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ORIGINAL ARTICLE

Fine-mapping qFS07.1 controlling fiber strength in upland cotton (*Gossypium hirsutum* L.)

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Abstract

Key message **qFS07.1 controlling fiber strength was fine-mapped to a 62.6-kb region containing four annotated genes. RT-qPCR and sequence of candidate genes identified an *LRR RLK* gene as the most likely candidate.**

Abstract Fiber strength is an important component of cotton fiber quality and is associated with other properties, such as fiber maturity, fineness, and length. Stable QTL qFS07.1, controlling fiber strength, had been identified on chromosome 7 in an upland cotton recombinant inbred line (RIL) population from a cross (CCRI35 × Yumian1) described in our previous studies. To fine-map qFS07.1, an F_2 population with 2484 individual plants from a cross between recombinant line RIL014 and CCRI35 was established. A total of 1518 SSR primer pairs, including 1062, designed from chromosome 1 of the *Gossypium raimondii* genome and 456 from chromosome 1 of the *G. arboreum* genome (corresponding to the QTL region) were used to fine-map qFS07.1, and qFS07.1 was mapped into a 62.6-kb genome region which contained four annotated genes on chromosome A07 of *G. hirsutum*. RT-qPCR and comparative analysis of candidate genes revealed a leucine-rich repeat protein kinase (*LRR RLK*) family protein to be a promising candidate gene for qFS07.1. Fine mapping and identification of the candidate gene for qFS07.1 will play a vital role in marker-assisted selection (MAS) and the study of mechanism of cotton fiber development.

Introduction

Cotton is the world's most widely cultivated fiber crop. Upland cotton (*Gossypium hirsutum* L., $4n = 4x = 52$) dominates the production of cotton fiber, accounting for about 95% of the world's total production (Chen et al. 2007). Modern textile mills are adopting more efficient spinning technologies that rely on high speed and automation to achieve higher performance (Bradow and Davidonis 2000). Pursuing a high yield of good quality fiber is the ultimate goal of cotton breeding (Arpat et al. 2004; Lee et al. 2007), but it is still a challenging job for cotton breeders because of the complex genetic foundation governing fiber development.

Fiber quality traits are genetically complex quantitative traits, controlled by multiple genes and easily affected by environment, so it takes a long time to improve fiber quality through the traditional genetic improvement methods. Marker-assisted selection (MAS) is a more economical and efficient method than the traditional breeding based on phenotypic selection (Lande and Thompson 1990). Researchers have expended a great deal of effort on mapping quantitative trait loci (QTL). A recent meta-QTL analysis suggested that 1075 QTL in intraspecific *G. hirsutum* and 1059 QTL in interspecific (*G. hirsutum* × *G. barbadense*) populations has been detected for yield, fiber quality, seed quality, and stress tolerance (Said et al. 2015). Several new reports have added new cotton fiber quality QTL (Tang

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et al. 2015; Tan et al. 2015; Fang et al. 2014a; Shang et al. 2015).

Currently, only a few studies of fine-mapping cotton fiber quality QTL have been reported. Su et al. (2013) fine-mapped a fiber strength QTL QFS-D11-1 into an interval of 0.6 cM on chromosome 21 using introgressed lines from the cross between TM-1 (*G. hirsutum* L.)×H102 (*G. barbadense* L.). Cao et al. (2015) anchored qFL-chr.7 for fiber length and qFS-chr.7 for strength to a 0.36-cM interval, and anchored qFM-chr.7 for fiber micronaire to a 0.44-cM interval using a *G. barbadense* introgressed line. Islam et al. (2016) used an upland cotton intraspecific F_3 population to validate qFBS-c3, qSFI-c14, qUHML-c14, and qUHML-c24 related to fiber bundle strength, short fiber index, and fiber length, respectively, and spanned 4.4, 1.8, and 3.7 Mb of physical distance in the *G. raimondii* reference genome. Liu et al. (2016) identified a major QTL controlling multiple fiber quality traits using an upland cotton intraspecific population and mapped the QTL into 0.28 cM interval with a 5.3 Mb physical distance on chromosome A06 in *G. hirsutum*. Yang et al. (2016) detected and validated a stable fiber strength QTL (qFS-c9-1) within a ~2.98-Mbps genome region through four interspecific hybridization populations. However, all these QTL identified had confidence intervals too large for marker-assisted selection (MAS) and map-based cloning. Therefore, it is important to fine-map fiber quality QTL and identify QTL candidate genes in cotton.

Cotton fibers are seed trichomes that derive from individual cells of the epidermal layer of the seed coat. Fiber development consists of four overlapping stages: fiber initiation, cell elongation, secondary wall deposition, and maturation (Kim and Triplett 2001). Many studies have highlighted the stage-specific transcription of genes involved in fiber initiation, elongation, and secondary cell wall formation. For example, *GhMYB25* (Machado et al. 2009), *GhMYB25-like* (Walford et al. 2011), *GhSusA1* (Jiang et al. 2012), *GbPDF1* (Deng et al. 2012), *GhHD-1* (Walford et al. 2012), *GhFLA1* (Huang et al. 2013), *GhVIN1* (Wang et al. 2014), *GhCFE1A* (Lv et al. 2015), and *Gh14-3-3* (Zhou et al. 2015) have been demonstrated to have definite roles in cotton fiber initiation, and *GhPIP2* (Li et al. 2013), *GhHOX3* (Shan et al. 2014), *GhCaM7* (Tang et al. 2014), *GhPAG1* (Yang et al. 2014), and *GhCPC* (Liu et al. 2015a) play roles in controlling cotton fiber elongation. Fiber strength is a key component of fiber quality in cotton, and it is closely related to secondary cell wall synthesis (Fang et al. 2014b). So far, several genes related to secondary cell wall synthesis have been reported, such as *GhMYB7* (Huang et al. 2016), *GbEXPATR* (Li et al. 2016), *GhMYB1* (Sun et al. 2015), *GhCesA4* (Kim et al. 2011), *GbTLP1* (Munis et al. 2010), and *GhADF1* (Wang et al. 2009). However, only a few genes directly related to fiber strength have been reported. Therefore, an effective strategy to unravel the relationship between fiber quality traits and genes remains to be developed.

