

Supplementary data

Induction of cotton ovule culture fiber branching by a combination of three genes of *BRANCHLESS TRICHOMES*, *SIAMESE1*, and *STICHEL*

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Supplementary Methods

Plasmid constructs

Both of the two *CaMV35S* (*35S*) promoters in pCAMBIA1301(CAMBIA) were replaced by the *SCFP* promoter (Hou et al., 2008). The *GUS* in pCAMBIA1301 was replaced by *GaSIM1* cDNA at the *NcoI*–*NheI* site; the cDNA of *Arabidopsis STI* cDNA was cloned into the *XhoI* site (to replace the hygromycin resistance gene), forming *SCFP::STI:35S poly A/SCFP::GhBLT:Nos poly A*. The *CaMV35S* promoter of pBI121 was replaced by *SCFP* promoter at the *HindIII*–*XbaI* site, forming *SCFP::GUS* construct, then the *GUS* coding region was replaced by *GaBLT* cDNA. PCR was performed to amplify the fragment containing *SCFP* promoter, *GaBLT* cDNA, and *Nos poly A* terminator. Next, the fragment was inserted into *SCFP::STI:35S poly A/SCFP::GaSIM1:Nos poly A* at the *KpnI* site, forming *SCFP::STI:35S poly A/SCFP::GhBLT:Nos poly A/SCFP::GaSIM1:Nos poly A*. The *SCFP::GUS: Nos poly A* region of the *SCFP::GUS* construct was obtained using PCR, and then the PCR fragment was inserted into the above construct at the *SmaI* site, forming *SCFP::STI:35S poly A/SCFP::GhBLT:Nos poly A/SCFP::GUS:Nos poly A/SCFP::GaSIM1:Nos poly A*.

The intron-containing hairpin (ihp) construct with the pHANNIBAL/pART27 system was used to create RNA interference (RNAi) (Wesley et al., 2001). To produce transgenic cells expressing a *GaBLT*-RNAi construct, a 500-nucleotide *GaBLT* C-terminal coding region was amplified. The PCR product was cloned in both sense and antisense directions into pHANNIBAL. The construct was then subcloned into pART27, and introduced into *Agrobacterium* strain LBA4404.

The entry clone of full-length *GaBLT* was fused with the N-terminal of GFP in the pCAMBIA1302 (CAMBIA) vector. Full-length *Arabidopsis STI* replaced the *GUS* coding region of pBI121, then the PCR fragment (with supernumerary *EcoRI* recognition site at two ends) of *35S::STI:NOS-ter* was subcloned into the intermediate pCAMBIA1302 at the *EcoRI* by the use of In Fusion technology (Clontech) to create *35S::STI-35S::GaBLT-GFP*.

Hou L, Liu H, Li JB, Yang X, Xiao YH, Luo M, Song SQ, Yang GW, Pei Y.
2008. SCFP, a novel fiber-specific promoter in cotton. *Chinese Science Bulletin* **53**, 2639-2645.

Wesley SV, Helliwell CA, Smith NA, Wang MB, Rouse DT, Liu Q, Gooding

PS, Singh SP, Abbott D, Stoutjesdijk PA. 2001. Construct design for efficient, effective and high - throughput gene silencing in plants. *The Plant Journal* **27**, 581-590.

