AFLP MARKERS IN COTTON

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Abstract

The application of biotechnology for genetic manipulation at the molecular level requires that specific genes be identified and characterized. Genetic mapping with DNA markers has become one of the essential tools for revealing the genetic basis of both qualitative and quantitative traits in crop plants. The objectives of this research are: 1) to develop a suitable protocol for a non-radioactive AFLP method in cotton and 2) to identify the chromosomal location of the AFLP markers in cotton. Inter-specific monosomic and monotelodisomic F_1 cytogenetic deficient stocks between TM-1 (*G. hirsutum*) and Pima 3-79 (*G. barbadense*) were used to identify chromosomal locations of the AFLP markers. We used a modified method of AFLP using non-radioactive silver staining to develop DNA markers using an AFLP kit from Life Technologies Inc. and a staining kit from Promega Inc.. We have developed a silver staining method for the AFLP technique in cotton which will provide several advantages over the techniques that require the use of radioactive dNTPs because: 1) radiolabeled compounds and X-ray film were not used; 2) it is less expensive, simpler and faster with direct detection of fragments (e.g. no autoradiography); 3) resolution of DNA fragments over a larger size range than observed with RFLP or RAPDs because of the use of 6% polyacrylamide non-denaturing gels; 4) cloning of DNA fragments from the gel does not require careful alignment of an autoradiograph with the gel, rather silver stained bands can simply be removed from the gel and the DNA fragments can be purified by additional amplification and electrophoresis. Our results from the screening of monosomic F_1 plants indicated the presence of four AFLP markers ranging from 400 bp to 560 bp on chromosome 16 (primer pair E-AGC and M-CTC), a 420 bp AFLP marker on chromosome 10 (E-AGC and M-CTT primer pair), a 450 bp AFLP marker on chromosome 17 (E-AGC and M-CTT primer pair) and a 320 bp AFLP marker on chromosome 25 (E-AGC and M-CTA primer pair).

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:483-483 (1997)
National Cotton Council, Memphis TN