In our previous study, a major QTL for fiber strength, qFS07.1, was identified on chromosome 7 in five environments in upland cotton, with the favorable allele contributed by Yumian1 (Tan et al. 2015). To fine-map qFS07.1, we crossed CCRI35 with recombinant inbred line RIL014 with the Yumian1 allele for the qFS07.1 region to produce a fine-mapping population, and designed SSR primers according to the *G. raimondii* and *G. arboreum* genomes (Paterson et al. 2012; Li et al. 2014). RT-qPCR and molecular cloning were utilized to analyze gene expression and gene structure, identifying candidate genes related to fiber quality.

Materials and methods

Plant materials and fiber quality measurement

In our previous studies, CCRI35 and Yumian1 were chosen to establish a recombinant inbred line (RIL) population (Tan et al. 2015). CCRI35 is a high yielding and disease resistant cultivar bred by the Chinese Academy of Agricultural Sciences (Tan et al. 2015). Yumian1, a high fiber quality cultivar, especially characterized by high fiber strength, was developed from a multiple-cultivar intermating program (Zhang et al. 2009).

To fine-map qFS07.1 on chromosome 7, we selected one recombinant line, RIL014, characterized by superior fiber quality as one parent. For RIL014, the QTL region on chromosome 7 is derived from Yumian1 whereas the other regions with alleles affecting fiber quality are derived from CCRI35. We crossed CCRI35 with RIL014 in the summer of 2012 at Southwest University, Chongqing, China. F_1 plants were self-pollinated to obtain F_2 seeds in the winter of 2012 in Sanya, Hainan, China. The F_2 population and parents were grown in Chongqing, in the summer of 2013. $F_{2:3}$ individuals derived from F_2 recombinant individual plants were planted in the summer of 2014 at Southwest University, Chongqing, China.

All naturally opened bolls from the F_2/$F_{2:3}$ individual plants and parents were hand-harvested to gin fiber. Fiber samples were evaluated for fiber quality traits using the HVI (High Volume Instrument) system at the Supervision Inspection and Testing Cotton Quality Center, Anyang, China. Data were collected on fiber elongation (FE, %), fiber upper half mean length (FL, mm), fiber micronaire reading (FM), fiber strength (FS, cN/tex), and fiber length uniformity ratio (FU, %).

Marker development

Cotton genomic DNA was extracted from young leaves of F_2/$F_{2:3}$ individuals and parents, using a modified CTAB method (Zhang et al. 2005). To fine-map qFS07.1, a total of 1062 SSR primer pairs were designed from chromosome 1 of *G. raimondii* (Paterson et al. 2012) and 456 SSR primer pairs were designed from chromosome 1 of *G. arboreum* (Li et al. 2014). These primer pairs were named 'SWU' and were synthesized by Invitrogen Co. Ltd. (Shanghai, China) and Beijing Genomics Institute Genomics Co. Ltd. (Beijing, China).

The newly developed SSR markers were first screened for polymorphism between the mapping parents and those showing clear polymorphism were used to genotype the fine-mapping population. PCR amplification and product testing were performed according to Zhang et al. (2005). Clear polymorphic DNA bands on the gels were used for scoring and genotyping. Loci detected were named with the primer name.

QTL analysis

The genetic linkage map of the QTL region was constructed using JoinMap 4.0 (Van Ooijen 2006). Recombination values were converted into Kosambi genetic distances (cM) (Kosambi 1944). The interval mapping method of MapQTL6.0 (Van Ooijen 2009) was used to estimate additive effects and phenotypic variance explained. Linkage groups were created using MapChart 2.2 (Voorrips 2002). The precise position of QTL was determined by comparing the phenotypic means of $F_{2:3}$ recombinant plants within the QTL region.

Validation of candidate genes by real-time quantitative RT-qPCR

Total RNA was extracted from the developing cotton fibers [10-, 15-, 20-, and 25-day post anthesis (DPA)] of CCRI35 and Yumian1 using the RN38 EASYspin Plus plant RNA kit (Aidlab Biotechnologies Co., Ltd) according to the manufacturer's protocol. First strand cDNA was synthesized from total RNA by priming with oligodT primer using Thermoscript Reverse Transcriptase (Invitrogen, Carlsbad, CA). RT-qPCR reactions were carried out in quantitative real-time PCR kits (Bio-Rad), with a final volume of 20 ul containing 10 ul iTaq™ SYBR® Green Supermix with ROX (Bio-Rad Laboratories), 1 mM forward and reverse primers, and 0.1 mM cDNA template. Reactions were performed using an iQTM 5 Multicolor Real-Time Quantitative PCR Detection System (Bio-Rad, USA). The values from triplicate reactions were averaged, and the Ct values were determined and compared with the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Cloning and sequence analyses for candidate genes

Genomic DNA from parents CCRI35 and Yumian1 was used to amplify the DNA sequences of candidate genes. PCR reactions were performed in a total volume of 25 µl according to the PrimerSTAR® Max DNA Polymerase manufacturer's instructions. The products were electrophoresed through 1% agarose gels and extracted using a gel extraction kit (GeneMark). The purified PCR products were then used for "+A base" reactions with EasyTaq® DNA polymerase (#AP111, Transgen Biotech). The 50-µl reaction volume included 2.5 U Taq polymerase, 0.2 mM of dNTP, 5 µl of 10×Buffer (KCl), and the purified PCR products. The mixture was heated to 72 °C for 30 min, and the PCR products were subsequently used for TA cloning using TAKARA kits. Single bacterial colonies were validated by PCR, and triplicate positive clones of each cotton line were sequenced by Invitrogen Co. Ltd. (Shanghai, China) and Beijing Genomics Institute (BGI) Co. Ltd. (Beijing, China).