Supplementary figures

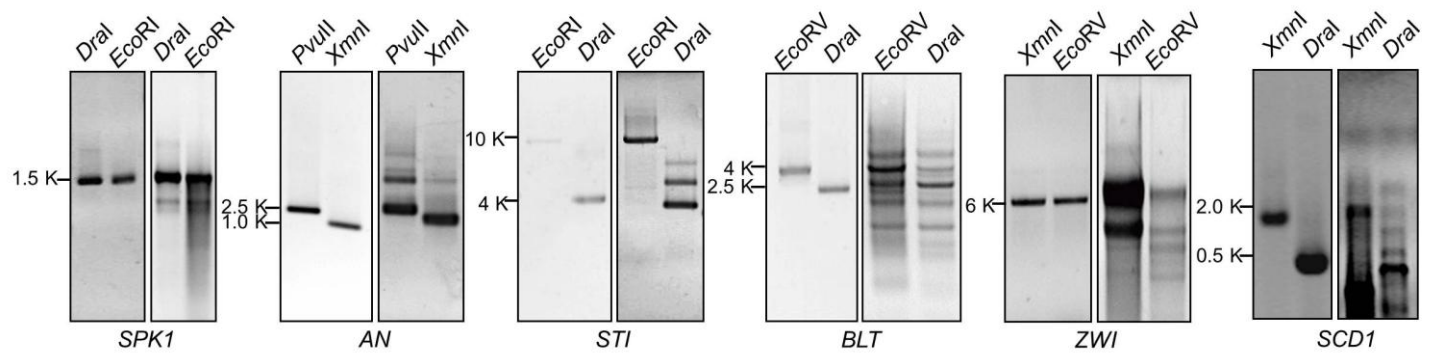


Fig. S1. Southern blots with high stringency (on the left side of each pair box, the probes are strongly complementary to *G. arboretum*-related genes) and low stringency (on the right side of each pair box, the probes are strongly complementary to *Arabidopsis*-related genes), respectively. Relative sizes of the hybridizing bands are indicated in kb (K).

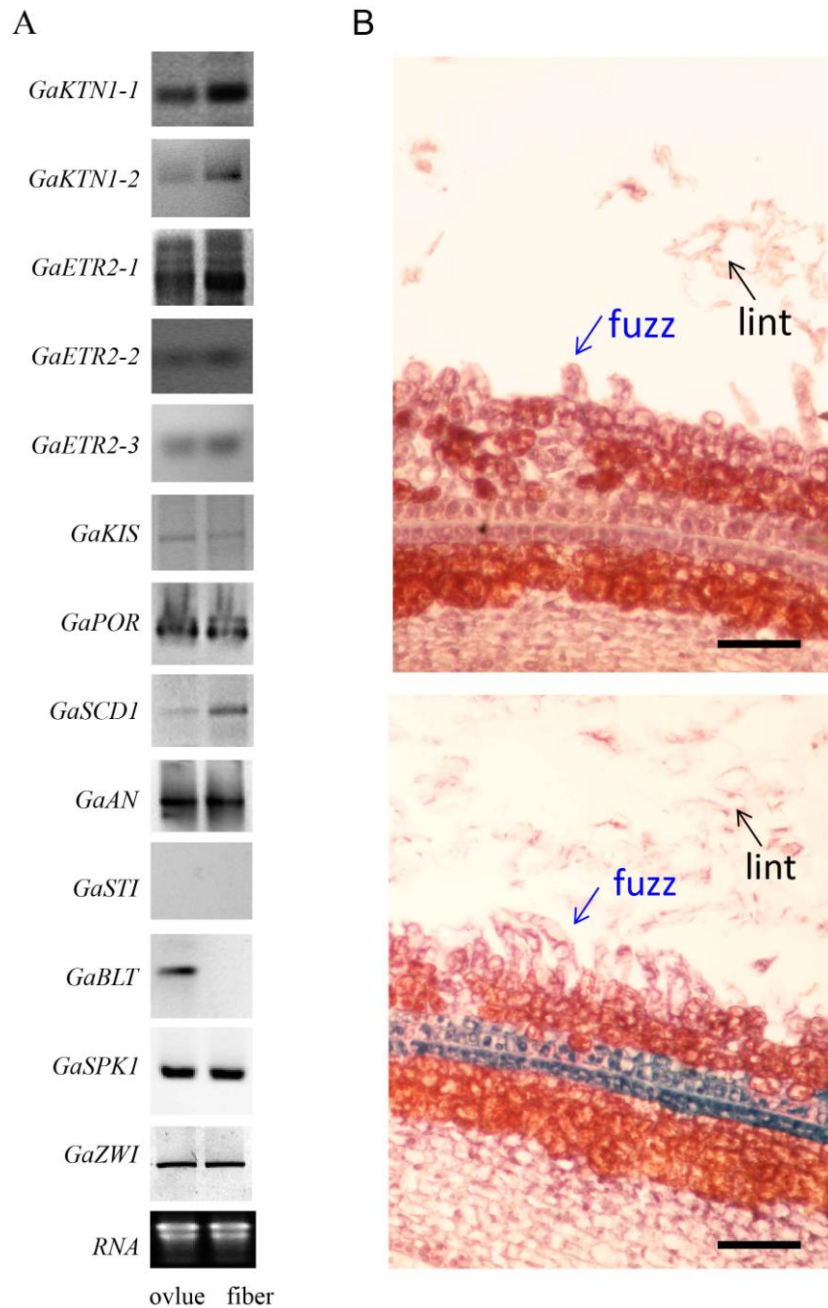


Fig. S2. Characteristics of the homologous genes controlling *Arabidopsis* trichome branching in *G. arboreum*.

