棉花纤维特异/优势表达基因的染色体定位

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摘要：棉纤维是研究植物细胞伸长和细胞壁建成以及纤维素生物合成的优良模型，迄今为止，已经分离了许多纤维特异/优势表达的基因。为了便于这些基因的图位克隆使其能够应用于棉花纤维品质的改良中，本研究采用分离群体定位法和 Blast 分析法对这些基因进行染色体定位。利用陆地棉、海岛棉 BC_1 种间分离群体，将 *GhCFE* 定位在第 6 染色体，*GhGLP*1-250 定位在第 19 染色体。Blast 分析将 11 个基因定位到棉花染色体上。这些基因与棉纤维的伸长和细胞壁的合成相关，与这些基因连锁标记的获得有助于棉花纤维长度和强度的分子标记辅助选择。

关键词：棉花；纤维特异/优势表达基因；Blast 分析；染色体定位
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Chromosome Mapping Fiber Specific/Enriched Genes in Cotton

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Abstract: Cotton is one of the most important economic crops in the world, and developing cotton fiber is a perfect experimental model for studying the mechanism of cell elongation, wall development and cellulose biosynthesis in plants. Up to now, many genes that expressed specifically or preferentially in developing cotton fiber have been isolated. In order to facilitate map-based cloning of these genes and make them useful in cotton fiber breeding, genetic mapping was performed to chromosome localization of these genes by experimental and Blast analysis. *GhCFE* and *GhGLP*1-250 were mapped to chromosome 6 and 19, respectively, in the [(Emian22 × 3-79) × Emian22] BC_1 population. Eleven genes were mapped corresponding chromosomes by Blast analysis. These mapped genes are involved for cotton fiber elongation and cell wall synthesis, and markers linked to these genes will be helpful in marker assisted selection for cotton fiber length and strength.

Key words: cotton; fiber specific/enriched gene; Blast analysis; chromosome mapping

棉花纤维是由棉花胚珠外珠被表皮层的单细胞发育而成，属于种子表皮毛，是最长的植物细胞之一，形状为直线形，而许多植物的表皮毛是有分枝的，如拟南芥叶表皮毛。棉纤维细胞的分化和发育过程可分为纤维原始细胞的分化与突起、纤维细胞的伸长或初生壁的加厚、次生壁的加厚和脱水成熟四个时期[1]。棉纤维作为一类单细胞，细胞伸长的同时不进行分裂，数以万计的细胞定

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时且同时快速伸长。棉纤维细胞成为研究植物细胞伸长的优良模型。

棉花纤维的发育过程中有大量基因活跃表达。近十年以来，棉花纤维的发育吸引了众多研究者的目光。棉纤维是一类高度活跃的细胞，棉纤维表达基因通过参与特定生物学途径直接或间接影响纤维的发育。分离和识别纤维特异或优势表达基因不仅可以深入认识棉纤维伸长和纤维素生物合成的分子机理，还可以为改良棉纤维品质的遗传工程奠定物质基础。迄今为止，已经分离的纤维特异/优势表达基因已多达几十个[2~37]（表1）。为了便于这些基因的图位克隆使其能够应用于棉花纤维品质的改良中，本研究利用实验和生物信息学方法对这些基因进行染色体定位。

1 材料和方法

1.1 实验定位方法

利用原始文献提供的 GenBank 登录号从 GenBank 数据库下载相应基因的核酸序列。利用 Primer premier 5 软件[38]设计相应基因的特异引物（表1），引物设计的参数：引物长度为 18～30 个碱基，GC 含量 50%～70%，Tm 值 55～65℃，PCR 产物长度为 200～700 bp。用于纤维特异/优势表达基因定位的群体为 Zhang 等[39]所用的陆地棉和海岛棉种间 BC_1 作图群体，即[（鄂棉 22 × 3-79）×鄂棉 22]的 141 单株。PCR 反应体系为：DNA 模板 60 ng，上游、下游引物各 1 μmol·L^{-1}，200 μmol·L^{-1} dNTPs，1 × Reaction buffer，1.5 mmol·L^{-1} $MgCl_2$，*Taq* DNA 聚合酶 1 U（购自 MBI 公司），用 ddH_2O 加至 20 μL。PCR 反应程序为：94℃变性 3 min；94℃变性 1 min，55～60℃退火 1 min，72℃延伸 1 min，共 34 个循环；最后是 72℃延伸 7 min。扩增产物用 1.5%的琼脂糖电泳检测确定其是否成功扩增；成功扩增的产物用 6% 的变性 PAGE 胶分离，电泳后银染。利用软件 MapMaker3.0b[40]将表现多态性的基因整合到 BC_1 遗传连锁图上。

