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PRIMER NOTE

Isolation and characterization of highly polymorphic microsatellites in tea (*Camellia sinensis*)

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Abstract

Relatively little is known about the diversity and origins of tea. The highest value tea products are sold on the basis of their region of origin but there are currently no methods available to verify the claims made on packages. We have developed 15 microsatellite loci for tea. These have been evaluated for polymorphism in a set of tea clones to determine their usefulness for authentication purposes. The majority of the microsatellites developed proved to be highly polymorphic both between and within different geographical origins and offer the potential to investigate the population genetics and genetic origins of tea.

Keywords: *Camellia*, diversity, identity, microsatellite, tea

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World production of processed tea exceeded three million tonnes (dry weight) in 2002 and represents an annual market of ~US$4.5 billion (FAOSTATS, www.fao.org). The two tea species, 'China type', *Camellia sinensis* and 'Assam type' *C. assamica* originate around Southwest China, Myanmar and Northeast India (Assam), but the native range is obscured by a history of cultivation and introduction by man (Sealy 1958). Europeans obtained tea plants from China in the 18th and 19th centuries and established plantations in their colonies. Following the discovery that native tea plants in Assam were more suited to cultivation there (Griffiths 1967), China type plants were supplanted with Assam types, although hybridization had by then occurred and no plants were considered 'true' Assam (Banerjee 1992). Subsequent dispersal of tea cultivation involved large quantities of tea seed, often of mixed provenances, although there were preferences for certain seed stocks, particularly Assam types. Vegetative propagation (clones) began to replace seed propagation in the 1960s and probably reduced genetic diversity within tea cultivation (Visser 1976). The historical processes in each tea producing region — particularly the predominance of local clones — gives an opportunity to study the allelic differences between regions.

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Tea diversity has been studied with random amplified polymorphic DNA (RAPD) markers (Wachira 1995; Chen *et al*. 1998; Liyanage *et al*. 2001; Wachira *et al*. 2001; Kaundun & Park 2002) and amplified fragment length polymorphism (AFLP) markers (Wachira *et al*. 2001; Balasaravanan *et al*. 2003). However, both of these techniques generate dominant markers and in the case of RAPDs there are serious questions concerning reproducibility between laboratories. DNA extraction from processed tea has been demonstrated (Mahipal Singh & Ahuja 1999), but the DNA is unsuitable for RAPDs and AFLP. In order to investigate the possibility of detecting the region of production by genetic analysis of processed tea, we have developed a set of microsatellite loci. These markers should also provide a tool for assessing population structures throughout the range of tea material.

A microsatellite-enriched library was constructed according to the enrichment protocol of Edwards *et al*. (1996) and screened to develop microsatellite primers and test for optimal annealing temperature as described in Haddrill *et al*. (2002).

To evaluate polymorphism of the microsatellites, 10 ng of DNA samples were amplified in 12.5 μL reactions containing: 2.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 0.2 mM dNTP, 0.1 U *Taq* DNA polymerase (Promega), 0.5 μM forward (labelled with a fluorescent ABI dye) and reverse primer. The polymerase

Table 1 Development and screening of 15 microsatellite loci for tea (*Camellia sinensis*). #, number of tea genotypes assessed; T_a, annealing temperature for the primer pair; the size range of alleles for these genotypes is based on automated Genotyper data; alleles, the number of different alleles detected among the 14 or 15 genotypes assessed; the Polymorphic Informtion Content (PIC) values are calculated from these data

