

Cotton *beta*-glucosidase gene *GhBG1A* coordinates fiber transition from elongation to secondary cell wall deposition

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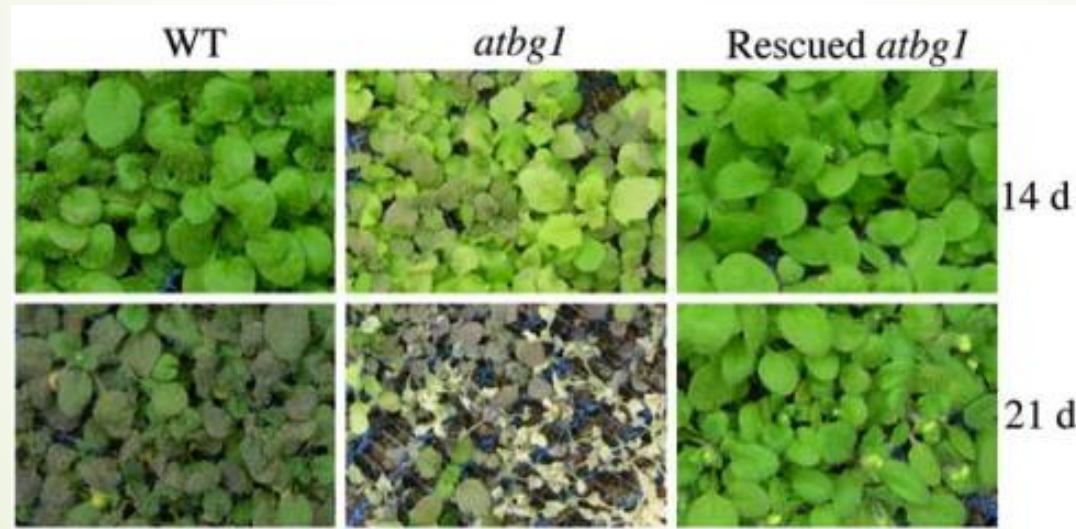
State Key Laboratory of Crop Genetics and Germplasm Enhancement

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Background

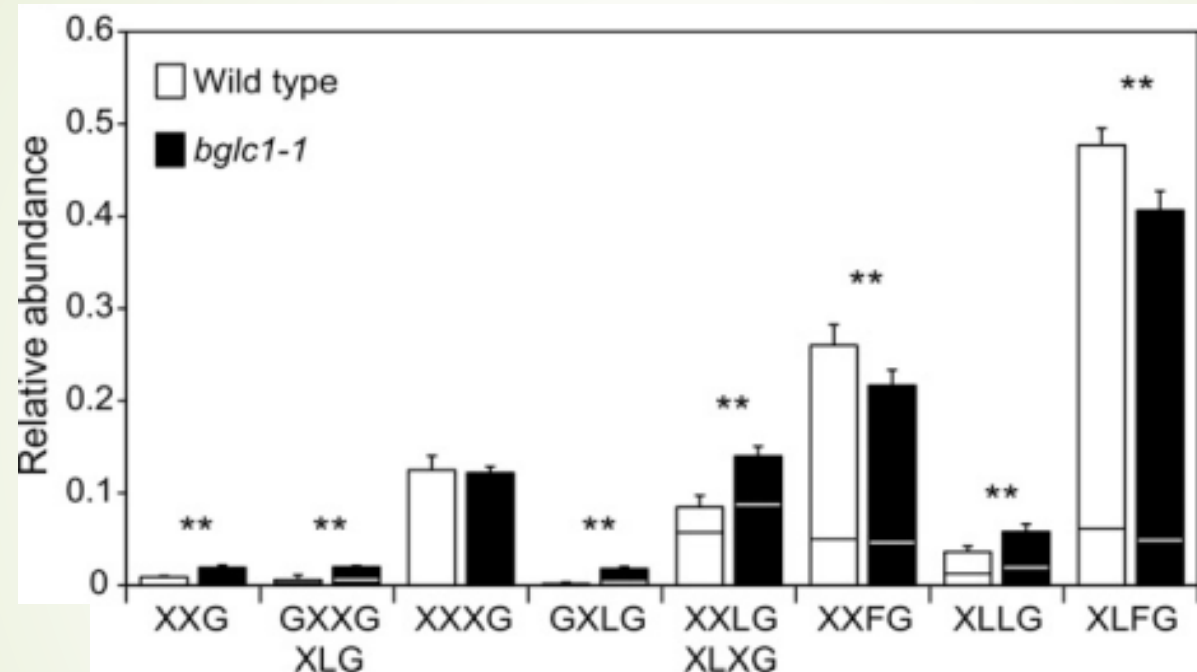
(1) **Beta-glucosidases** (β -D-glucofuranoside glucohydrolases, E.C. 3.2.1.21) are enzymes that hydrolyze glycosidic bonds to release nonreducing terminal glucosyl residues from glycosides and oligosaccharides.

(2) In plants, β -glucosidases play various functions, including biotic defense, abiotic stress response, phytohormone activation, release of aromatic compounds and so on.