(A) Northern blots showing genes expression in fibers and ovules. RNA used in the analysis was derived from a balanced mix of total-RNA samples from 3 to 25 DPA fibers or ovules.

(B) Longitude-sections of 5 DPA cotton seed hybridized with sense (up) and antisense (down) RNA probes for *GaBLT*. Blue-dark indicates hybridization signal. Bars = 20 μ m

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 CAAAGAAAATACAAAACAATGTAGAAGGTA

probe1
 probe2
 probe3
 probe4
 probe5
 probe6
 probe7

Fig. S3. Genomic sequence of *ψGaSTI*.

The target sequences in the Northern blots are underlined. The theoretical coding sequence is uppercased.

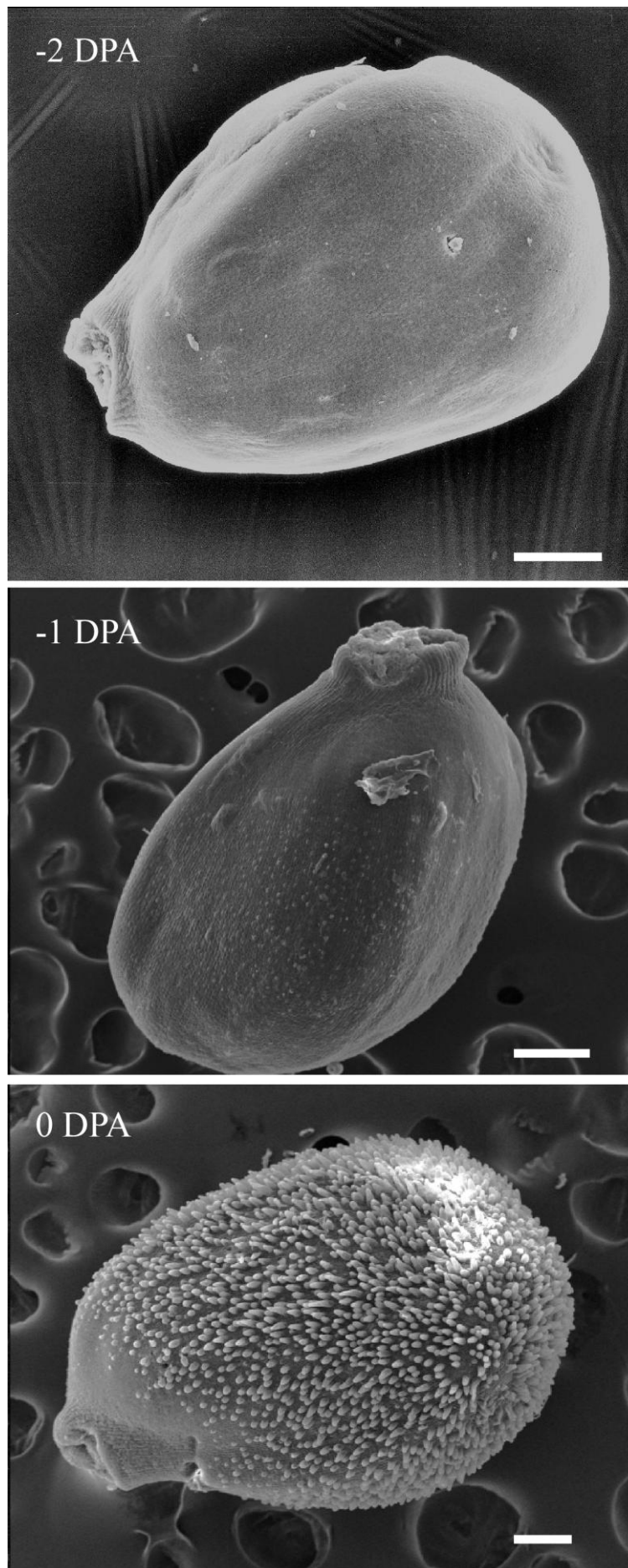


Fig. S4. Histological observation of DPL971 seeds at different developmental stages. Bars = 200 μm.