All DNA sequences related to upland cotton line TM-1 were downloaded from the Phytozome website (https://phytozome.jgi.doe.gov/pz/portal.html). Gene structural profiles were determined based on alignment analysis of DNA (NCBI, http://www.ncbi.nlm.nih.gov/spidey/spideyweb.cgi) as well as structural prediction from the FGENESH website. DNA and amino acid sequences were aligned with the DNAMAN program.

Results

Phenotypic variation for fiber quality traits in parental and mapping populations

Basic statistics describing fiber quality parameters for F_2 and $F_{2:3}$ populations were presented in Table S1. Fiber quality traits showed near-normal phenotypic distributions in F_2 and $F_{2:3}$ populations (Fig. S1). Based on 1 year of data collected from 2484 individuals, significant correlations were detected among the five fiber quality traits ($p=5\%$), except between fiber elongation and fiber micronaire (fineness) (Table 1).

High-density genetic map

All polymorphic SSR primer pairs were used to genotype the F_2 population of 2484 individuals (Table S2). Combined with the original primers mapped on chromosome

Table 1 Correlation coefficients between five fiber traits in the F_2 population of a cross between CCRI35 and RIL014

Traits	FL	FU	FM	FE
FU	0.277**			
FM	−0.457**	0.093**		
FE	−0.605**	−0.350**	0.045	
FS	0.637**	0.182**	−0.459**	−0.671**

FL fiber length, *FU* fiber uniformity, *FM* fiber Micronaire, *FE* fiber elongation, *FS* fiber strength

**Significant correlations at the 0.01 level

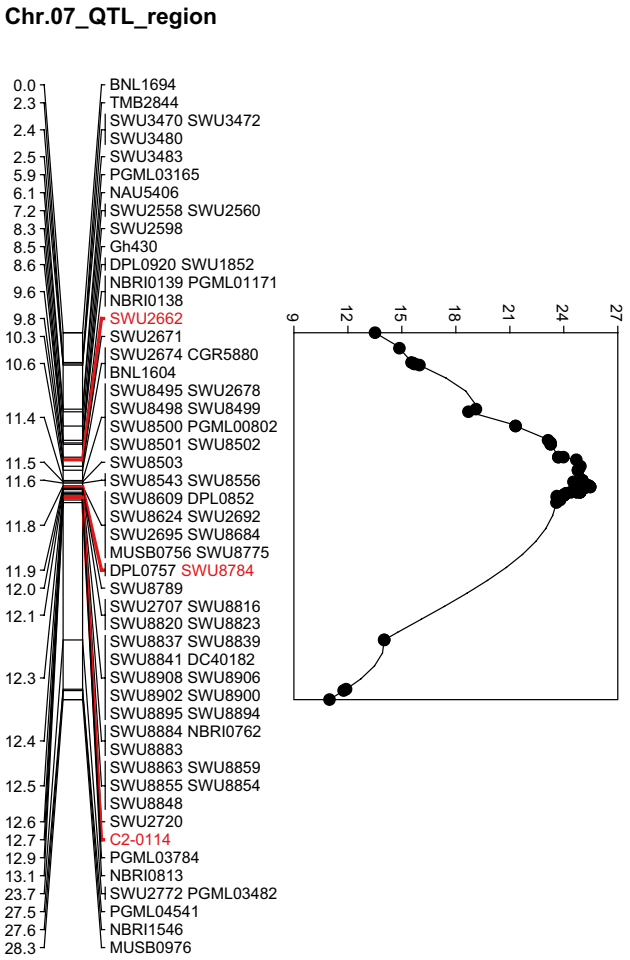

Fig. 1 Genetic map and LOD score for qFS07.1 for fiber strength on cotton chromosome 07, based on 2484 F_2 plants in 2013. The nearest marker of qFS07.1 is SWU8784, LOD score 25.47. The confidence interval for the position of the QTL from the peak LOD to less than 1, qFS07.1, was mapped into the interval of 2.83 cM flanked by SWU2662 and C2-0114

7, we constructed a high-density genetic map of the QTL region with 75 loci, spanning 28.3 cM (Fig. 1).

To check the accuracy and precision of the local genetic map, we compared it with the physical maps of the corresponding chromosome segments. The physical positions of SSR markers were based on aligning the primer sequences to A07 and D07 of *G. hirsutum* (Zhang et al. 2015) reference genomes (Table S3). The locus order on the local genetic map is completely consistent with the order on the physical maps from A07 and D07 of *G. hirsutum* (Fig. 2), which indicated that the local genetic map is accurate and suitable for QTL mapping.

QTL mapping of fiber quality in F_2 and $F_{2:3}$ populations

Based on the fiber quality traits of the F_2 population with 2484 individuals and the local genetic map, qFS07.1 was mapped into an interval of 2.83 cM flanked by SWU2662 and C2-0114. The additive effect of qFS07.1 was 1.17 cN/tex, and the phenotypic variation explained by qFS07.1 was 10.2% (Fig. 1; Table 2). The favorable allele of qFS07.1 was contributed by RIL014, whose QTL region on chromosome 7 is derived from Yumian1.

SSR markers within the interval flanked by SWU2662 and C2-0114 were used to further genotype 2571 $F_{2:3}$ individuals derived from 207 F_2 recombinant plants. Out of 2571 $F_{2:3}$ individuals, 904 individuals with more than 15 gram fiber were measured for fiber property and QTL analysis mapped qFS07.1 into a 0.33-cM interval flanked by SWU8775 and SWU2707 within the fine-map linkage group Gh_A07. The additive effect of qFS07.1 reached to 1.50 cN/tex and the phenotypic variation explained by qFS07.1 was 16.5% (Table 2; Fig. 3a).