Locus	Primer sequences (5′–3′)	Repeat motif	EMBL Accession no.	#	Size range (bp)	T_a (°C)	Alleles	PIC value
CamsinM1	F: GAATCAGGACATTATAGGAATTAA R: GGCCGAATGTTGTCTTTTGT	$(GT)_{16}$	AJ621786	15	280–300	50	9	0.88
CamsinM2	F: CCTCTGGTGGTCCTACACCT R: AAAGCCTTGATGCCTTTCG	$(GT)_{17}$	AJ621787	15	240–260	55	10	0.91
CamsinM3	F: GGTGTGGTGTTTTGAAGAAA R: TGTTAAGCCGCTTCAATGC	$(CA)_{18}$	AJ621788	14	190–210	65	8	0.86
CamsinM4	F: ACATTCAAGCANTCCACATATGTGAAA R: CCTGNTGCAGGACTGTCTATAGATGA	$(GA)_{19}$	AJ621789	15	358–370	60	5	0.72
CamsinM5	F: AAACTTCAACAACCAGCTCTGGTA R: ATTATAGGATGCAAACAGGCATGA	$(GT)_{15}(GA)_8$	AJ621790	15	170–205	60	10	0.81
CamsinM6	F: TGTTTTCTTAGGGTTGGATAAAGG R: TTTTGTTGTAATGACGAAAATTC	$(TG)_{12}(T)_{15}$	AJ621791	15	280–300	55	11	0.89
CamsinM7	F: TGGTAAGGGTCCTAAGAGGTACAC R: TTCCAATCTTTTTCTATAACATCTGC	$(GT)_{16}$	AJ621792	15	210–235	55	10	0.87
CamsinM8	F: CCATCATTGGCCATTACTACAA R: CCATATGTGTGTGAATGATAAAACC	$(CA)_{17}((TA)_5$	AJ621793	15	154–170	65	10	0.92
CamsinM9	F: CTCATGGAGTCCAAGGAAGC R: AAAGCAGTCTGGAACCTTGC	$(CT)_{15}(CA)_{12}$	AJ621794	15	170–205	55	7	0.88
CamsinM10	F: TTACATCTCTTTTGCAGCTGTCGG R: CTTCGGGAACTTCTGCTTCATC	$(GT)_{16}$	AJ621795	14	170–195	65	8	0.86
CamsinM11	F: GCATCATTCCACCACTCACC R: GTCATCAAACCAGTGGCTCA	$(CA)_{12}$	AJ621796	14	82–160	65	9	0.81
CamsinM12	F: CATTATCGTCACTTGCAAAGAGGT R: CGAGAAGAAGAGCTCTATTGGTT	$(GT)_{12}(GA)_{18}$	AJ621797	14	135–190	65	12	0.89
CamsinM13	F: CACATTGTGGCGTGTTATTAATTT R: ACATTGGCTATCTCTCATCATGG	$(TG)_{13}$	AJ621798	14	160–246	60	13	0.92
CamsinM14	F: TGGACTTGGAAGGACTGAGG R: ACAAAGCTCAACCTGCCATT	$(GA)_{16}$	AJ621799	14	236	65	1	0
CamsinM15	F: CAACTTGAGCATCAAACGTCA R: TGAAGCTGTGGGAGATGTCA	$(CT)_{13}(CA)_{23}$	AJ621800	14	222	55	1	0

chain reaction (PCR) protocol used the optimized annealing temperature as determined above and given in Table 1. The PCR products were detected on the ABI PRISM 3100 Genetic Analyser, capillary array using Genescan polymer 3100 POP-4, loaded with 0.5 μL product, mixed with 9.5 μL GeneScan-500 size standard and Hi-Di Formamide (1 : 20), denatured at 95 °C for 2 min and ice cooled before loading.

Assessment of microsatellites was made using DNA from a set of clones derived from Kenya (six), South India (four), Malawi (three), Japan (one) and Assam (one). Most came from plants maintained in the University of Cambridge Botanic Gardens. DNA preparation was based on a modified Dellaporta method (Dellaporta *et al*. 1983). Approximately 0.2 g of dried leaf tissue was incubated in 0.75 mL extraction buffer (0.1 M Tris, 0.5 M $NaCl_2$, 0.05 M EDTA, 1% PVP, 10 mM 2-mercaptoethanol, 1% sodium dodecyl sulfate) in a 1.5 mL microfuge tube at 65 °C for 60 min. Samples were then centrifuged in a Biofuge Pico (Heraeus) microfuge at 16 060 *g* for 10 min. The supernatant was removed, and DNA precipitated with an equal volume of propan-2-ol and centrifuged in a Biofuge Pico (Heraeus) microfuge at 16 060 *g* for 20 min. The supernatant was discarded and the pellet resuspended in 100 μL TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) and diluted to 10 ng/mL.

Table 1 presents basic data on the microsatellites amplified and the results of screening 14 or 15 different genotypes with these primer sets. As can be seen, Polymorphic Information Content (PIC) values are generally high, with the number of different alleles ranging from (excluding CamsinM14 + 15) 5 to 13. This is the first report of a set of microsatellites developed from tea. The development of this set of highly polymorphic tea microsatellites should allow a base-line of genetic information to be generated for tea that is comparable world wide and would allow the accumulation of genetic information on the origins of tea.

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