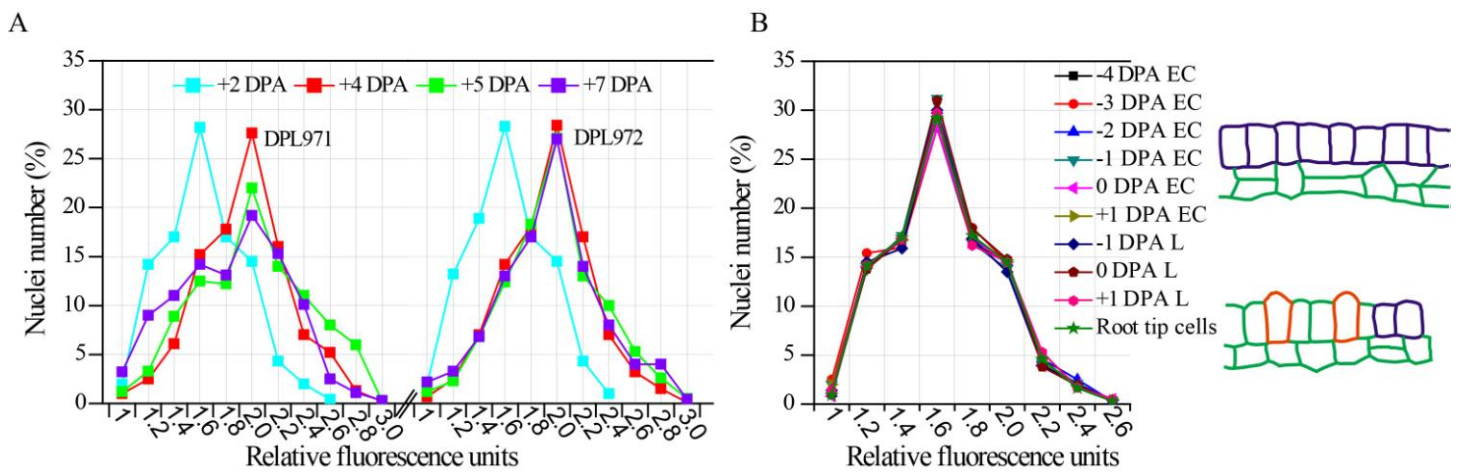


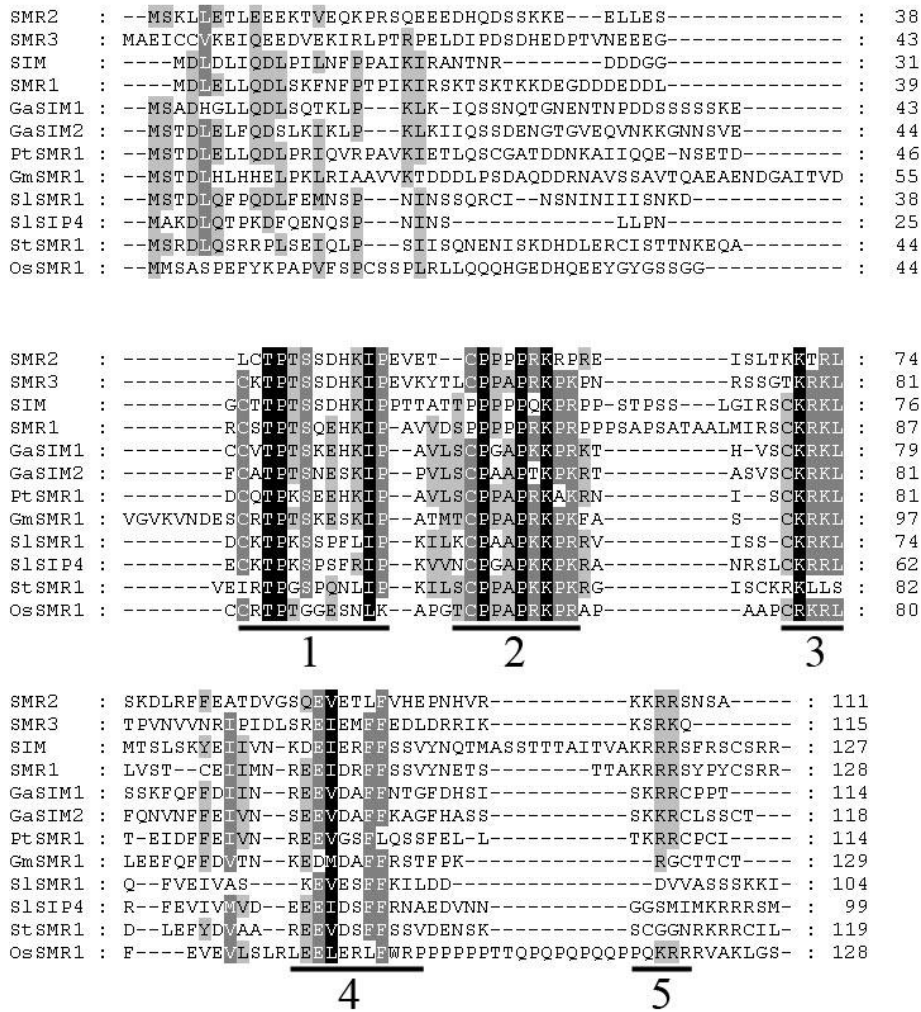
Fig. S5. Fiber nuclear DNA content at different developmental stages.

(A) Histograms showing the fluorescence of isolated nuclei from fiber cells at 2, 4, 5, and 7 DPA labeled with Hoechst 33258, expressed as a percentage of the total number of nuclei measured. The Relative fluorescence units (RFU) of DPL971 at 2, 4, 5, and 7 DPA was 1.62 ± 0.06 , 1.95 ± 0.07 , 1.90 ± 0.06 , and 1.82 ± 0.06 , respectively. The RFU of DPL972 at 2, 4, 5, and 7 DPA was 1.60 ± 0.06 , 1.92 ± 0.07 , 1.95 ± 0.06 , and 1.93 ± 0.09 , respectively. The number of nuclei measured were all more than 400, and came from at least 7 ovules of different bolls.