QTL substitution mapping

Out of 904 $F_{2:3}$ individuals tested for fiber quality traits, 149 plants each had one recombinant event which took place between marker SWU8775 and SWU2707. These recombinants were grouped into nine classes based on their breakpoints (Fig. 3b). Comparing the genotypes of recombinant classes with those of parents, G1 to G5 had lower fiber strength, not distinguishable from CCRI35. Classes G6 to G8 had higher fiber strength, not distinguishable from RIL014. The recombinant groups G3, G4, G5, G6, and G8 delineated qFS07.1 to a 0.17 cM region between SWU8775 and SWU8789.

Predicted genes in the QTL region

The 0.17-cM region between SWU8775 and SWU8789 corresponds to 62.6-kb genome region on chromosome A07

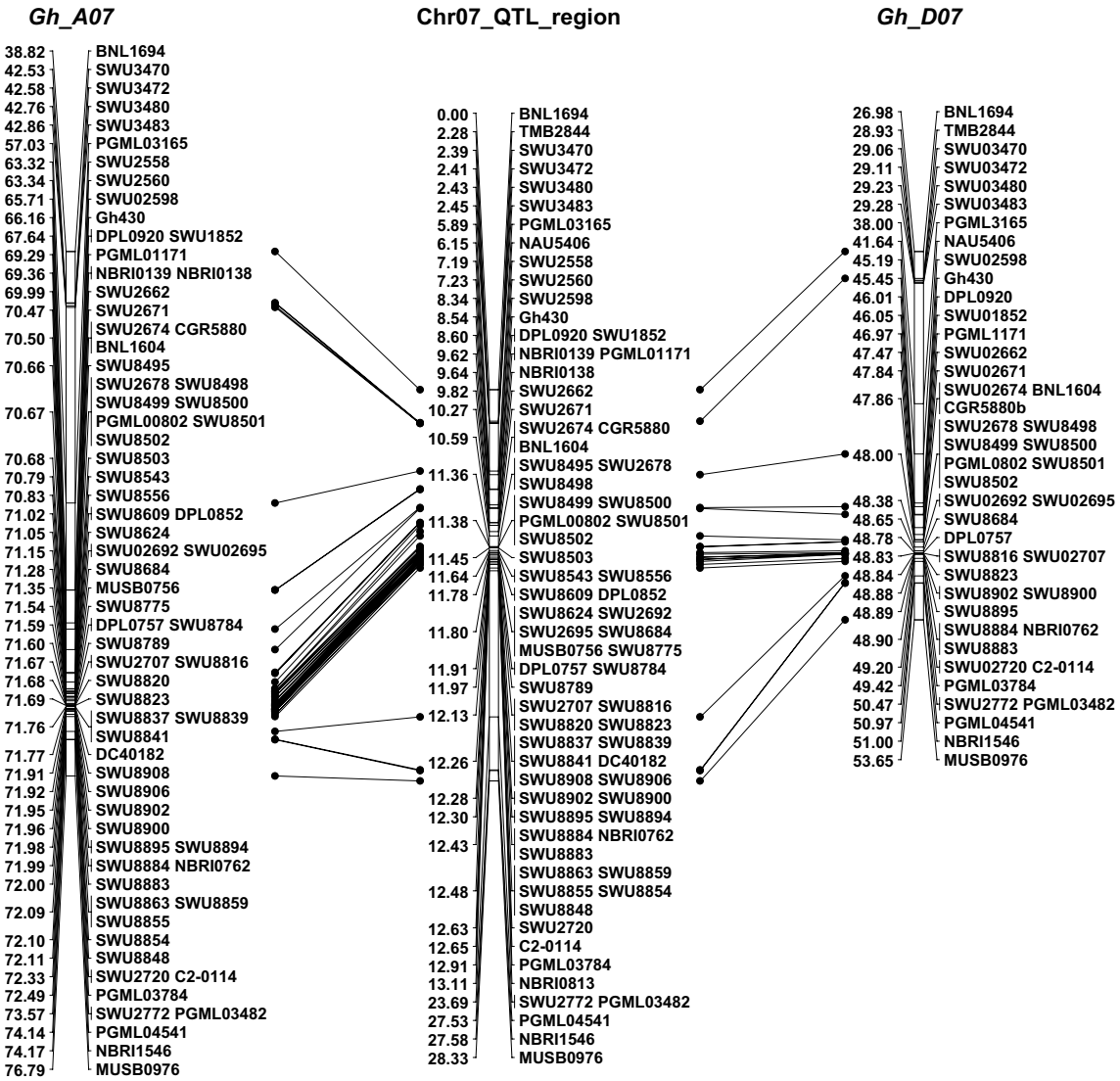

Fig. 2 Comparison of genetic and physical maps in the qFS07.1 QTL region. Chr07_QTL_region is the QTL region genetic map on chromosome 07 constructed in this study; Gh_A07 and Gh_D07 are the physical maps, based on the physical positions of SSR markers, aligning the primer sequences to A07 and D07 of *G. hirsutum* (Zhang et al. 2015) reference genomes, respectively

Table 2 QTL analyses for qFS07.1 in the F_2 and $F_{2:3}$ generations derived from the cross between CCRI35 and RIL014

QTL	Population	Nearest marker[a]	LOD	R^2(%)[b]	A[c]
qFS07.1	F2	SWU8784	25.47	10.2	1.17
	F2:3	SWU8789	35.09	16.5	1.5

[a]Marker where QTL peak is located

[b]% Of total phenotypic variation explained by the QTL

[c]Additive effect of the marker linked to the QTL peak. Positive signs indicate favorable alleles from RIL014

of the *G. hirsutum* genome. According to the chromosome A07 of *G. hirsutum* reference genome annotation (Zhang et al. 2015), the 62.6-kb genome region contains four predicted genes: histidine-containing phosphotransfer factor 5 (*AHP5, Gh_A07G1748*), leucine-rich repeat protein kinase family protein (*LRR RLK, Gh_A07G1749*), receptor-like protein 45 (*RLP45, Gh_A07G1750*), and nudix hydrolase homolog 23 (*NUDIX23, Gh_A07G1751*) (Fig. 4). Coding sequence comparisons between chromosomes of polyploid *G. hirusutum* revealed some asymmetry between genomes. *Gh_A07G1748* and *Gh_A07G1751* exist only in the A genome of *G. hirsutum* and the D genome reference sequence contained no homoeologous counterparts. The D genome reference sequence of *G. hirsutum* only contained