(Lee et al., 2006, Cell)

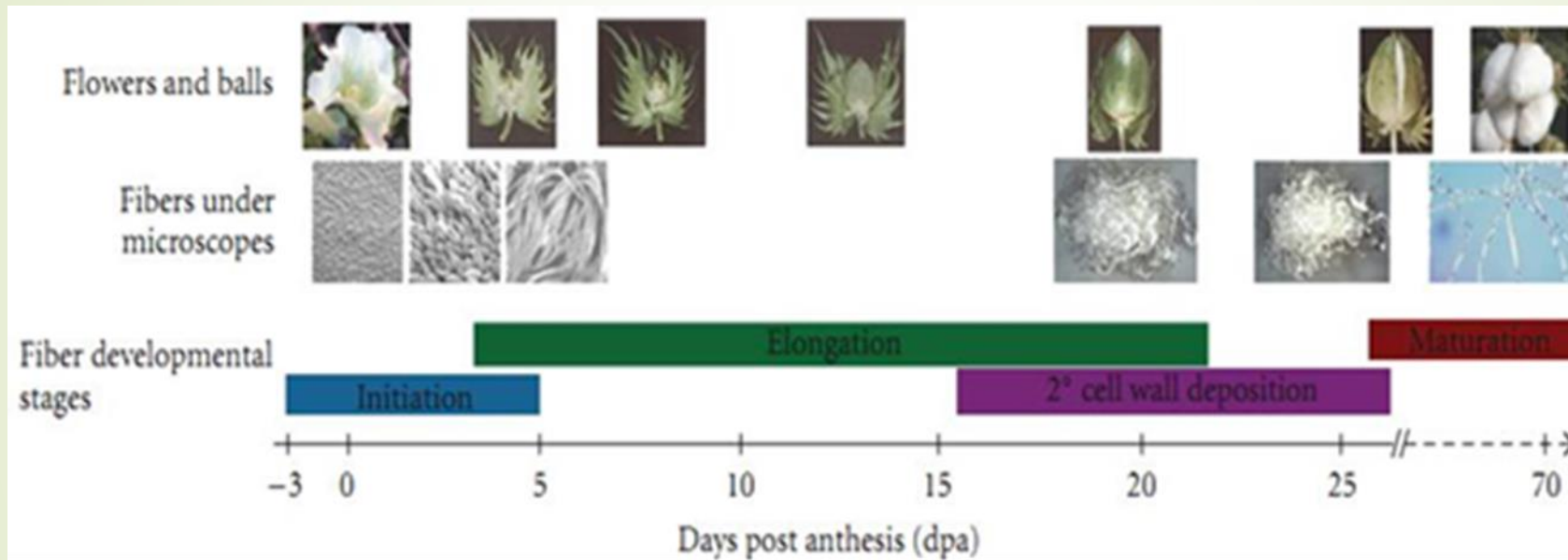
(3) Involved in degradation of oligosaccharides generated in cell wall turnover process



(Sampedro et al., 2017, Plant physiol)

However, the precise functions of β -glucosidase in cell wall development is unclear.

2. Cotton fiber is an excellent experimental system to study functions of β -glucosidase in cell wall development.



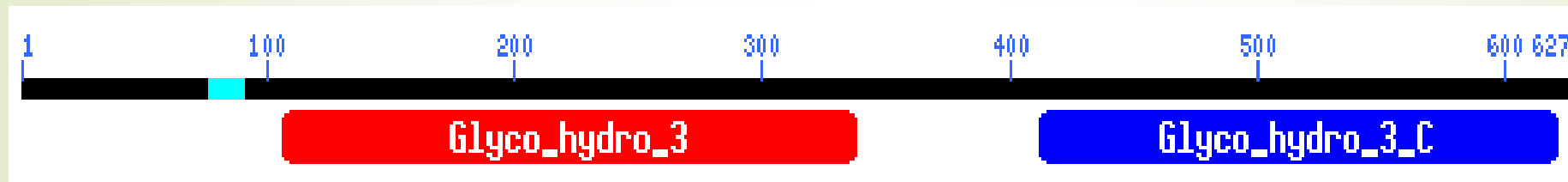
(Wilkins and Arpat, 2008)

Results

1. Isolation of *GhBG1* gene

(1) In previous studies, *GhBG1*, a gene encoding beta-glucosidase, was found highly expressed in cotton fiber cDNA library.

(2) After cloning, sequence analysis revealed that GhBG1 protein contains 627 amino acids, belonging to glycosyl hydrolase family 3 (GH3), with two conserved domains, N-terminal Glyco_hydro_3 domain and C-terminal Glyco_hydro_3_C domain.