1.2 生物信息学定位方法

为了能够将在作图群体中没有表现多态性的基因定位到棉花染色体上，采用 Blast 分析进行相应基因的定位。从 CMD 网站上（http://www.cottonmarker.gov）下载已经定位的分子标记的序列，建立本地化数据库。从 NCBI 网站上（http://www.ncbi.nlm.nih.gov）下载 Blast 分析程序“BlastAll”用于序列匹配分析，序列匹配的标准为 E 值$<1 \times 10^{-15}$。

2 结果与分析

2.1 棉花纤维特异/优势表达基因的实验定位

用 72 对特异引物扩增两亲本鄂棉 22 和 3-79。扩增产物用琼脂糖电泳检测后发现，11 对基因引物没有特异扩增，它们是 *GhMYB25*，*GhHOX1*，*CFL1*，*GaRDL1*，*GhRDL1*，*GhSAC25*，*GhCNX*，*GhFAD3*，*GhBDC1*，*GhBDC2* 和 *Rac13*。51 对基因引物没有多态性。8 个多态性基因引物（*F10*、*F14*、*ACO2*、*Fb-B6*、*GhEXP2*、*GhEF1-like*、*GhPRP5*、*GhTua5*）表现为母本显性，因而在 BC_1 群体中不表现分离。两个基因的引物（*GhCFE* 和 *GhGLP1*）表现共显性差异，可用于群体分离。*GhCFE* 在 BC_1 群体中产生一个位点，连锁分析表明该位点定位于第 6 染色体（图1）。*GhGLP1* 产生两个共显性位点，其中 *GhGLP1-250* 定位于第 19 染色体（图1），*GhGLP1-280* 没有定位到棉花染色体上。

表1 已经克隆的棉花纤维发育特异或优势表达基因及其相应的上下游引物

Table 1 Genes that specifically or preferentially expressed in cotton developing fiber and corresponding primers

基因	上游引物	下游引物	参考文献
E6[b]	GAATGCCTACGAGTCCACTAAGC	CATCACAAGAAATTAACCGCAAG	[2]
H6[a]	CAACGATCTGAGCTTGTCTCGAT	TGAAACATTACAGAGCTATTGCATC	[3]
Fb-B6[b]	TGGCAGGTCCATATCACAAG	GTACCAGACTCATCGTTGAATTG	[4]
Rac13[b]	ACTGAGTTATAGAGGAGCTGATGTG	TTGTAATGACATGTTTGTAGGGAAC	[5]
FbL2A[a]	GATCGGTAGCCACACCGTCTC	CTCCTTGTGAATTTCAGGCTTCTC	[6]
ceLA1[a]	CCTCTATGGTATGGCTTTGGAGGT	AAGAGTGGTCCCCAAGCTTCGTA	[7]
celA2[a]	GAGGCCATTCACGTAATTAGCT	ACCATACCAAAGTGGACAGTGAC	[7]
SSS3[b]	GCTAACCTCGTAGTTGTAGGTGGT	GAACTGATTCAGCCAGCTTACG	[8]
ACP[a]	CTCTCTCCAATGGCTTCTATTGCT	CCAACCAACTGCGATCCTTG	[9]
GhEX1[a]	GTCTGCAACTCCATTTTCCTTG	CTGGACATAGGTAGCCATCCTGT	[10]
GhTua1[b]	CTGTTGCCACCATCAAGACCA	CTACTCGTCAAATCTATTGCAGTCC	[11]
GhTua2[b]	GTATCAACTACCAACCACCCACTG	CTAGGGTCAAATAACCACATGAATC	[11]