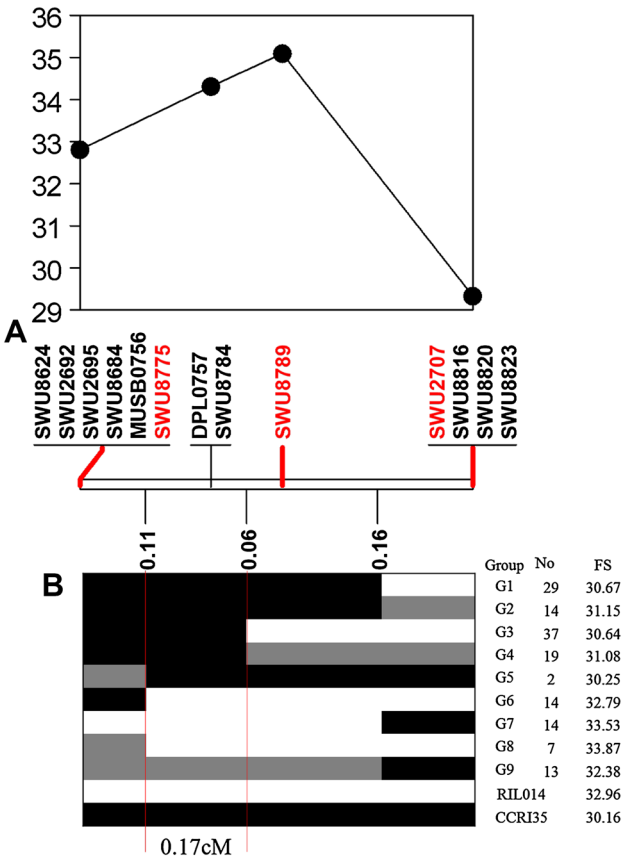

Fig. 3 Fine-mapping and substitution mapping of the qFS07.1 QTL region. **a** qFS07.1 fine-mapping based on 2571 $F_{2:3}$ individual plants in 2014. **b** Graphical genotypes and fiber strength for $F_{2:3}$ recombinants derived from 2571 individuals. The *white, black*, and *gray bars* represent genotypes of RIL014, CCRI35, and heterozygotes, respectively. The nearest marker of qFS07.1 is SWU8789, LOD score 35.09. The confidence interval for the position of the QTL from the peak LOD to less than 1, qFS07.1, was mapped into a 0.33-cM interval flanked by SWU8775 and SWU2707

Gh_D07G1953 (homoeologous to *GhA07G1749*) and *Gh_D071954* (homoeologous to *GhA07G1750*) in the QTL region.

RT-qPCR analyses of the genes within the QTL region

The four annotated genes within the QTL region were tested for expression levels by RT-qPCR using fiber tissue from four developmental stages (10, 15, 20, and 25 DPA) in Yumian1 and CCRI35. Through the sequence alignment of A genome and D genome of *G. hirsutum* (Zhang et al. 2015), single-locus specific primers of the four candidate genes were designed at the divergent coding region (Table S4). The relative expression levels in both parents Yumian1 and CCRI35 were shown in Fig. 5. The expression levels of *AHP5* did not show significant differences between Yumian1 and CCRI35 during the four developmental stages tested. *LRR RLK* showed gradually increasing expression from 10 DPA to 25 DPA, especially in CCRI35 which experienced about a three-fold increase compared with Yumian1 in the 20 and 25 DPA stages. The gene *RLP45* showed a variable expression pattern in CCRI35 across the four fiber development stages, whereas it showed stable expression in Yumian1 through the four stages. NUDIX23 showed significantly differential expression at 10 DPA and 25 DPA between Yumian1 and CCRI35.

Comparative analysis of candidate genes within the QTL region

To investigate the gene structure of candidate genes in the QTL region, primer pairs (Table S4) were designed to amplify the genomic DNA sequences and the gene upstream region (~2000 bp) from CCRI35 and Yumian1.

