Brief Communication

Genetic variation in MYB5_A12 is associated with fibre initiation and elongation in tetraploid cotton

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The MYB gene family, which is predominantly composed of the R2R3-MYB family, is large and functionally diverse in plants including cotton (Gossypium hirsutum L.) (Guan et al., 2014; Tian and Zhang, 2021; Walford et al., 2011; Wan et al., 2016; Wang et al., 2019, Wu et al., 2018). In Arabidopsis, the R2R3-MYB protein AtMYB5, which belongs to the GL1 branch in the phylogenetic tree, is a component of the MYB-bHLH-WD40 protein complex that directly regulates the expression of GL2 to control the fate of seed coat outer epidermal cells and plant hairs (Gonzalez et al., 2009). However, the role of the homologue of AtMYB5 in cotton is currently unknown.

The sequence of AtMYB5 from Arabidopsis was used to search for homologous genes in the sequenced TM-1 genome with sequence variation and interspecific expression difference, resulting in the identification of the homologous R2R3-MYB-encoding gene Gb_A12G1020 on chromosome A12. This gene, designated as GbMYB5_A12, was classified into the GL1 functional subgroup (Figure 1a), and showed a high level of similarity (75% identity) to AtMYB5, with a typical nuclear localization (Figure 1b).

To understand the transcriptional pattern of GbMYB5_A12 in relation to fibre initiation, we compared its transcript levels between G. hirsutum (SG747) and G. barbadense (Giza75) fibres at different stages. The transcript levels of MYB5_A12 were higher in G. barbadense than in G. hirsutum at −3 to 0 DPA (the fibre initiation stage) in ovules and at 5 to 10 DPA (the early elongation stage) in fibres (Figure 1c). These data indicated that the transcript level of MYB5_A12 may be associated with the natural variation of the fibre between G. hirsutum and G. barbadense.

To reveal if this MYB5_A12 gene is genetically involved in fibre development, a co-localization analysis was conducted to determine whether the location of MYB5_A12 on TM-1 chromosome A12 was in or near fibre trait-related quantitative trait loci (QTLs) identified through analyses of interspecific G. hirsutum × G. barbadense populations (Said et al., 2015a; Said et al., 2015b). GhMYB5_A12 was localized within an FL QTL hotspot, that is, Fl_{QTL_Hotspot_A12} in QTL cluster I (Figure 1d).

We detected a nonsynonymous SNP, MYB5_A12 (i.e., T413G), which is a nucleotide transversion at the 413th nucleotide in MYB5_A12 between G. hirsutum and G. barbadense. To determine whether this SNP was associated with fibre traits, we conducted high-resolution melting (HRM) analyses for an interspecific backcross inbred (BIL) population of 146 lines derived from a backcross between SG747 as the recurrent parent and Giza75. A correlation analysis was performed for the BILs tested in five replicated field tests. The presence of the MYB5_A12 allele from Giza75 was significantly positively correlated with FS in five tests, with FL in four tests and with LP in three tests (Figure 1e). When the BILs were divided into two homoygous groups based on the two MYB5_A12 alleles, the MYB5_Gb genotype showed significantly increased FL by 1.12 mm, and LP by 1.41%, as compared with the MYB5_Gh genotype (Figure 1f).

We speculated that the positive effect of the GbMYB5_A12 allele on FL and LP may be related to its high transcript level driven by its promoter. Therefore, we compared the promoters of GbMYB5_A12 and GhMYB5_A12 to identify cis-elements involved in regulating gene expression. A cis-element TFmatrixID_0063 that bound by a TEM transcription inhibitor to negatively control trichome initiation in Arabidopsis (Matias-Hernandez et al., 2016) was identified in the MYB5_A12 promoter region. Compared with the core sequence of TFmatrixID_0063 in G. hirsutum (GATTTGT), that in G. barbadense showed two SNP sites (AATGTTAT) (Figure 1g).

We determined whether the sequence variation in the TFmatrixID_0063 between the promoters of GbMYB5_A12 and GhMYB5_A12 resulted in differences in TEM binding. In the yeast one-hybrid (Y1H) assay, GhTEM2 (Gh_A03G0840) strongly bound to the Gh_TFmatrixID_0063, whereas no binding signal was observed for Gb_TFmatrixID_0063 in selective SD/-Leu medium (Figure 1h). In the dual-luciferase assays, the signal of the LUC reporter triggered by GhTEM2+GbMYB5_A12-pro was much weaker than those triggered by GhTEM2+GbMYB5_A12-pro and controls, suggesting that GhTEM2 transcriptionally represses GhMYB5_A12 but not GbMYB5_A12 (Figure 1i). These results showed that GhTEM2 directly binds to the GbMYB5_A12 promoter to inhibit its transcription.

We further determined whether the transcript levels of the two MYB5_A12 alleles were correlated with those of TEM2 in G. hirsutum and G. barbadense fibres. As expected, we detected opposite trends in the transcript levels of MYB5_A12 and TEM2 at

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the fibre initiation and early elongation stages in *G. hirsutum*. In *G. barbadense*, the transcript levels of the corresponding alleles did not show a significant negative correlation (Figure 1j). These results confirmed the negative relationship between the two genes in *G. hirsutum* due to the high affinity of the promoter of *GhMYB5_A12* for the negative regulator TEM2 but no relationship between the two genes in *G. barbadense* because of the mutation in the cis-elements region of *GbMYB5_A12* promoter.

To confirm the role of the *GhMYB5_A12* gene in fibre development of cotton, we obtained and analysed two independent *GhMYB5_A12*-overexpressing homozygous transgenic T3
lines (OE-1 and OE-2) (Figure 1k). As expected, the transcript levels of GhMYB5_A12 at the fibre initiation and elongation stages were significantly higher in the two transgenic lines than in the CRI 24 (WT) (Figure 1l). The number of fibre initial cells in the 0 DPA ovules, FL and LP was significantly increased in OE-1 and OE-2, as compared with CRI 24 (Figure 1m and n). These results were consistent with the association between the SNP marker for MYB5_A12 and the two traits—FL and LP.

To further explore the role of GhMYB5_A12 in the fibre development network and to determine whether there was a difference in function between the GhMYB5_A12 and GbMYB5_A12 proteins, six proteins that were screened from a fibre and ovule mix prey library were selected for protein–protein interaction analyses. These results suggested that GhMYB5_A12 and GbMYB5_A12 both participated in the fibre development network by interacting with EGL3 and HOX3 in the same way and confirmed that the sequence variation (MYB5_413) does not affect the functionality of the two MYB5_A12 proteins (Figure 1o).

In summary, in the BILs, the interspecific allelic MYB5_A12 was shown to similarly interact with two fibre-related proteins, that is, HOX3 and EGL3. More importantly, the TEM transcription inhibitor (GhTEM2) strongly bound to the Gh_TFmatrixID_0063, whereas no binding signal was observed for the Gb_TFmatrixID_0063. Overall, the sequence variation in the cis-elements resulted in the difference of transcriptional levels of the two alleles, which are linked with the natural variation of fibre development (Figure 1p). Moreover, our findings also provided useful information for using genetic engineering technology to directly edit specific G. hirsutum genes or for directional selection of progenies of G. barbadense × G. hirsutum hybrids to improve the fibre quality of G. hirsutum.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

J. Yu, J. Zhang and S. Yu conceived the study. Q. Ma and H. Sun assisted in the experiments. W. Pei, M. Wu, X. Zang, G. Liu, J. Song and B. Jia performed the field cultivation. N. Wang performed the experiments and wrote the manuscript. J. Zhang and J. Yu revised the manuscript. All the authors read and approved the final manuscript.

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