PRIMER NOTE

Microsatellite markers for Ceiba pentandra (Bombacaceae), an endangered tree species of the Amazon forest

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Abstract

From a genomic library enriched for AG/TC repeats, eight polymorphic microsatellite markers were developed for Ceiba pentandra, a pan-tropical forest tree. Polymorphism was evaluated using a panel of 74 adult trees. Using automated fluorescence detection, a total of 112 alleles was detected with an average of 14 alleles per locus. All microsatellite loci showed very high levels of genetic information content, with expected heterozygosity ranging from 0.814 to 0.895. These microsatellite markers represent a powerful tool to investigate refined questions of mating systems, gene flow, family structure and population dynamics in natural populations of C. pentandra.

Keywords: Bombacaceae, Ceiba pentandra, microsatellite, tropical tree

Received 2 July 2002; revision received 9 September 2002; accepted 18 December 2002

Ceiba pentandra (Bombacaceae), the kapok tree, is an emergent, fast-growing tree species with pan-tropical distribution. The species grows naturally in tropical America and Africa, whereas populations from Southeast Asia were probably introduced by man (Baker 1965). In the Amazon Basin, this giant tree occurs in the lowland, seasonally-flooded ‘várzea’ forest. Native populations of C. pentandra in the Peruvian and Brazilian Amazon are threatened because of intensive exploitation by the plywood industry.

The development of microsatellite markers provides a valuable tool for rapidly generating information on the patterns of genetic variation, gene flow and mating systems in natural populations to develop better strategies for sustainable management and conservation of tropical tree species. Here, we report the development and characterization of a highly informative battery of eight microsatellite markers for C. pentandra.

An enriched genomic library from a single C. pentandra tree was constructed with Tsp 509 digested DNA, following protocols described by Brondani et al. (1998). Recombinant clones in plasmid vectors were transformed into E. coli cells according to Collevatti et al. (1999), and colonies having simple sequence repeat (SSR) were identified by poly AG/TC probe hybridization. Positive clones were picked and sequenced using dye-terminator fluorescent chemistry, and resolved on an ABI 377 instrument. Primers to the SSR flanking regions were designed using the software PRIMER (Lincoln et al. 1991).

Out of 300 recombinant colonies, 49 (16%), identified as positive for AG repetitive sequence, were sequenced. As expected, AG repeats were confirmed for most of them. However, due to the complexity of the AG repeats inside the clone, primers flanking the SSR region were designed for 22 of them. Following microsatellite marker screening by silver nitrate detection in polyacrylamide gels, eight loci were selected based on a higher level of allelic variation, low stutter and robustness of interpretation in a sample of six individual plants.

To carry out detailed genetic analysis, forward primers labelled with HEX, 6-FAM and NED fluorescent dyes were synthesized to allow multiplexed electrophoresis and allele scoring. PCR amplification was carried out in a 13 µL volume containing 7.5 ng genomic DNA, and 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl₂ (pH 8.3), 0.2 mM of each

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CP1  ggACTCCTAggCTCTgCTTACT  (AG)$_{27}$  182–236  65  17  0.845  0.360  0.040  0.711  AF503157
CP12  CTTgAggTgTCACgATgCCCT  (CT)$_{22}$  81–133  53  17  0.871  0.777  0.027  0.758  AF503158
CP13  ACCACTgAAgCgAAgCTCCTACg  (AG)$_{30}$  105–125  65  11  0.834  0.667  0.044  0.695  AF503159
CP15  CCACgAggAAgCgAAgCTCCTACg  (TC)$_{19}$  91–109  65  10  0.814  0.690  0.058  0.655  AF503160
CP18  gATTgTCTCCTCCTgCTCAT  (CT)$_{25}$  117–153  65  15  0.873  0.592  0.027  0.760  AF503161
CP19  gATgAggATgAAgCTCCTACg  (GA)$_{25}$  117–141  69  12  0.833  0.810  0.043  0.698  AF503162
CP20  GCATATAggAggCTgCTCAT  (CT)$_{18}$  142–184  65  15  0.895  0.860  0.019  0.797  AF503163
CP22  TAATTCTTCgAACTgCTgAAgA  (GA)$_{18}$  249–271  63  15  0.823  0.727  0.051  0.670  AF503164

Acknowledgements

The authors would like to acknowledge the Ministry of Education CAPES master and doctoral fellowships to A.A.M. and R.P.V.B., respectively, the Brazilian National Research council — CNPq master, doctoral and research fellowships to M.K., F.A.G. and D.G., respectively, and the CNPq/PADCT grant # 620059–97.4 to D.G.

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