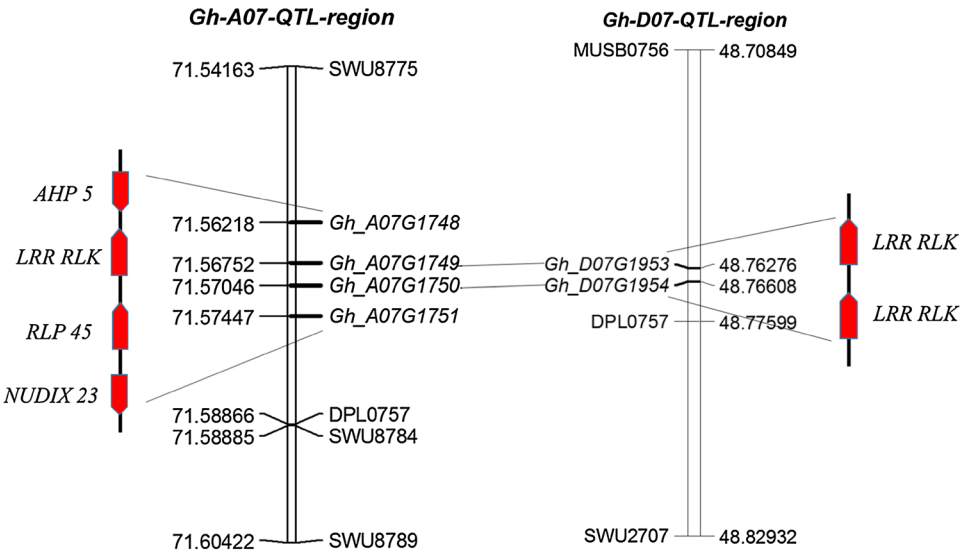

Fig. 4 Annotated genes within the 62.6-kb qFS07.1 QTL region on A07 and D07 of *G. hirsutum*. *Gh_A07G1748* (histidine-containing phosphotransfer factor 5, *AHP5*); *Gh_A07G1749* (leucine-rich repeat protein kinase family protein; *LRR RLK*); *Gh_A07G1750* (receptor-like protein 45, *RLP 45*); *Gh_A07G1751* (nudix hydrolase homolog 23, *NUDIX 23*). *Gh_D07G1953* (leucine-rich repeat transmembrane protein kinase, *LRR RLK*); *Gh_D07G1954* (leucine-rich repeat transmembrane protein kinase, *LRR RLK*)

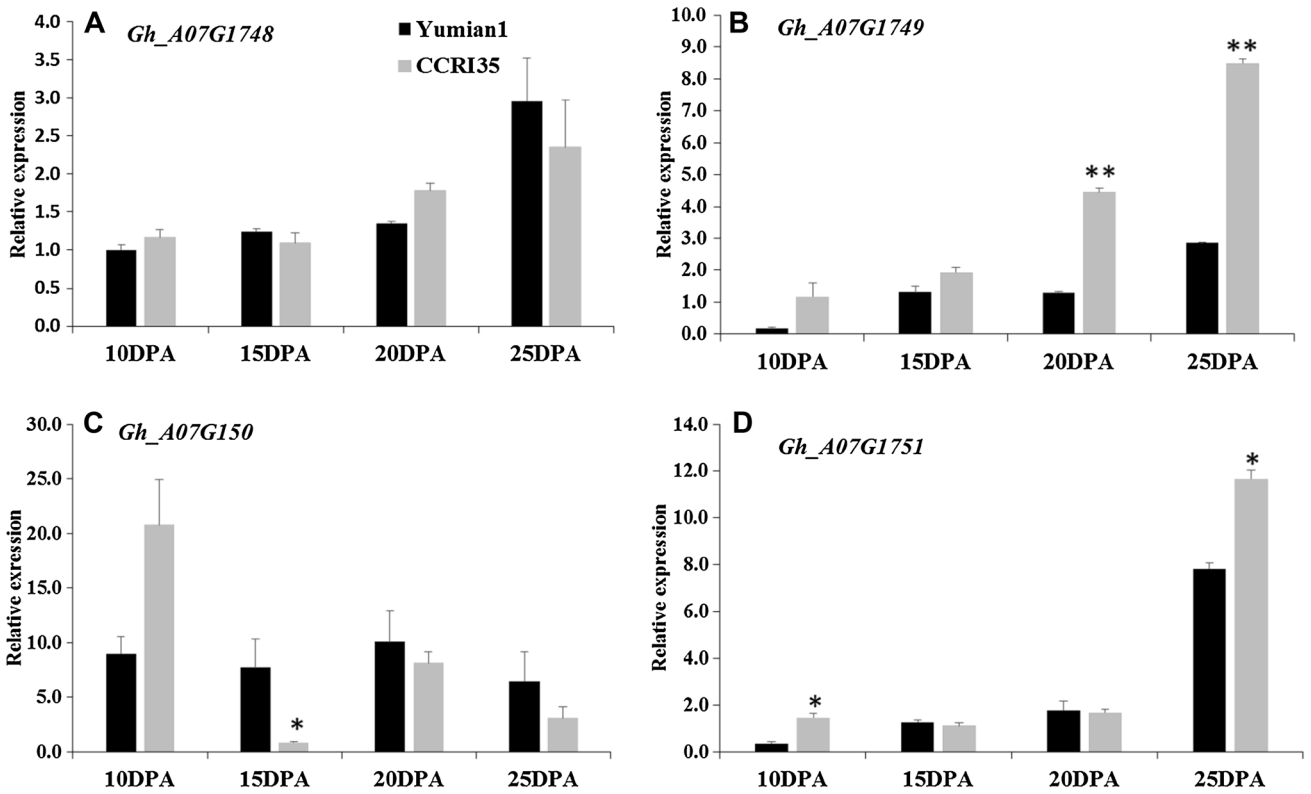

Fig. 5 RT-qPCR expression assay of genes in the QTL region during fiber development. **a** Histidine-containing phosphotransfer factor 5 (*AHP5, Gh_A07G1748*), **b** leucine-rich repeat protein kinase family protein (*LRR RLK, Gh_A07G1749*), **c** receptor-like protein 45 (*RLP45, Gh_A07G1750*), and **d** nudix hydrolase homolog 23 (*NUDIX23, Gh_A07G1751*). The *grey bars* are CCRI35, and the *black bars* are Yumian1. All data were normalized to the expression level of actin. *Error bars* indicate the standard deviation of three biological replicates. * and ** Denote significantly differential expression at $p < 0.05$ and $p < 0.01$, respectively

