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利用基因芯片技术筛选棉纤维伸长相关基因

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Identification of Fiber Length-Related Genes Using Cotton Oligonucleotide Microarrays

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摘要 从回交近交系(backcross inbred lines, BIL)群体中选取纤维长度差异较大的两个系NMGA-062 (33.03 mm)和 NMGA-140 (25.87 mm)，利用Affymetrix棉花基因芯片，分析其开花后10 d (DPA, days post anthesis)棉纤维伸长相关基因表达谱。在24 029条转录本中，两材料间差异表达的转录本有7 282条，占总数的30.31%；其中差异表达倍数在2倍或2倍以上的转录本有3 993条，占筛选转录本总数的16.62%，功能分类表明这些转录本主要包括功能预测基因(15.57%)、翻译、核糖体结构相关基因(13.54%)和翻译后修饰、蛋白质转换相关基因(9.29%)3大类。为了验证芯片数据的可信性，8个差异表达显著的基因(*Ghi.10655.1.S1_s_at*, *ACO1*, *ARF1*, *SAHH*, *TUA6*, *TUA7*, *β-tub1*, *β-tub10*)被用于实时荧光定量PCR。两种检测手段表现出一致性。随后，利用实时荧光定量PCR对3个与棉纤维相关基因(*ARF1*, *β-tub1*, *β-tub10*)在纤维发育不同时期(5、10、15、20和25 DPA)的表达模式进行了研究，结果表明，3个基因在纤维伸长发育时期(10和15 DPA)大量表达，推测这3个基因可能与棉纤维伸长有重要关系。

关键词： 基因芯片 纤维伸长相关基因 差异表达基因 实时荧光定量PCR

Abstract: *Gossypium barbadense* L. is known for its superior fiber quality including long fiber and their quantitative trait loci (QTL) have been reported. However, little is known about the molecular genetic basis of fiber quality traits. The objective of the present study was to identify differentially expressed genes in the rapid fiber elongation stage (10 days post-anthesis, 10 DPA) using a comparative microarray analysis between two backcross inbred lines (BIL) with contrasting fiber lengths. The two BIL lines, NMGA-062 (33.03 mm) and NMGA-140 (25.87 mm), were selected based on a 3-year field trial in four environments. The Affymetrix Cotton GeneChip was then used to perform a transcriptome analysis of 24 029 transcripts in developing fibers (10 DPA). Among the transcripts 7 282 (30.31%) showed a significant differential expression (DE) and 3 993 (16.62%) showed 2-fold or higher levels of expression changes between the two BIL lines. Through quantitative RT-PCR analyses on different plant organs and developing fibers of 10 DPA, eight selected DE genes, including *Ghi.10655.1.S1_s_at, ACO1*, *ARF1*, *SAHH*, *TUA6*,*TUA7*, *β-tub1*, and *β-tub10*, all displayed similar results to theses of the microarray analysis. This indicated that the comparative microarray results were biologically reproducible. Quantitative RT-PCR analyses were also performed at five fiber development stages from 5 to 25 DPA on *ARF1*, *β-tub1*, and *β-tub10*. The results indicated that they were all highly expressed in a period of fast fiber elongation and primary cell wall synthesis (at 10–15 DPA), implicating their roles in fiber elongation. This study represents the first investigation using a microarray analysis to compare differential gene expressions between near-isogenic lines with contrasting fiber quality. It provided a list of putative candidate genes for further studies in identifying genes responsible for fiber traits and developing molecular markers for marker-assisted breeding.

Keywords: Affymetrix microarray Fiber length-related genes Differential expressed genes Quantitative RT-PCR

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