GhTua3[b]	AGTGCGGTATCAACTACCAACCA	GACATCGTAAGCACACTCAATCATAC	[11]
GhTua4[b]	CCATCAAGACCAAACGCACAAT	ACATCCCTCAATACTCCTCCTCATC	[11]
GhTua5[b]	ACGCATTGACCACAAGTTTGATC	GCATAATTTGAGAAACCTCACACG	[11]
AnnGh1[b]	GCAGGCTTATCATGCTCGTTAT	TTCATCCGTTCCTCGTCTATTG	[12],[13]
AnnGh2[b]	ATGCTCATCTGGCTAATGAAGCTAC	TCCAGCTCCGATCAAGGCAAGA	[12],[13]
CFL1[b]	GCTAGAAGTTGTGCTTCTGTTGGTG	GGATGGACAAGTCTGTTAACGCAA	[14]
F1[a]	GATGGAGCCATATATCTGCGTCT	AACTGCCAGGTAGGACAAAGAG	[15]
F4[a]	TTGACCAAAGATACAACCGACTA	CAATATCGGCCAATGCTTCTAC	[15]
F6[a]	AGTGATAAGGGAGAAGCCAGGA	GAAGTGAAAACAGCTCAACAGTGAC	[15]
F10[a]	AGACACCTATGGCTGAGGCAAC	CCATTATCATTAAAGACTTGAACCA	[15]
F11[b]	TGCTATCAACAAGGATTTGAAGG	ACCAAACAAACATAACTCACACCTC	[15]
F12[b]	TCTCCGTGAGGGTGTTACCACT	CTTCAATCCATGTCACCATAGC	[15]
F14[a]	ATAGGCATTTGGTGTTCAAGGAG	GGAAGTGAAGACGCCATAGCAT	[15]
F15[a]	GCTGTGCCTAGTCCTTCTACTATG	GTCTATAAGAAGAGACCAGTGATAGC	[15]
F16[a]	GAAGTGAATACCAGCATGAGAACT	GTCTATAAGAAGAGACCAGTGATAGC	[15]
GhExp1b[b]	CCTCAATGGTGCATTAGTCG	CCAGCTTCGTTATCAACACC	[16]
GhExp2[a]	CAACATGGTGTTGATAACCG	AGTTTGGTTCACTATTAGG	[16]
GhTUB1[b]	GGATGGATGAGATGGAGTTCACAG	CCTTCTTAGTCCCAGATCAAGCCAT	[17]
Gh-BTubL[b]	GAGTCACATGCTGCCTTCGCTT	CCTCCTAAACATCACTGTGAACTGTT	[18]
GhPFN1[b]	GCTCTAGAATGTCGTGGCAAACAT	CCTCCCACCTAAACAATC	[19]
GhMYB109[a]	GACCTCGTCATTAGACTTCATAAGC	TGGTCAGGAATCCAGAAAGTGTT	[20]
GhKCBP[a]	GAACATCTTACAAAAGATG	TTCTCCCAACAATTGATTAGTGT	[21]
GhGLP1[a]	AGAAGACAGCCACCGAAGATGA	TATTGAAATGCCGAGTCCGTTA	[22]
GhCTL1[a]	CCATTGGCTTGGGGTCTATGCTA	TCCTGCCTCTTCTCTCCCAACTC	[23]
GhCTL2[a]	AGTGCCCATCATTCATCAACCCTGA	CAAATCCATGAGGCTGATACTGAGC	[23]
GaMYB2[b]	CAGCATTGATGTGGACGAATTCT	CCAAGGAAACATGTTAATTACAAACT	[24]
GaRDL1[b]	ATACGTTTTCATCTGACAAGTTGC	CAGCTGCTATTGTATACTTTTGCA	[24]
GaHOX3[b]	ATTTCTTCAAAGATGAAAGAACTAGA	GGCTCGTAAAACGGATATACAGT	[24]
GhbZIPa	GGAGATCGTGAAAGAGGTCG	ATGCCCAAGTTGTTGGAGTC	[25]