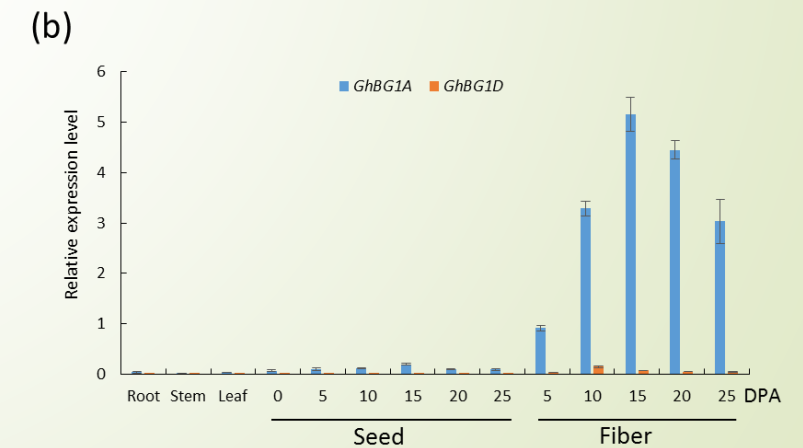
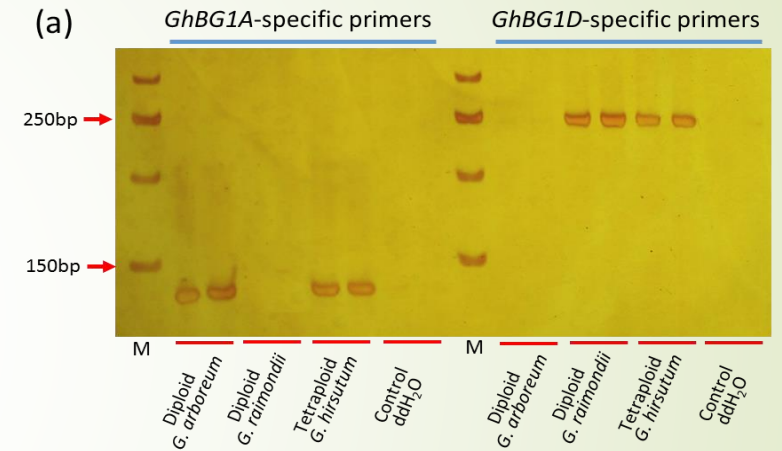
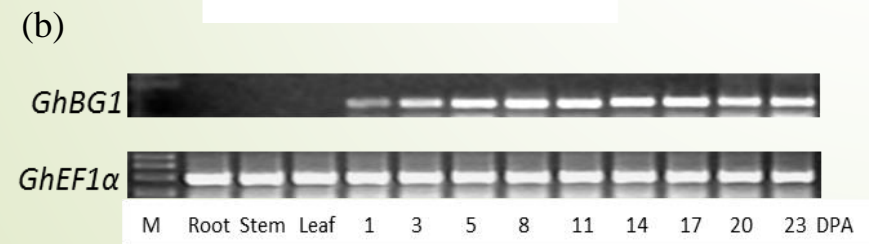
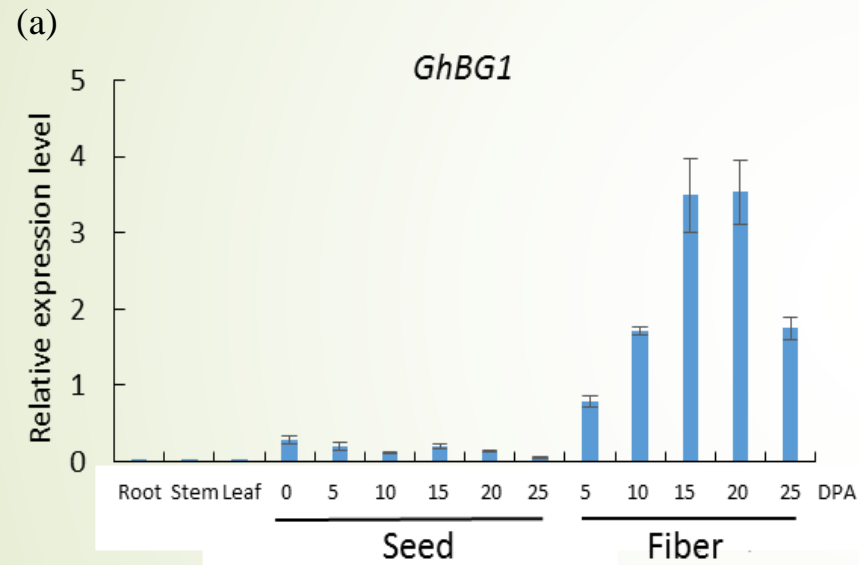


N-terminal Glyco_hydro_3 conserved domain: 102~339 amino acids;

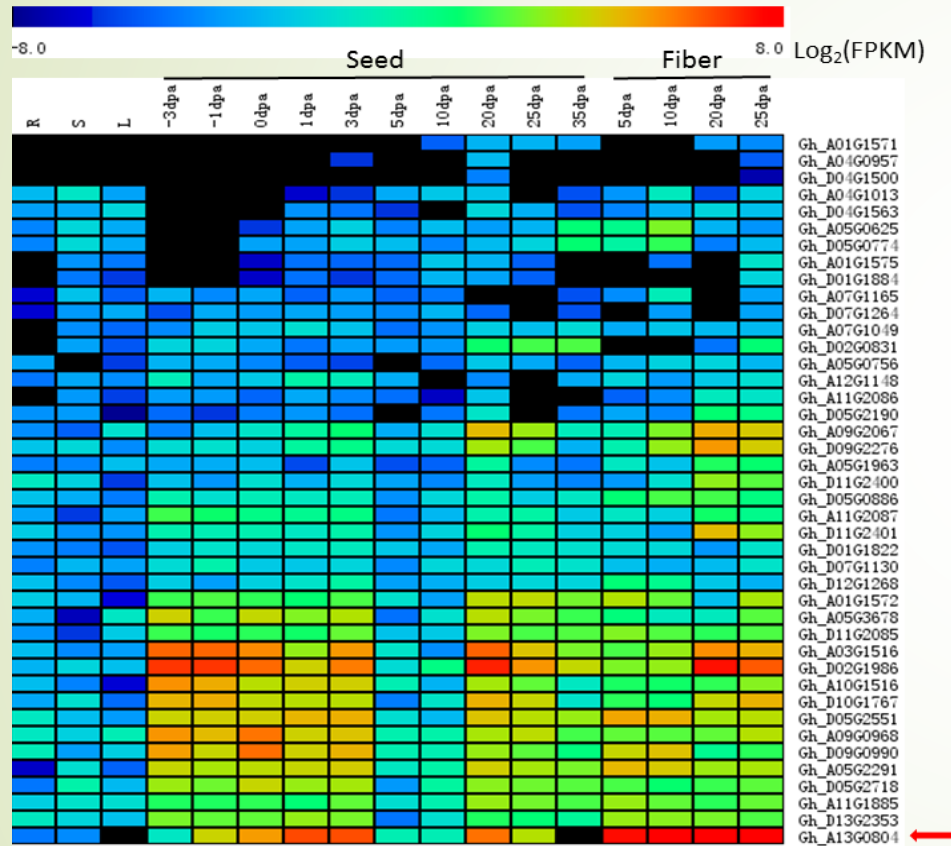
C-terminal Glyco_hydro_3_C conserved domain: 411~623 amino acids.