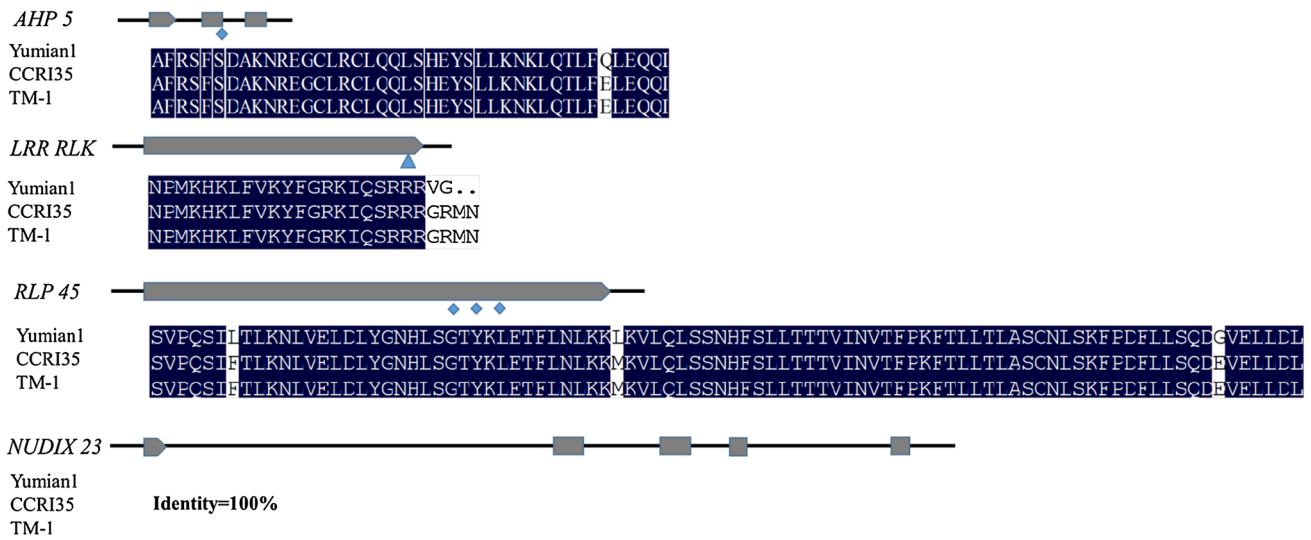

Fig. 6 Putative exons and divergent amino acids among parents (Yumian1 and CCRI35) and TM-1. *Blue diamonds* and *triangles* below the exons indicate the positions of non-synonymous SNPs and deletions, respectively. (Color figure online)

Genomic DNA sequences from CCRI35, Yumian1, and TM-1 were then used for the analysis of putative amino acid (aa) sequences deduced from the genomic interval containing these candidate genes (Fig. 6). The DNA sequences were also aligned and described in Fig S2. Annotation of *AHP5, LRR RLK, RLP45*, and *NUDIX23* from "TM-1" indicated that there were three, one, one, and five exons in the coding regions, respectively. The alignment of *AHP5* DNA sequence revealed a missense mutation in the second exon. Comparison of the nucleotide sequences of *LRR RLK* alleles from CCRI35 and Yumian1 revealed a 1-bp deletion resulting in a premature stop codon in Yumian1. The alignment of *RLP45* DNA sequences revealed multiple single base mutations, which caused missense mutations of aa sequences in Yumian1. The alignment of NUDIX23 DNA sequences in the five exons was consistent among the three cotton lines. The ~2 kb genome sequences in upstream region were obtained and compared between CCRI35, Yumian1, and TM-1 (Fig S3). In addition to some single-base differences in four candidate genes between CCRI35 and Yumian1, a 7 bp deletion of "CATGCTG" was detected locating at 250 bp upstream of the *LRR RLK* start codon in Yumian1 which was likely to be involved in the differential gene expression in Yumian1 and CCRI35. Although *RLP45* cannot be excluded completely the possibility, *LRR RLK* more likely contributed to the different fiber quality traits between CCRI35 and Yumian1.

Discussion

Mapping of complex traits using an efficient strategy

Fiber quality traits are unarguably the most important traits in commercial cotton production. The elucidation of specific favorable alleles related to fiber quality traits promotes more effective plant breeding. The increasing availability of genome sequences for species in the *Gossypium* genus (Paterson et al. 2012; Wang et al. 2012; Li et al. 2014, 2015; Zhang et al. 2015; Liu et al. 2015b; Yuan et al. 2015) has permitted anchoring of genetic maps and positioning of QTL on physical maps. Despite some positional cloning successes in plants, there is room for complementary approaches (Monclus et al. 2012). Integrating genome annotation and QTL position to prioritize candidate genes (Price 2006) has been used in *Populus* spp. (Monclus et al. 2012), *Glycine max* L. (Bolon et al. 2010), and *Triticum aestivum* L. (Barrero et al. 2015).

In the present study, to uncover key genes related to superior fiber traits in Yumian1, we integrated structural and functional genomics analyses. First, we mapped qFS07.1 to a 2.38-cM interval using an F_2 population. Second, using an $F_{2:3}$ population derived from F_2 recombinant individual plants, we mapped qFS07.1 by substitution mapping to a 0.17-cM interval containing four genes. Finally, we combined RT-qPCR for detecting expression patterns and gene cloning to determine alteration of genes structure. The results revealed an *LRR RLK* as a potential candidate gene responsible for superior fiber strength in upland cotton cultivar Yumian1. Improvement of upland cotton genome annotation and the reduction of QTL confidence intervals might help to refine this strategy of candidate gene discovery.

Stable QTL for fiber quality traits on Chr.07

In our previous studies, Tan et al. (2015) identified qFS07.1, qFL07.1, qFM07.1, qFU07.1, and qFE07.1 flanked by SSR makers DPL0757 and BNL1604 across multiple environments through the (CCRI35 × Yumian1) RIL population. Zhang et al. (2012) used a three-parent composite population in upland cotton (*G. hirsutum* L.) to identified QTL for fiber quality traits on Chr.07 between HAU1367 and HAU2282. In addition, Wang et al. (2013a) identified qFL-C7-1, qFU-C7-1, qFE-C7-1, and qFS-C7-1 flanked by SSR markers SHIN1447 and DPL0757 on Chr.07 using F_2 and $F_{2:3}$ family lines derived from a cross between Luyuan (LY343) and Lumianyan (LMY22). Sun et al. (2012) identified qFS-C7-1, qFM-C7-1, and qFL-C7-1 in F_2, $F_{2:3,}$ and RIL populations derived from an upland cotton cross between strains 0–153 and sGK9708. All these QTL are located in the interval that overlapped with that of qFS07.1 identified in the current study, based on the shared markers DPL0757, DC40182, and BNL1604. Therefore, qFS07.1 is a QTL of widespread importance for superior fiber strength in upland cotton.