GhManA2[a]	CAAGTGGGCGACGGCATT	TGTTGGTGATCCCTTTTACGG	[26]
GhACT1[b]	CCCTTGAATATTAAATAAATAAAAAAATA	TTGTGCTCAGTGGGGGTTCAACC	[27]
GhKCR1[b]	CACTTTGGGTTCTTTATCACTCTT	TTTATCTTCTTCACGCCTTCAT	[28]
GhKCR2[b]	TATCCTTTCTCACCCGCTCC	CACCACTTCCTC CTCCACCTC	[28]
GhRLK1[a]	GCTCTAGACGGACGAAGAAGCCCAAACT	CGAGCTCAAGCAGCAGCAATCAAGGC	[29]
GbFb3[b]	GGTGCAGGATTTCCAGCAGTAG	TTGCCATAATACACAAACCATCACT	[30]
GbMAPK[b]	AGAGCCCACTTGTCCATCTCCT	CCATCTTAAATCTCAACACCTCCAG	[30]
GhMYB25[a]	TCGATCCTGTCACCCACAAG	TTAGCAGCATCTTTAGGGTTACCA	[31]
GhRDL1[b]	GCTTGCCTAGCGCTTGCT	TGGCAGCTTATAGCTCCAATATTG	[31]
GhACT[a]	CCACTTGTCTGCGACAATGG	GAACACAGCCCTTGGAGCAT	[31]
GhSAC25[b]	GGCGACGACCTGCTACGT	GTCTACAAAGTATTTCGCGGTGACT	[31]
GhCNX[a]	GCATTTTGCGCTTCTTGTCTT	TCAGCGGCAATGCAGAGA	[31]
GhFAD3[a]	AAGCTACAAAAGCAGCGAAACC	AACGGAATCGGCCCTGAT	[31]
GhBDC1[b]	CCCGAATGTGGCTCTTTTCTT	TTTCAATGAAATGCAGGCTCAT	[31]
GhBDC2[a]	GCTAGCCGAGGTGAGACGAA	CGATGTGGCGAAGTCAATCA	[31]
GhNOD26[a]	GACACCGGCGGGTTTAGTAG	CGAAACCGCCACAAACAAC	[31]
GhEF1-like[a]	CAGGGCAGATTGGAAATGGA	GCAAACTTGACCGCAATGTG	[31]
GhSPP[b]	TGGATGCTTCAGCTCGGATA	CGACAACACATTCACTCCATCA	[31]
ACO1[b]	CGCCACTTGCCTGAATCTAAC	TGTGAGCCCTGAGTCCCTTG	[32]
ACO2[b]	TGAGGAGAGAGGAGCCACC	CCCTTAGCCCCTTGATTAGC	[32]
ACO3[b]	CACCAATGGCAAGTACAAAAGT	GCAAACAACACACATCTACGA	[32]
SMT1[b]	GGAGGCGAAGAGGAAGAGAG	GTTGTTTATCCCAGTGACCGA	[32]
DET2[b]	TCACTTTCTTCCTCTTTCCCTC	CACCAACACTTTATCAGCCCTA	[32]
GhCFE[b]	TGTGGCGGTTAAAGATGAC	GACTCAGCGACGGTTCAGA	[33]
GhAPX1[b]	TCCCTAACCTCACCTACGCT	CCTTCCTTCTCTCCCGACA	[34]
GhDET2[b]	GAAAATGGCCTCCGATCAGAC	CACCGAATCAATCATATGTGGC	[35]
GhPRP3[a]	GTACCATTGCCACCTCCGATG	TGTGATGGCAGAGTTCATTCTC	[36]
GhPRP5[a]	GAGAATTCTGAGAAGGAACCT	TAACTGAATGACAATAATTCAAG	[36]
GhHOX1[b]	ATTTTAGATGTAGGAGAGTGA	TTAGGGCCTATATAAGTTGGT	[37]
GhHOX3[b]	AGCAATGAGCGGCGAAGAT	ACCATAACCCAAATCAAATC	[37]
GhHOX4[b]	AGCACAGTCCACCATCTCTT	GATCATTTACATTCTTCCGAGTC	[37]