2. *GhBG1* gene functions in fiber development

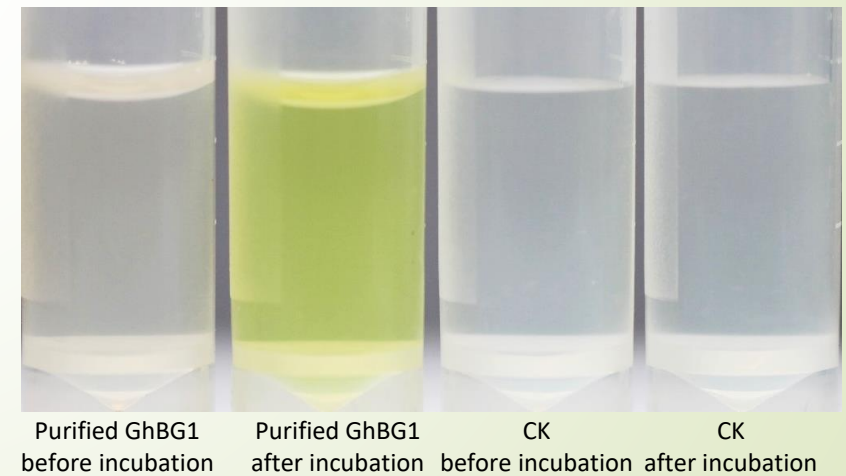
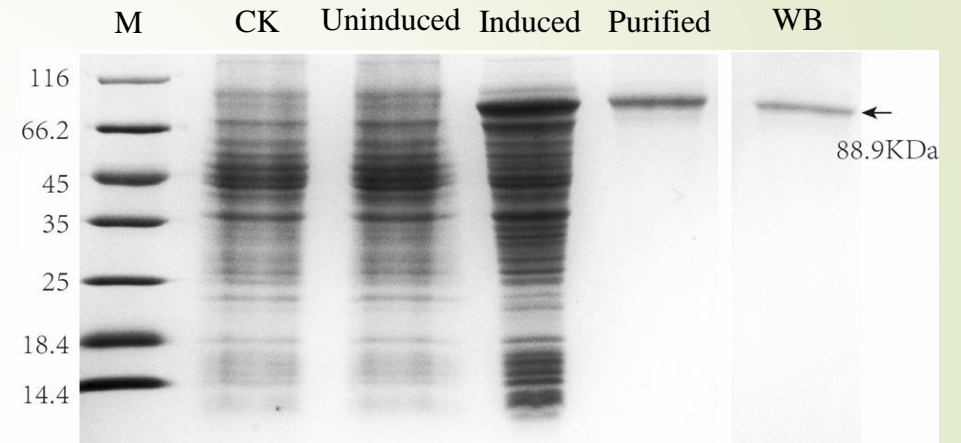
(1) *GhBG1* is preferentially expressed in cotton fibers, and *GhBG1A* plays more important roles in cotton fiber relative to *GhBG1D* in tetraploid *G. hirsutum* TM-1



(2) *GhBG1A* is the critical *beta*-glucosidase gene in *GH3* family for fiber development, and *GhBG1A* encodes a functional *beta*-glucosidase with cleavage-activity

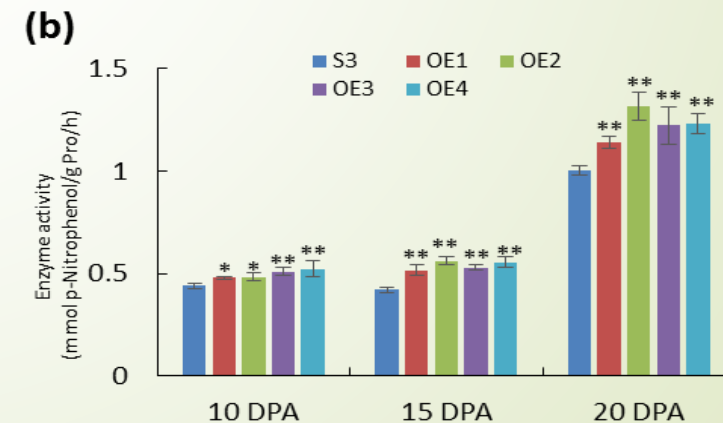
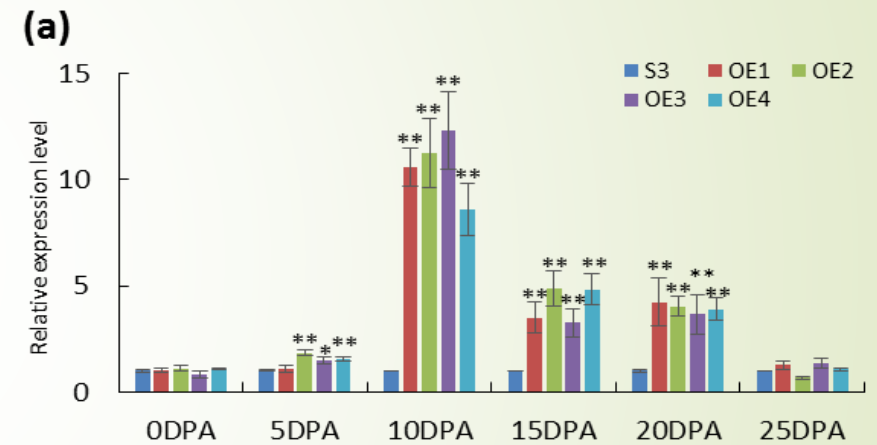
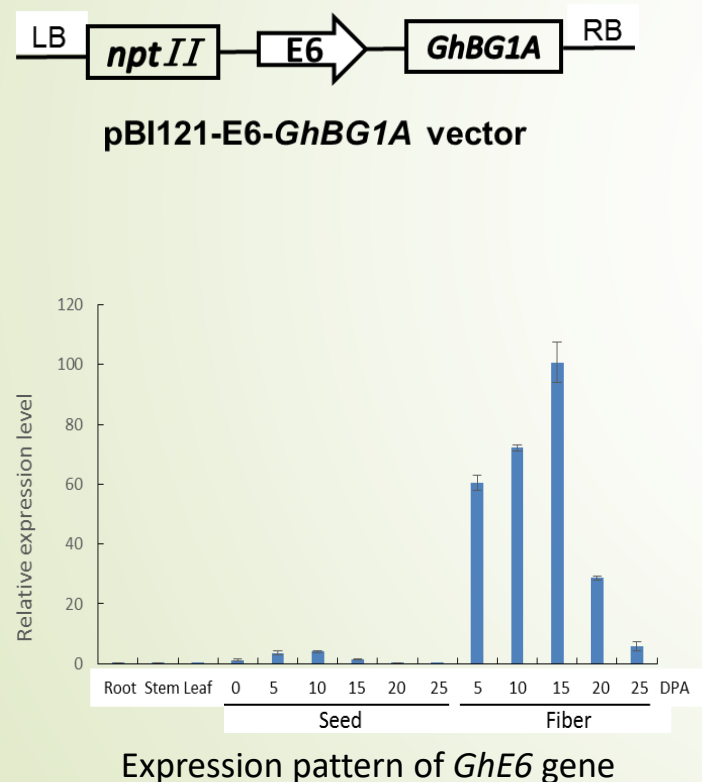


