2002 BELTWIDE COTTON CONFERENCES, ATLANTA, GA – JANUARY 8-12

GENOMICS RESEARCH IN COTTON

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Abstract

Conventional breeding has greatly contributed to cotton genetic improvement and thus, to cotton high fiber yield and quality and low production cost. However, a decline in cotton yield and quality has recently occurred. Cotton breeders are facing a significant challenge to continued genetic improvement of this crop. Studies in other crop plants and farm animals have demonstrated that genomics research promises to provide powerful tools for enhanced genetic improvement. Examples of such tools include portable DNA markers and cloned genes that are essential for marker-assisted selection in germplasm analysis and variety breeding, and genetic engineering in molecular breeding. These tools will allow breeders to simultaneously select and pyramid several agronomic traits into a single variety and to transfer agronomic genes from distantly related species into cultivated crops efficiently. Nevertheless, genomics research of cotton lags far behind those of other major crops such as maize, soybean and wheat. Without essential genomic tools, cotton breeding and consequently yields and quality will remain lagging behind other crops that are subject to modern genetic analyses. Therefore, we have initiated genomics research in the crop, with an emphasis on the following areas:

1. *Molecular mapping and cloning of genes for root-knot nematode resistance (RNR) in cotton* - Root-knot nematode is one of the most destructive pathogens in cotton and it is estimated that approximately $150 million of cotton yield potential is lost beltwide each year due to this pathogen. To genetically map and clone the RNR genes, we developed four large mapping populations segregating for the RNR genes: Deltapine 16 x Auburn 623 and reciprocal, Deltapine 16 x Wild Mexican Jack Jones (TX-2516), and Auburn 623 x Pima 6. Each population consists of at least 5,000 F2 and/or F3 seeds. Preliminary genetic analysis using the Deltapine 16 x Auburn 623 population showed that the RNR of Auburn 623 appears to be controlled by two major linked genes. Using ten pairs of NILs for RNR, RAPDs were generated using 700 decamer primers, from which six DNA fragments were identified that are likely to be closely linked to the RNR. We are now further verifying the linkage relationship of the RAPD fragments with the RNR and developing portable DNA (e.g., SSR and/or SNP) markers for the RNR that are suitable for marker-assisted selection of the RNR in cotton germplasm analysis and breeding.

2. *Genomics of disease resistance (R) genes* - Pathogens, including fungi, bacteria, nematodes and viruses, cause about 10% loss of total cotton yield potential beltwide each year. To facilitate disease management and studies of pathogen-host interactions, approximately 33 genes conferring resistance to different pathogens have been cloned from several plant species. Sequence analysis showed that approximately 75% of the cloned R genes encode the motifs of nucleotide-binding site (NBS) and leucine-rich repeat (LRR) that are highly conserved across different plant species. Using degenerate primers designed from the NBS-LRR motifs of the cloned R gene products, we amplified the NBS-LRR-encoding genes of the cultivated cotton (Auburn 634) genome and generated an NBS-LRR-encoding gene-rich library. From this library, we randomly selected 229 clones and sequenced. BLAST search showed that about 26% of the clones showed high identities to the previously cloned R genes and/or R gene analogs in the Genbank. Phylogenetic analysis allowed classification of these R gene-like clones into several classes. To determine the positions of the clones in the genome, 16 of them were selected and mapped to the TM-1 x 3-79 cotton genetic map. Although the 16 clones were mapped to 7 linkage groups of the map, over half of the clones (9) were mapped to a single linkage group, indicating that the NBS-LRR-encoding genes are genetically clustered in the cotton genome. These R gene-like clones have provided a useful tool for discovery, mapping and cloning of the genes conferring resistance to different pathogens in cotton.

3. *Development of a whole-genome, BAC/BIBAC-based, integrated physical/genetic map* - Physical mapping, i.e., reconstruction of a genome from arrayed large-insert bacterial artificial chromosome (BAC) and/or plant-transformation-competent binary BAC (BIBAC) clones of a species, is the centerpiece of modern genomics research. Whole-genome physical maps integrated with genetic maps will provide revolutionized tools and platforms for all kinds of genomics research, including large-scale gene mapping, cloning and target DNA marker development. Development of such an integrated physical map for cotton will accelerate its genomics research manifold. To develop a whole-genome, BAC/BIBAC-based integrated physical/genetic map of the cotton AD genome that is widely applicable to the cotton community, the Upland cotton genetic standard line TM-1 is being used. We constructed eight BAC and BIBAC libraries from four lines of Upland cotton, of which six were developed from the Upland cotton genetic standard line TM-1 and its NIL. The TM-1 and NIL libraries have average insert sizes ranging from 130 to 154 kb, collectively covering 17.4 x the AD cotton genome. This genome coverage of clones is sufficient to develop a whole-genome, robust integrated physical/genetic map of the cotton AD genome. To rapidly develop the physical map of the cotton AD genome, we have also developed essential strategies and techniques, and automated the fingerprint analysis procedure of BAC and BIBAC clones by using robotic workstations (Autogen 960), capillary sequencers (ABI 3100) and advanced computer programs. Several pilot experiments were conducted on the feasibility of development of the cotton physical map from the AD-genome TM-1 BAC and BIBAC libraries using the strategies, techniques and procedures developed. The result showed that they allowed not only assembly of BAC/BIBAC contigs according to their origin of A or D genome, but also sorting of the contigs of A-genome from those of D genome. This result indicates that it is feasible to develop a robust physical map of the AD genome cotton using the techniques and strategies that we developed. Using the newly developed automated procedure of BAC and BIBAC fingerprint analysis, approximately 1000 BACs could daily be fingerprint-analyzed on one ABI 3100 capillary sequencer, and the clones (about 150,000) covering 10 x cotton AD genomes could be analyzed within 5 – 6 months. These clones are sufficient to assemble a whole-genome physical map of the cotton AD genome using our advanced computer programs.