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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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 MgCl₂, 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

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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. The PCR products were detected on the ABI PRISM 3100.

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 worldwide and would allow the accumulation of genetic information on the origins of tea.

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<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer sequences (5’−3’)</th>
<th>Repeat motif</th>
<th>EMBL Accession no.</th>
<th>Size range (bp)</th>
<th>( T_a ) (°C)</th>
<th>Alleles</th>
<th>PIC value</th>
</tr>
</thead>
</table>
| CamsinM1  | F: GAATCAGGCACTTATAAGAAATTAA  
            R: GGGGAAATTGTCTTCTTTTGT | (GT)\(_{16}\) | AJ621786           | 15              | 280−300         | 50      | 9         | 0.88      |
| CamsinM2  | F: CCTCTGCGGTCCTACTACCT  
            R: AAACCTTGATCTCCCTTGCC | (GT)\(_{17}\) | AJ621787           | 15              | 240−260         | 55      | 10        | 0.91      |
| CamsinM3  | F: GTCTGGTGTTTTTGAAAGAA  
            R: TTTAAAGGCCTCCTGAACC | (CA)\(_{18}\) | AJ621788           | 14              | 190−210         | 65      | 8         | 0.86      |
| CamsinM4  | F: ACATTCAACCACTTCAATATTG AA  
            R: CCTGNTCTCAGACTCTGATATGA | (GA)\(_{19}\) | AJ621789           | 15              | 358−370         | 60      | 5         | 0.72      |
| CamsinM5  | F: AAACCTCCAAACCCACTCGGTA  
            R: ATTATTGGCTCGAAAGCAGGA | (GT)\(_{15}\)(GA)\(_{18}\) | AJ621790           | 15              | 170−205         | 60      | 10        | 0.81      |
| CamsinM6  | F: TGGTTTTTTTACAGTTTAAAGG  
            R: TTTTTATGTAATGAGAAATACTC | (TG)\(_{12}\)(T)\(_{15}\) | AJ621791           | 15              | 280−300         | 55      | 11        | 0.89      |
| CamsinM7  | F: TGGTAAGGCTTCTCACCACAC  
            R: TTTTACCCTCTTTTCTAAACTCTGC | (GT)\(_{16}\) | AJ621792           | 15              | 210−235         | 55      | 10        | 0.87      |
| CamsinM8  | F: CCATCAAATGCTAAAGAAGAC  
            R: CCAATATTTCTCTGTAAAGTAAAAACC | (CT)\(_{15}\)(CA)\(_{12}\) | AJ621794           | 15              | 170−205         | 55      | 7         | 0.92      |
| CamsinM9  | F: TTATACCTCTCTTTTGCACCTGCG  
            R: CTCGGACAAATCTCTCTGATC | (GT)\(_{16}\) | AJ621795           | 17               | 140−195         | 65      | 8         | 0.86      |
| CamsinM10 | F: GCATCACTTCCACACCTACCC  
            R: GTGTCACCAACGCTGCTCTCA | (CA)\(_{12}\) | AJ621796           | 15               | 82−160          | 65      | 9         | 0.81      |
| CamsinM11 | F: CAATTCATTTTGCACACCTTCC  
            R: CCTGATCTCTTCTCTCTCT  | (CA)\(_{12}\) | AJ621797           | 15               | 135−190         | 65      | 12        | 0.89      |
| CamsinM12 | F: CCATCCATCTTCATCTTCA  
            R: CAGAAGAAAGGGCTTTATGGTT | (GT)\(_{12}\)(GA)\(_{18}\) | AJ621798           | 15               | 160−246         | 60      | 13        | 0.92      |
| CamsinM13 | F: CACATGGCTGGCCTTATCAATTCTT  
            R: ACGTTGCTACTCCTCTCATTGAG | (TG)\(_{13}\) | AJ621799           | 14               | 236              | 65      | 1         | 0         |
| CamsinM14 | F: TGACCTGTTGCAAGGACTGTTG  
            R: CAAAATGTCACCTCGGATC  | (GA)\(_{16}\) | AJ621800           | 14               | 222              | 55      | 1         | 0         |

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References