42 GH3 β -glucosidase members were isolated from tetraploid *G. hirsutum*, and transcriptome analysis showed that *GhBG1A* is the most highly expressed one in cotton fibers

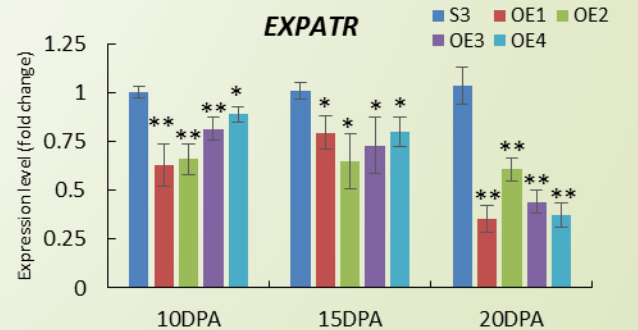
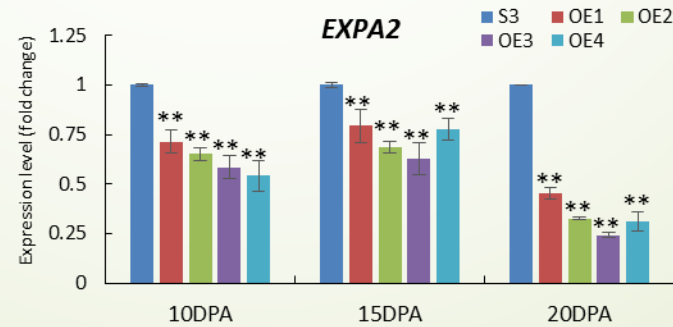
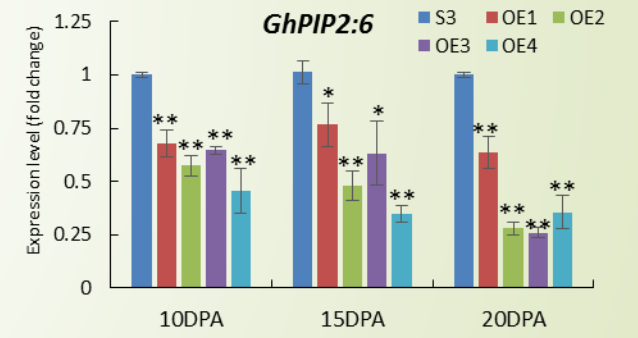
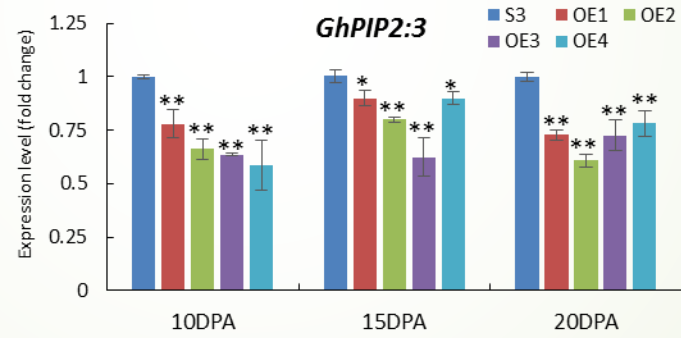
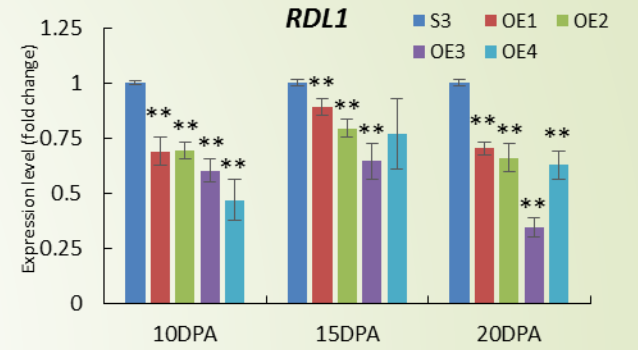
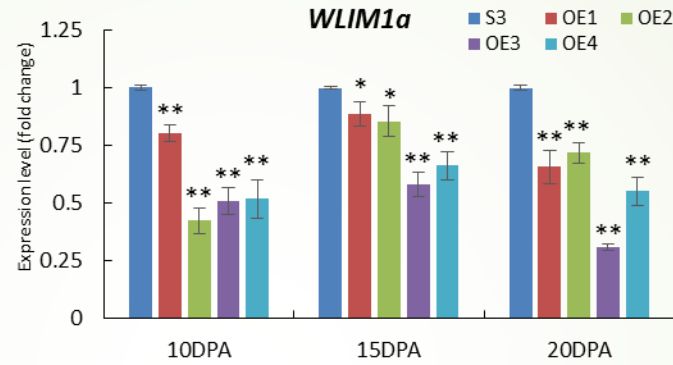
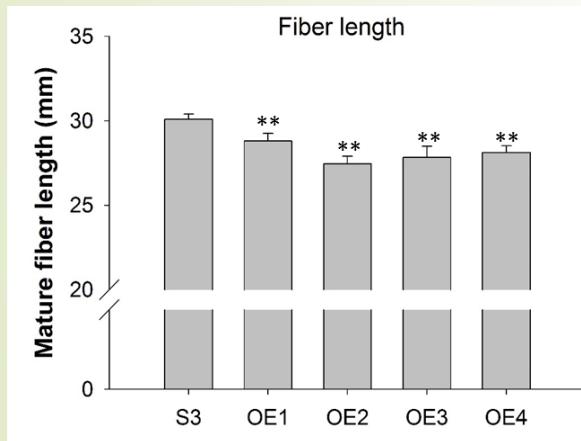