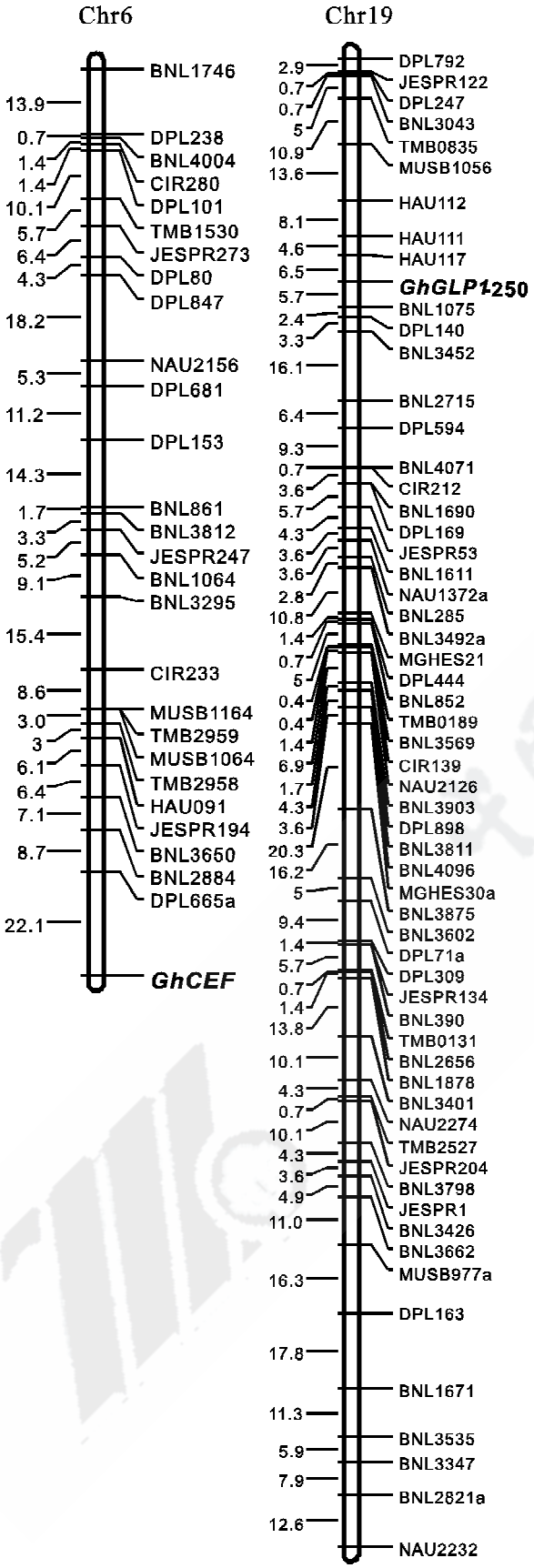

图 1 *GhCFE* 和 *GhGLP*1-250 在棉花基因组的染色体定位

Fig. 1 Chromosome mapping of *GhCFE* and *GhGLP*1-250 in cotton genome

2.2 棉花纤维特异/优势表达基因的 Blast 分析

由于大多数基因引物在群体中没有表现多态性，利用基因序列对已经定位的棉花标记进行 Blast 分析。结果表明，11 个基因定位到棉花染色体上(表 2)。*H*6 基因有两个同源标记，分别位于第 3 和 14 染色体上，而这两个染色体属于同源染色体。SSS3 位于第 6 染色体的一个区间，跨度为 6.8 cM[41]。*F*6、*GhTua*1、*GhTua*2、*GhTua*3、*GhTua*4 和 *GhTua*5 各有一个同源标记，但这些标记都有两个同源位点，位于同源染色体上。

