MOLECULAR MARKER BASED GENETIC ANALYSIS OF QTLs USING RECOMBINANT INBRED LINES IN COTTON

H. Tan and J. X. Wu
Mississippi State University
Mississippi State, MS
S. Saha, J. N. Jenkins and J. C. McCarty
USDA/ARS
Mississippi State, MS
R. G. Cantrell
New Mexico State University
Las Cruces, NM

Abstract

Primary challenges in molecular mapping of quantitative trait loci (QTLs) are in: 1) constructing an agronomically acceptable mapping population which can detect small linkage effects free of distorted segregation and 2) developing an efficient molecular marker system which can detect large numbers of polymorphic markers. Recombinant inbred lines (RI) have recently been used as a powerful tool to construct linkage maps in many crop species. The specific objectives of this project are: 1) to develop a useful mapping population of *Gossypium hirsutum* L. RI lines with diverse agronomic and fiber traits; 2) to optimize an efficient molecular marker system that can rapidly score a large number of polymorphic markers; and 3) to identify a large number of polymorphic markers between the two parents of the RI lines to eventually construct a linkage map of molecular markers and QTLs. About 600 RI lines were derived without artificial selection from 96 individuals of an F_2 population from an intraspecific cross (*Gossypium hirsutum*) of HS46 and MARCABUCAG8US-1-88 by bulk-selfing from F_3 through F_6. Individual plants were selfed in the F_6 generation to produce the RI lines. All of the 598 RI lines were grown in the field this summer to record agronomic and fiber traits. Genomic DNAs were isolated from freeze-dried leaf tissue of the parents using a DNeasy spin column (QIAGEN, Germany) following manufacturer's protocol. SSR and AFLP methods were used to develop DNA markers. The SSR markers were received from Research Genetics, Huntsville, AL and the PCR method was used in amplification of the genomic DNAs of the parents as per manufacturer's protocol in the presence of fluorescently labeled base or primer. The AFLP analysis was done between the two parents using the AFLP Selective Amplification Module for large plant genomes from PE Applied Biosystems (Foster City, CA 94404). Capillary electrophoretic analysis (CE) of fluorescently labeled amplified DNA markers were visualized as peaks on electropherograms using the automated PE-Applied Biosystems ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA). Our results demonstrated that we have from a single cross 598 RI lines that are very diverse in many agronomic and fiber traits. The results also revealed the presence of significant additive and dominance QTL effects in the segregating population indicating the potential of genetic improvement for many of these traits in the RI lines. We used a DNA marker method, which is non-radioactive, highly sensitive and less laborious. This method is sensitive enough to detect one-two base pair DNA fragment size differences between alleles of a marker. Automatic sample loading, digitized output of DNA band position, automated data collection, and no need for gel preparation makes CE attractive to process large numbers of samples for analysis. We were able to rapidly detect at least 197 polymorphic SSR and AFLP markers between the two parents of the RI population. Many of these polymorphic markers will be used in our ongoing molecular mapping program of QTLs in cotton.

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