3. Overexpression of *GhBG1A* in fiber repressed fiber elongation but promoted cellulose deposition

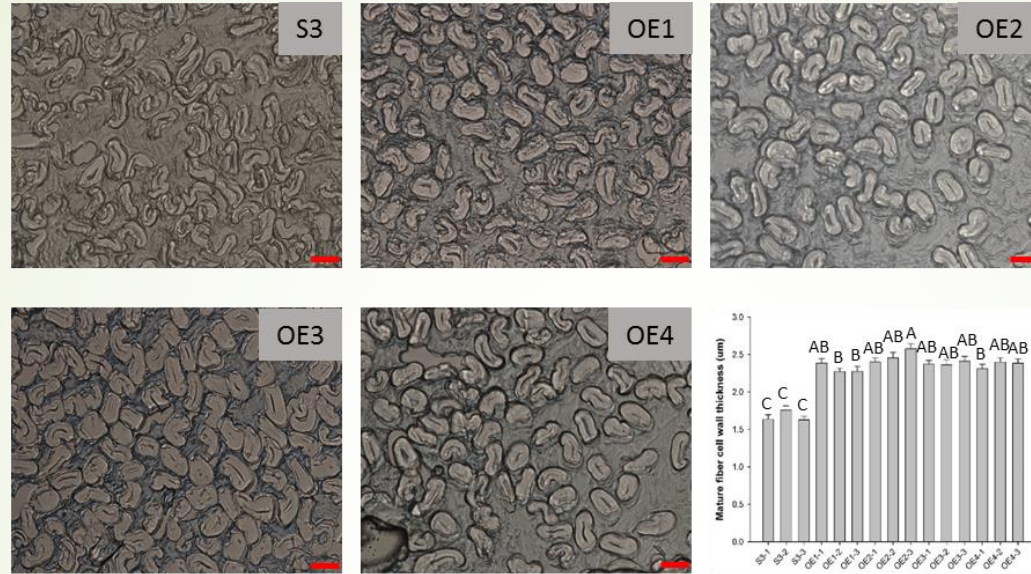
(1) Overexpression construct of *GhBG1A* was made, and expression level and enzyme activities were increased in transgenic fibers



(2) Fiber length was significantly decreased in transgenic fibers, and marker genes in fiber elongation process was significantly reduced.



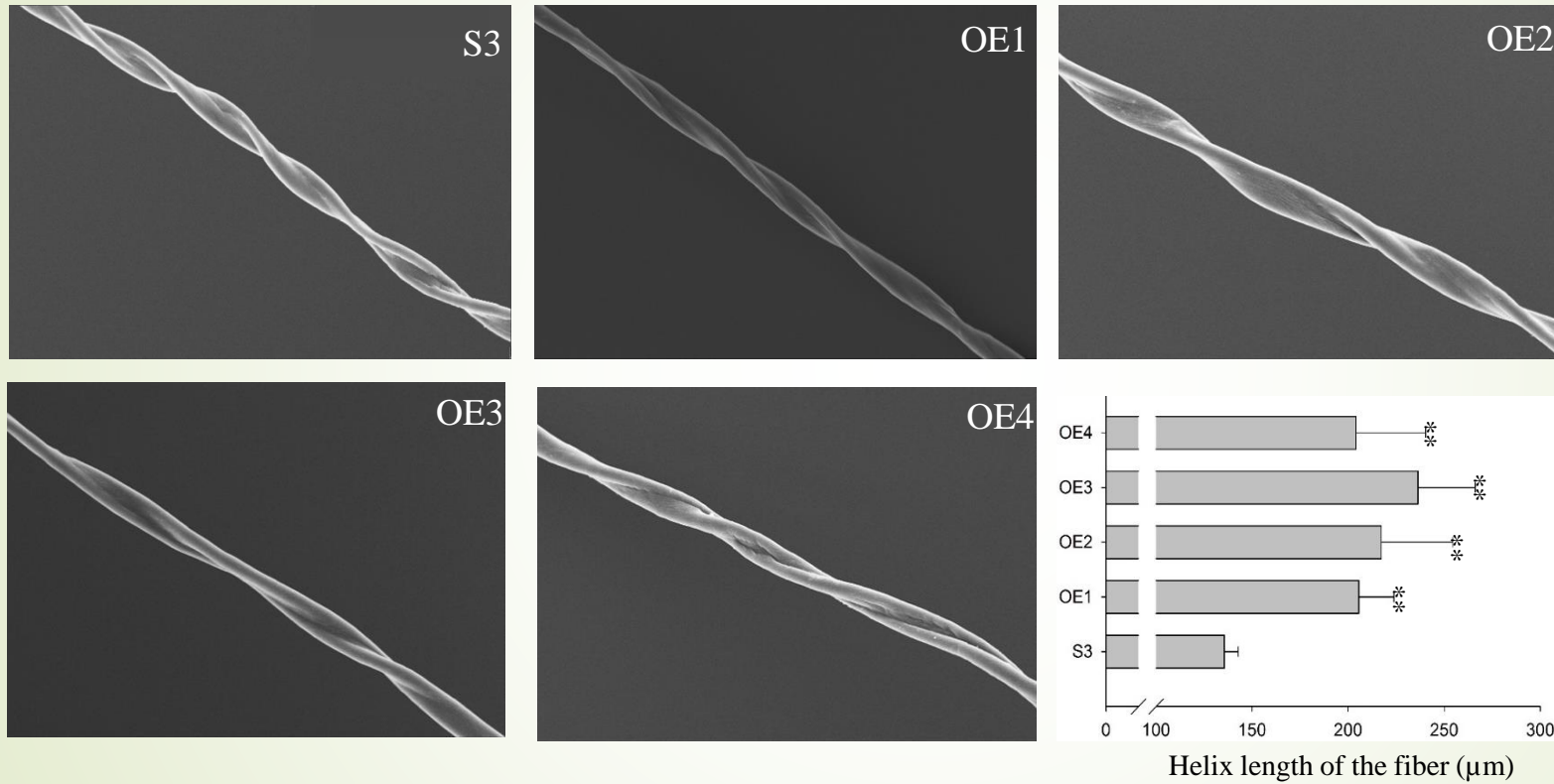
(3) Cell wall thickness and cellulose content were increased in transgenic fibers