(B) Histograms showing the fluorescence of isolated nuclei from different epidermal cells types before 2 DPA. EC, epidermic (unswelled) cells; L, lint cells

Red cells in the right panel are schematic diagrams of fiber initial cells and blue demonstrated non-fiber epidermal pavement cells. The two type cells were respectively collected by laser-capture microdissection. None of the pairwise comparisons among these samples is significant ($P > 0.05$, Kruskal-Wallis one-way ANOVA and Dunn's test), approximately 1.63.

A



B

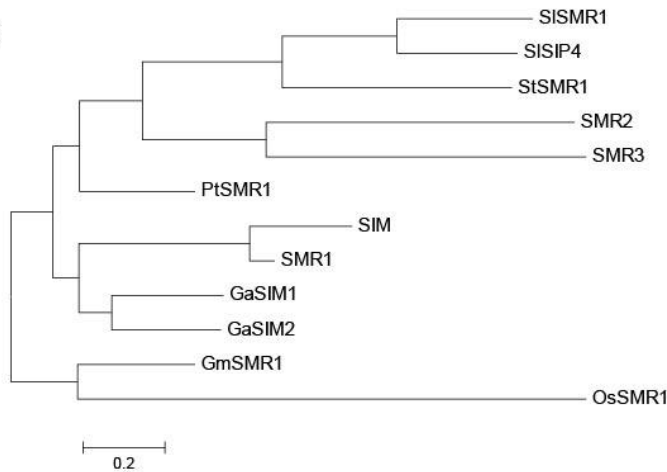


Fig. S6. Alignment and evolutionary tree of GaSIM related plant proteins.

