These genetically different landraces (Rodrigues 2001) may yield hybrid vigour in inter-racial crossings.

The Pampa Hermosa landrace (Yurimaguas, Peru) supplies the seeds used in the expansion of heart-of-palm plantations throughout Latin America, as it has a high frequency of spinelessness and is very productive. However, most of the components of heart-of-palm yield have low heritabilities, so molecular marker-assisted selection will be a powerful tool to assure genetic gain in improvement programmes.

Microsatellite loci were isolated from an (AG)-enriched genomic library constructed with *Mse*I-digested DNA from a single individual of *B. gasipaes* (Brondani *et al*. 1998). The polymerase chain reaction (PCR) products containing the inserts were sequenced using dye-terminator fluorescent chemistry in a Mega BACE 1000 sequencing system (Amersham Bioscience). The efficiency of library enrichment for AG/TC was verified in hybridization plates; 29.1% of the colonies were positive for the presence of microsatellites. Of the 291 positive clones sequenced, 115 contained repetitive sequences, predominantly GA and CT, varying in size from nine to 25 repeats. Of these, 65 (56.5%) sequences were appropriate for the design of primers in the flanking regions and 25 sequences were selected for primer pair design (PRIMER3; Rozen & Skaletsky 2000). Following primer screening on 4% polyacrylamide gels stained with silver nitrate, 10 primer pairs were selected considering both the variability and robustness of the amplified products. For genetic characterization of these loci, DNA was extracted from 62 individuals of *B. gasipaes* from a single population in the Pampa Hermosa landrace using DNAzol (Invitrogen, Inc). The PCR was performed with fluorescent labelling of the fragments (Schuelke 2000) with modifications. The PCR mixture (10 μL) contained 1× PCR buffer (10 mM TrisHCl, 50 mM KCl, pH 8.4), 250 μM dNTP, 2.5 mM $MgCl_2$, 0.25 μM primer forward tail M13, 0.375 μM primer forward M13 label with FAM, Hex or TAMRA, 0.5 μM primer reverse, 1.5 U *Taq* DNA polymerase (CENBIOT/RS), dH_2O and 25 ng of genomic DNA. The amplifications were performed in a PTC-100 thermal cycler (MJ Research) as follows: 94 °C for 2 min; 25 cycles of 94 °C for 10 s, 10 s at a primer-specific annealing temperature (Table 1) and 72 °C for 30 s; 10 cycles of 94 °C for 10 s, 50 °C for 10 s and 72 °C for 30 s; and final elongation at 72 °C for 30 min. Fluorescently labelled PCR products were analysed in the Mega BACE 1000. Alleles were sized using an ET-ROX-400 size standard (Amersham Pharmacia). Data collection and analysis were performed using GENETIC PROFILER AND FRAGMENT PROFILER software (Amersham Bioscience). The allele frequencies and observed and expected heterozygosities were calculated for each locus using ARLEQUIN (Schneider *et al*. 2000).

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Table 1 Characteristics of 10 microsatellite loci of *Bactris gasipaes* (Pampa Hermosa landrace, Yurimaguas, Peru, $n = 62$)

Accession no.	Locus	Repeat motif	Primer sequence (5′–3′)	T_a (°C)	Size range (bp)	A	D_L	D	H_O	H_E
AY629152	Bg02-04	$(GA)_8TA(GA)_{15}$	F: GCAAATTTTGGAGAGGGGTA R: CCCAGCGTTCTTCACTTTCT	64	118–178	19	0.89	0.91	0.84	0.89
AY629153	Bg02-05	$(GA)_{16}$	F: CCAACCTTGGTTCAAAGCAT R: CATTCTCCCATCTCCCTCAC	64	179–213	11	0.75	0.76	0.74	0.76
AY629154	Bg02-06	$(CT)_{14}$	F: CTTGCGCCCCTGACATAC R: GCTGAGTTCCGAGGAATTTG	58	89–121	8	0.77	0.78	0.68	0.78
AY629155	Bg02-08	$(CT)_{12}(CA)_9(GA)_3$	F: CTTTGTCCAGTGGCTCCTTC R: AAGGGAGAATCAGGGAGGAA	64	190–222	15	0.81	0.82	0.42	0.82
AY629156	Bg02-09	$(GA)_{17}$	F: CGCAGCAGCAGCAATAAATA R: TCCAGCAACTTTCAGTCGAG	58	160–194	13	0.83	0.85	0.79	0.84
AY629157	Bg02-10	$(CT)_{10}TT(CT)_5$	F: GATTGGGTCCAGATCCTCTTT R: GTGGCACACATGGGGTTC	64	146–178	10	0.74	0.76	0.68	0.75
AY629158	Bg02-11	$(GA)_{11}GG(GA)_5$	F: GGAGGCAACACAAATGGAGT R: GCCAGCTCTGGTTGAGCTAC	58	130–174	15	0.87	0.88	0.74	0.88
AY629159	Bg02-12	$(GA)_9CA(GA)_5$	F: CAGCAAGATAAATGGGTGCAT R: CGGATTCAAGTTTCCACACC	58	141–177	13	0.73	0.75	0.66	0.75
AY629160	Bg02-19	$(CT)_{23}(CA)_6$	F: GCGTTCAGACTTGCATACACA R: CCCACATGCAGGAGTCGTAT	58	149–205	21	0.92	0.94	0.76	0.93
AY629161	Bg02-24	$(GA)_{17}$	F: AAACCTGATCCGATTGGCTA R: CACCACCACCACCTCCAC	64	119–155	19	0.88	0.89	0.85	0.89

T_a, annealing temperature; A, number of alleles; D_L, estimated discriminating power; D, calculated discriminating power; H_E, expected heterozygosity; H_O, observed heterozygosity.

All microsatellite marker loci were highly polymorphic, with a mean of 14.6 alleles per locus. The mean expected heterozygosity was 0.83 and eight of the 10 microsatellites had expected heterozygosities greater than 75% (Table 1). The estimated discriminating power (D_L) and calculated D (Tessier *et al*. 1999) were high for all microsatellites, with D_L values greater than 0.75 in eight of the loci and nearly as high in the other two, similar to the values obtained by Martinez *et al*. (2002). These microsatellites will be useful in studies of population genetics and germplasm characterization of this important peach palm landrace.

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