Cellulose content (% fiber dry weight)

Lines Stages	S3	OE1	OE2	OE3	OE4
15 DPA	7.23 ± 0.65%	10.18 ± 0.51%**	9.09 ± 0.38%*	12.66 ± 0.94%**	10.66 ± 0.54%**
20 DPA	19.85 ± 2.13%	29.90 ± 4.26%*	28.00 ± 1.05%**	26.49 ± 2.14%*	27.73 ± 3.20%*
25 DPA	48.44 ± 1.74%	57.12 ± 1.55%**	53.85 ± 0.90%**	54.01 ± 1.03%**	54.73 ± 0.91%**
30 DPA	66.84 ± 2.20%	78.55 ± 3.64%**	75.75 ± 3.72%**	77.87 ± 3.52%**	78.28 ± 2.43%**
40 DPA	87.58 ± 2.93%	93.19 ± 3.74%	92.40 ± 4.93%	94.01 ± 3.02%	92.57 ± 2.97%
Mature	93.06 ± 1.92%	97.58 ± 2.60%	99.42 ± 3.34%	98.88 ± 3.53%	98.72 ± 3.57%

(4) Fiber twist was extended in transgenic fibers.



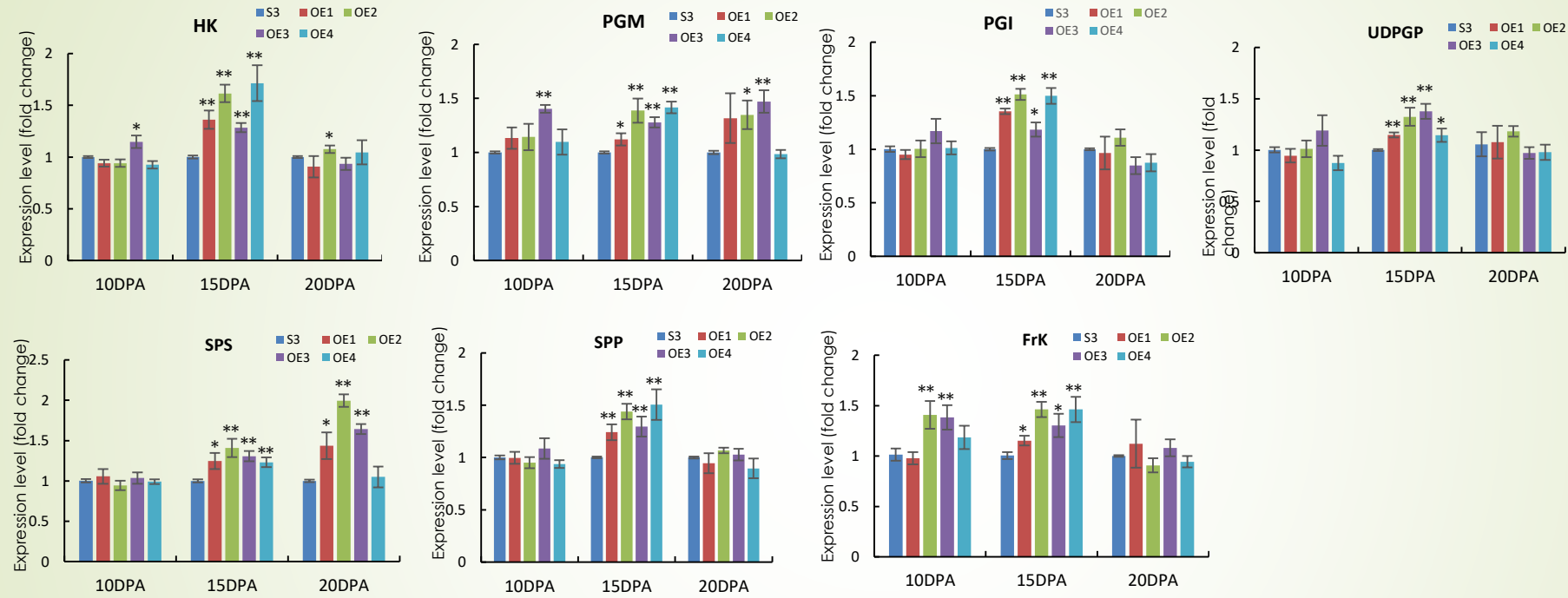
4. Overexpression of *GhBG1A* alters sugar metabolism and promotes expression of genes related to cellulose biosynthesis