(A) Alignment of conceptual translation of GaSIMs reading frame and related plant proteins. The regions numbered 1 to 5 denote conserved domains (Churchman *et al.*, 2006). SIM, CAB85553; SMR1, BAC42937; SMR2, AAF18255; SMR3, CAB85979; *Solanum lycopersicum* (Sl) SMR1, AI780963; SlSIP4, AAG43410; *S. tuberosum* (St) SMR1, BM110486; *Oryza. sativa* (Os) SMR1, AAK20052; *Populus tremula* (Pt) SMR1, BU815024; *Glycine max* (Gm) SMR1, AW704877. All of these accession numbers are from the National Center for Biotechnology Information database.

(B) A maximum likelihood tree was constructed in MEGA 5.05.

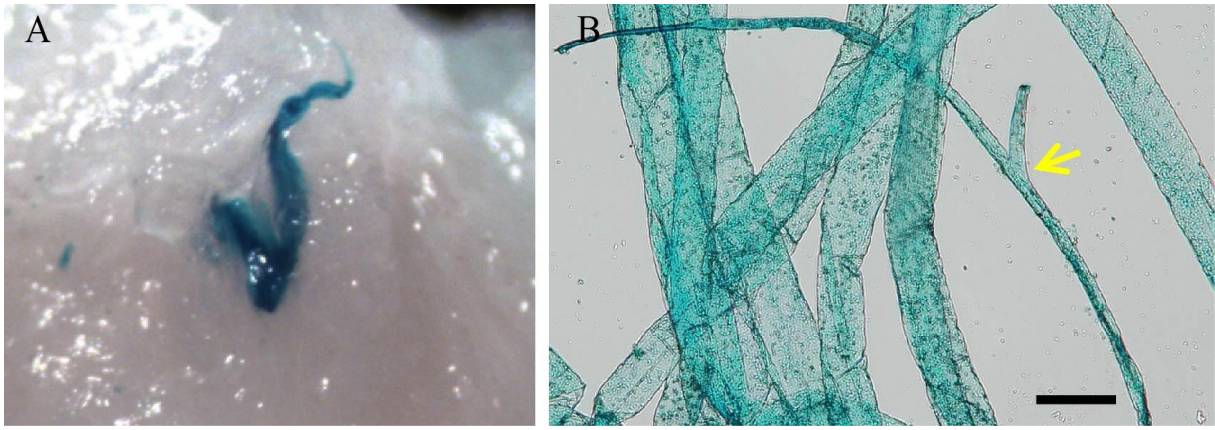


Fig. S7. Co-expression of *GaBLT*, *GaSIM*, *STI* can induce fiber-branching phenotype.

Ovules were collected at 2 DPA and bombard with a construct to target express *GaBLT*, *GaSIM1*, *Arabidopsis STI*, and *GUS* in fiber cell, followed by *in vitro* culture for 5 day. Fibers showing transgenic expression, indicated by GUS blue. A showing a tuft GUS stained fibers. B, a higher magnification of the fiber-tuft in A.

Yellow arrow shows branching site. Bar = 30 μm .