3 讨论与结论

72 对基因引物中，11 对没有特异扩增，可能是引物设计的位置不当，扩增区间内有大内含子的存在导致产物远大于期望片段或偏离目标片段的多个产物。51 对引物在海岛棉和陆地棉之间没有多态性，说明这些基因在二者之间比较保守。由于本研究中所用的定位群体是回交群体，导致两亲本之间有多态性的显性标记无法定位，采用遗传信息量大的 F_2 群体可以实现这些标记的连锁定位。

两个表现共显性标记的基因中，*GhCFE* 定位于第 6 染色体。*GhCFE* 是一个棉纤维优势表达的基因，在纤维伸长期高效表达，该基因的功能尚不明确[33]。*GhGLP*1 产生两个共显性位点，只有 *GhGLP*1-250 定位于第 19 染色体；*GhGLP*1-280 没有定位到棉花染色体上，随着该图谱的不断丰富，该位点可能会定位到相应染色体上。*GhGLP*1 是一个棉纤维特异表达的基因，编码萌发素(germin)类似蛋白(GLP)，参与棉纤维的伸长过程[22]。这两个基因的定位有助于其在棉花纤维品质改良中的应用。

通过 Blast 分析，11 个基因找到了已经定位的同源标记。*F*1 基因为 3′，5′-二磷酸核酸酶，参与纤维细胞阳离子流的渗透调节[15]。*F*6[15]和 *E*6[2]基因参与次生壁的加厚。*H*6 基因的产物为富含脯氨酸的蛋白，参与次生壁的发育和构架[3]。*celA*2 基因编码纤维素合成酶的催化亚基，参与次生壁的生物合成[7]。*SSS*3 基因产物为蔗糖合成酶，参与细胞壁的蔗糖合成[8]。*GhTua*1-5 为一个基因家族，序列相似性很高，编码 α-微管蛋白，与纤维伸长有关[11]。这些基因同源标记的获得对于棉花纤维长度和强度的分子标记辅助选择提供了一个便捷途径。

Blast 分析的 11 个基因中，除 *GhTua*5 外，其

它基因在本研究的作图群体中没有表现多态性。Blast 分析的方法克服了实验群体的局限,为这些基因的定位开辟了一个新的途径。Xu 等[47]通过 Blast 分析发现棉花第 5、10、14 和 15 染色体是纤维发育相关基因的富集染色体。本研究中的多个基因都位于这些染色体上,证实了 Blast 分析方法的有效性。此外,Blast 分析方法也有助于棉花功能基因组学分离的其它基因的染色体定位。

表 2　棉花纤维特异/优势表达基因的 Blast 定位

Table 2　Chromosome assignment of fiber specific/enriched genes in cotton via Blast analysis

基因	同源标记	E 值	染色体		参考图谱
F1	BNL1161	2×10^{-23}	C10		[42],[43],[44],[39],[41]
F6	NAU3574	0	C10	C20	[41]
E6	NAU2128	0	C6		[41]
H6	NAU2190	0	C14		[41]
	MGHES66	1×10^{-127}	C3		[39]
celA2	NAU3902	6×10^{-26}	C5		[41]
SSS3	NAU2278	7×10^{-69}	C6		[41]
	NAU2156	2×10^{-66}	C6		[39],[41]
	NAU1272	2×10^{-26}	C6		[41],[45],[46]
GhTua1	GA__Ea0004N06	4×10^{-70}	C14	C23	[43]
GhTua2	GA__Ea0004N06	1×10^{-61}	C14	C23	[43]
GhTua3	GA__Ea0004N06	1×10^{-66}	C14	C23	[43]
GhTua4	GA__Ea0004N06	0	C14	C23	[43]
GhTua5	GA__Ea0004N06	1×10^{-17}	C14	C23	[43]

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