GENETIC MAPPING OF COTTON: ISOLATION AND POLYMORPHISM OF MICROSATELLITES

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Abstract

Polymorphic DNA markers have numerous applications in the cotton improvement programs, e.g., genetic mapping and tagging genes controlling agronomically important traits, marker-assisted selection, germplasm screening, pedigree analysis and cultivar identification. Microsatellites, also referred to as Simple sequence repeats (SSRs), are tandemly repeating di-, tri- or tetra- nucleotide units with high polymorphism information content (PIC) relative to that of other types of markers. However, the principal disadvantage of microsatellites as markers in plants is the high costs associated with isolation, sequencing and producing primer pairs. To isolate and characterize microsatellites, we have constructed a cotton genomic library enriched for $(GA)_n$ repeats. Sequence analysis of enriched libraries revealed that 116 clones out of 146 analyzed had sufficient 5' and 3' flanking sequence for PCR primer synthesis. The remaining 30 clones did not have sufficient 5' or 3' sequence. Furthermore, 12 redundant sequences were identified in 17 of the 116 clones. These redundancies were presumably derived from bias which occurred during the pre- or post-selective PCR amplification(s). Microsatellite repeats were between 16 and 60 with an average of 28 repeats in length. Shorter repeats are presumably represented in the cotton genome but selected against during the high stringency washes used in screening enriched libraries. Out of 99 non-redundant $(GA)_n$ microsatellite containing DNA sequences, 38 primer pairs were designed and synthesized. A few cotton genotypes were used to evaluate polymorphisms of selected $(GA)_n$ microsatellites. Out of 38 primer pairs tested, only 20 gave good amplification products. The remaining 18 primer pairs either produced unscorable complex microsatellite banding pattern or produced a number of "ghost" bands. Allele numbers are ranging from 3 to 9 among 10 genotypes tested. Although the degree of various microsatellite repeat polymorphism remains to be determined with large collection of cotton genotypes, the level of polymorphism with (GA)n repeats is high. It appears that a few microsatellite loci is sufficient to distinguish cotton genotypes. We plan to develop a core set of robust, and hypervariable microsatellites for cotton genotyping in the next two years.

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