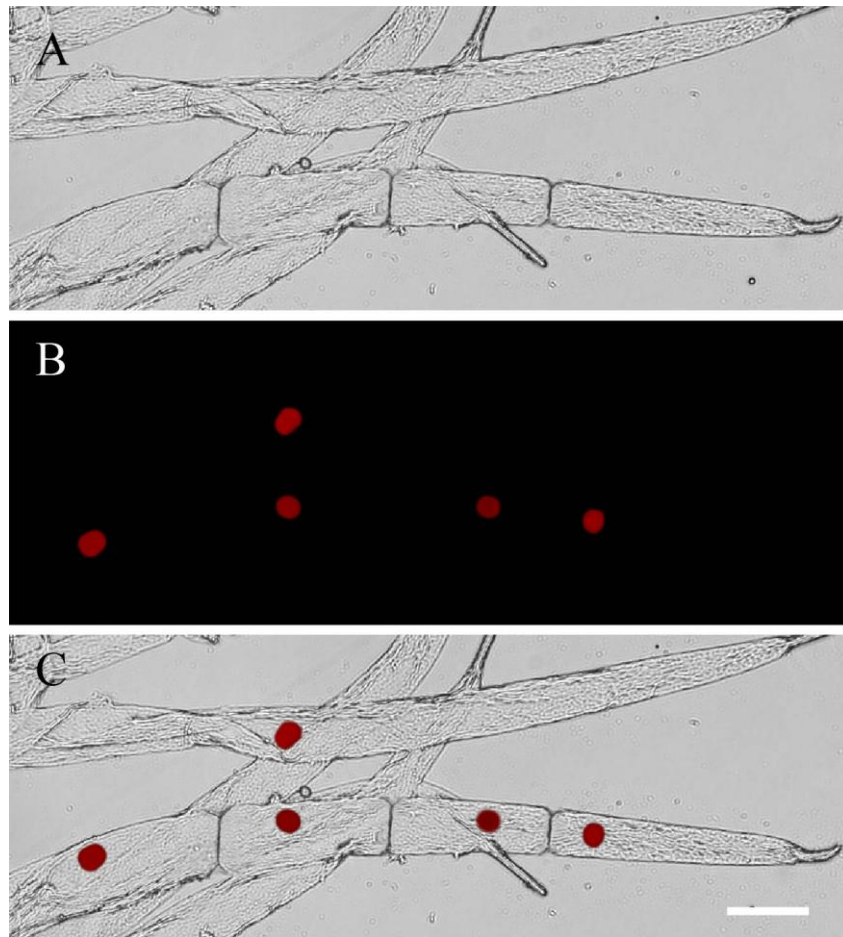


Fig. S8. Fibers with multicelled and unicelled phenotype.

(A) Light micrograph. (B) Propidium Iodide (PI) image. (C) Overlay.

5 DPA Ovules were removed from the boll and fixed in methanol:glacial acetic acid (3:1). Then rinsed with 1×PBS, followed by PI(50 µg/ml) staining (10 min). PI images were acquired using a CCD camera (Olympus DP72) attached to anOlympus microscope (BX53). The equipment was operated with cellSens standard software (ISO 200; exposure time 380 ms). Bar = 30 µm.

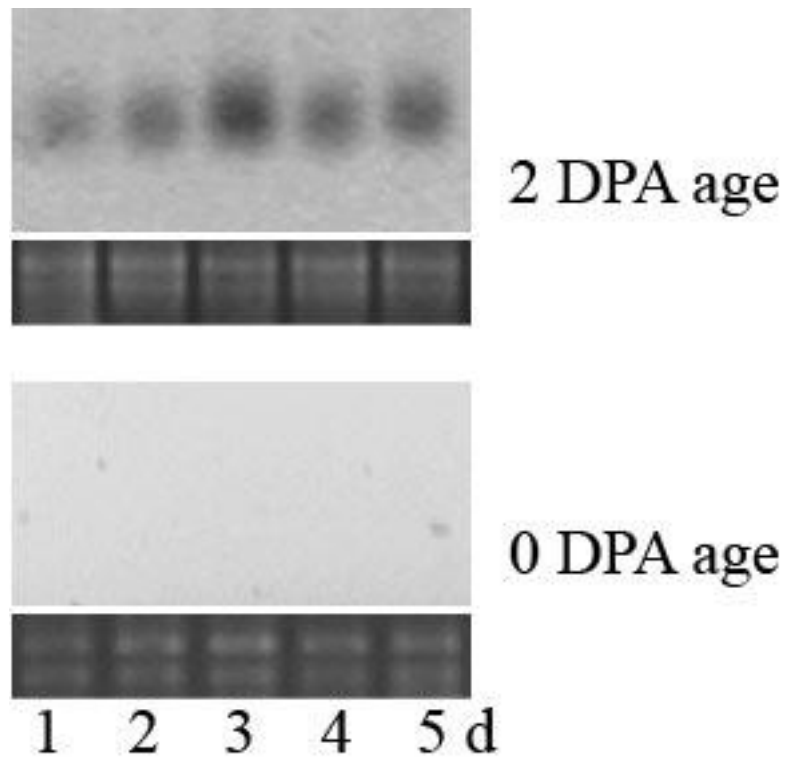


Fig. S9. Northern blots with antisense-*GaBLT* probe showing *GhBLT* mRNA levels in cultured fibers. The ovules of *G. hirsutum* cultivar MD51 ne were put into culture at 2 DPA (2 DPA age) or 0 DPA (0 DPA age) for 1 to 5 days, and then recover fiber RNA for detection.