Functional annotation of genes in the QTL region

In this study, qFS07.1 was fine-mapped to a 62.6-kb region on chromosome A07 of the *G. hirsutum* genome, which contained five annotated genes (*AHP5, LRR RLK, RLP45*, and *NUDIX23*). Histidine-containing phosphotransfer factors, designated *AHPs*, are involved in signal transduction mechanisms, by which plant cells appear to respond to certain hormonal stimuli, including cytokinin. Compared to wild-type plants, *AHP2*-overexpressing plants showed inhibitory effects on root and hypocotyl elongation (Suzuki et al. 2002). *AHP2, AHP3*, and *AHP5* are very closely related to each other and showed similar expression profiles (Suzuki et al. 2000). Receptor-like kinases (*RLKs*) play crucial roles in various signaling processes during plant growth and development, hormone perception, self-incompatibility, symbiont recognition, and pathogen responses (Morris and Walker 2003). The largest subfamily of these genes is the leucine-rich repeat (LRR) receptor-like

kinases (*LRR-RLKs*) (Li et al. 2005), comprising extracellular LRR domain, a transmembrane region, and a kinase domain that have recently been reported as a novel signaling pathway that regulates plant cell wall integrity maintenance (Hamann 2015). Two *LRR RLKs, FEI1* and *FEI2*, have been reported to play a vital role in cellulose deposition during elongation of root tips and seeds in Arabidopsis (Harpaz-Saad et al. 2011; Xu et al. 2008). Other *LRR RLKs* are suggested as regulators of secondary cell wall (SCW) formation in *Arabidopsis* (Wang et al. 2013b) and poplar (Song et al. 2011). The amino acid sequences of receptor-like proteins (*RLPs*) contain the LRR domain, typically, which differ from *RLKs* in that they lack the cytoplasmic kinase domain and only have a short cytoplasmic tail. In several plant species, *RLP*s have been found to play a role in disease resistance and plant development (Wang et al. 2008). Nudix proteins are postulated to control the cellular concentration of these compounds (Kraszewska 2008). Nudix hydrolases in *Arabidopsis* control a variety of metabolites and are pertinent to a wide range of physiological processes (Ogawa et al. 2008).

The functional role of RLKs in fiber development

In this study, sequence alignment showed that an *LRR RLK* (*Gh_A07G1749*) has a 1-bp deletion resulting in a premature stop codon of the Yumian1 allele that has superior fiber, and significantly lower expression (3.4- and 3.0-fold at 20 and 25 DPA, respectively) of this mutant allele was also observed. Zhao et al. (2013) revealed that *GbRLK* is involved in the drought and high salinity stresses pathway by activating or participating in the ABA signaling pathway. However, the previous literature indicated that the first peak of ABA content of the fiber occurred during fiber elongation and the second during secondary wall thickening (Davis and Addicott 1972; Gokani et al. 1998). Yang et al. (2001) also suggested that in vitro studies, ABA (50 μmol L^{-1}) markedly enhanced the accumulation of dry matter and cellulose in the fiber cell wall during secondary wall thickening. Niu et al. (2016) revealed that six genes from the *CrRLK1L* family, which is a subgroup of the receptor-like kinase (RLK), were highly associated with fiber development. Li et al. (2005) reported that a plasma membrane bound cotton *LRR RLK* named *GhRLK1* was induced during the active SCW synthesis stage. Islam et al. (2016) revealed that one RLK gene (*thaumatin-like protein 3*) has a premature stop codon and significantly higher expression (3.3- and 5.8-fold in 15 and 20 DPA, respectively) in MD90ne with inferior fiber, which may indicate feedback regulation that attempts to compensate for the lack of a functional protein.

This study fine-mapped a QTL to small genomic regions containing four candidate genes. Of those genes, only *LRR RLK* (*Gh_A07G1749*) had sequence differences in the promoter and coding region. Either the promoter (a 7 bp deletion presumably causing lower expression of Yumian1 allele) or the coding sequence (a premature stop codon attenuating its transcription by a feedback loop or attenuating its function by omitting part of an active domain to translation) appeared to be the cause of the superior fiber of RIL014 compared to that of CCRI35. It could be that both genetic factors could play a role in the phenotype, and they each deserve further study. Regardless of the causative genetic lesion, these findings suggest RLK signaling pathways to be engaged in mediating a combination of cell elongation and SCW biosynthesis during cotton fiber development.

The potential of qFS07.1 in MAS

Stable and major QTL for fiber quality are important to molecular breeding. The present study mapped qFS07.1 controlling fiber strength into a 0.17-cM interval with a 62.6-kb physical distance on chromosome 7 in *G. hirsutum*, and the presence of qFS07.1 led to an increase in fiber strength of approximately 3.0 cN/tex. Compared with the previously studies about fine-mapped QTL for fiber quality traits (Su et al. 2013; Cao et al. 2015; Islam et al. 2016; Liu et al. 2016; Yang et al. 2016), it thus appears that qFS07.1 fine-mapped in this study has the largest, single effect, and the smallest confidence interval within the genome. Therefore, qFS07.1 for fiber strength would have the potential to improve cotton fiber qualities through MAS and promote the investigation of the genetic mechanisms of high fiber strength.

Author contribution statement ZSZ conceived the experiment, designed SSR markers, analyzed the data, and revised the manuscripts. XMF contributed to genotyping, RT-PCR, clone, data analysis, and writing. XYL and XQW assisted in SSR genotyping, RT-PCR, and clone. WWW analyzed the sequencing data. DXL, JZ, DJL, and ZHT made crosses and conducted field tests. ZYT, FL, FJZ, MCJ, and XLJ contributed to DNA and RNA extraction. JWZ and JHY measured fiber properties. All authors reviewed and approved this submission.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors declare that this study complies with the current laws of the countries in which the experiments were performed.

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