(1) Sucrose content was increased in transgenic fibers

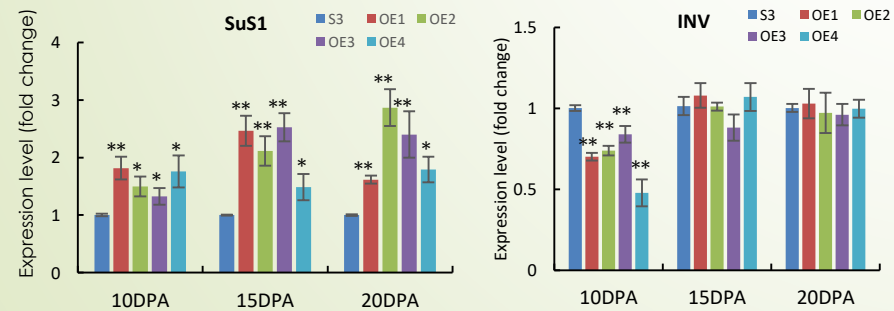
Fiber Stage	Lines	Fructose (% total sugar)	Glucose (% total sugar)	Sucrose (% total sugar)
10 DPA	S3	19.99 ± 1.78	20.57 ± 1.52	7.14 ± 0.58
	OE1	20.16 ± 1.34	19.64 ± 0.89	11.05 ± 0.67**
	OE2	22.27 ± 1.78	21.28 ± 1.32	12.13 ± 1.48**
	OE3	21.84 ± 1.10	21.77 ± 0.66	13.09 ± 1.64**
	OE4	21.42 ± 1.07	21.44 ± 0.73	13.21 ± 0.23**
15 DPA	S3	21.38 ± 0.24	22.89 ± 0.22	5.63 ± 0.24
	OE1	22.46 ± 1.59	23.74 ± 1.98	7.88 ± 0.79**
	OE2	23.54 ± 1.25	24.04 ± 1.45	7.09 ± 0.32**
	OE3	20.90 ± 0.65	22.65 ± 0.74	6.90 ± 0.39*
	OE4	21.13 ± 1.18	120.96 ± 1.19*	7.34 ± 0.31**
20 DPA	S3	17.03 ± 2.42	17.46 ± 1.70	4.84 ± 0.18
	OE1	17.82 ± 1.57	20.63 ± 1.36	6.03 ± 0.25**
	OE2	15.38 ± 0.92	14.11 ± 0.42	7.15 ± 0.29**
	OE3	15.73 ± 1.49	13.93 ± 0.90	7.93 ± 0.27**
	OE4	15.35 ± 0.84	14.08 ± 0.60	8.74 ± 1.44**

(2) Sucrose metabolic pathways changed in transgenic fibers

A

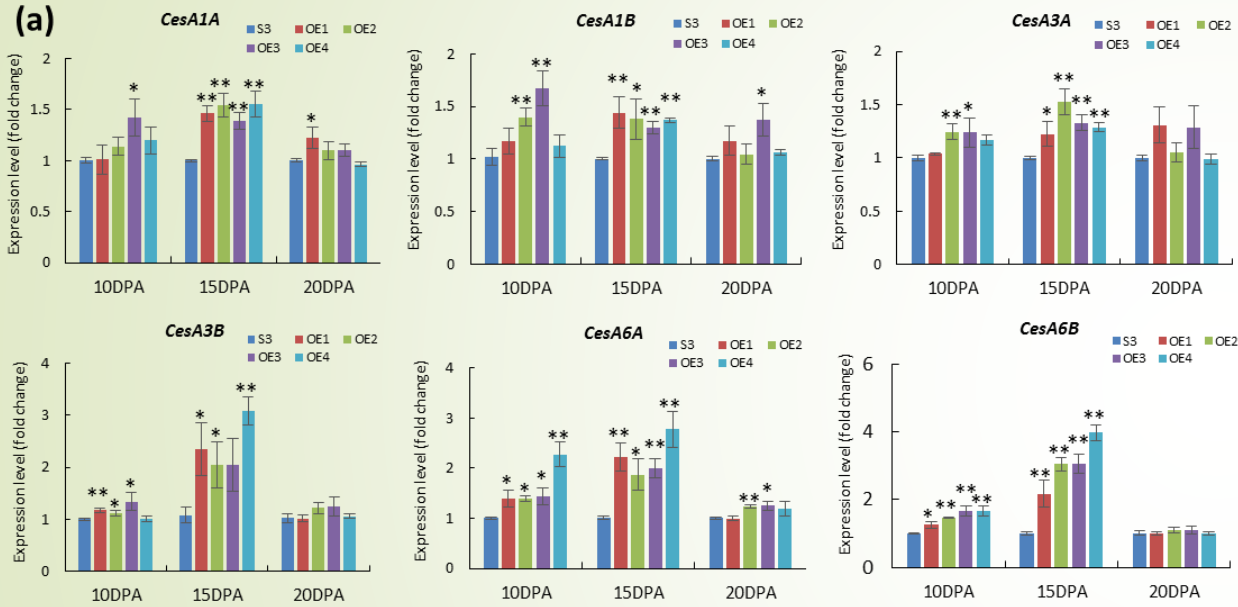


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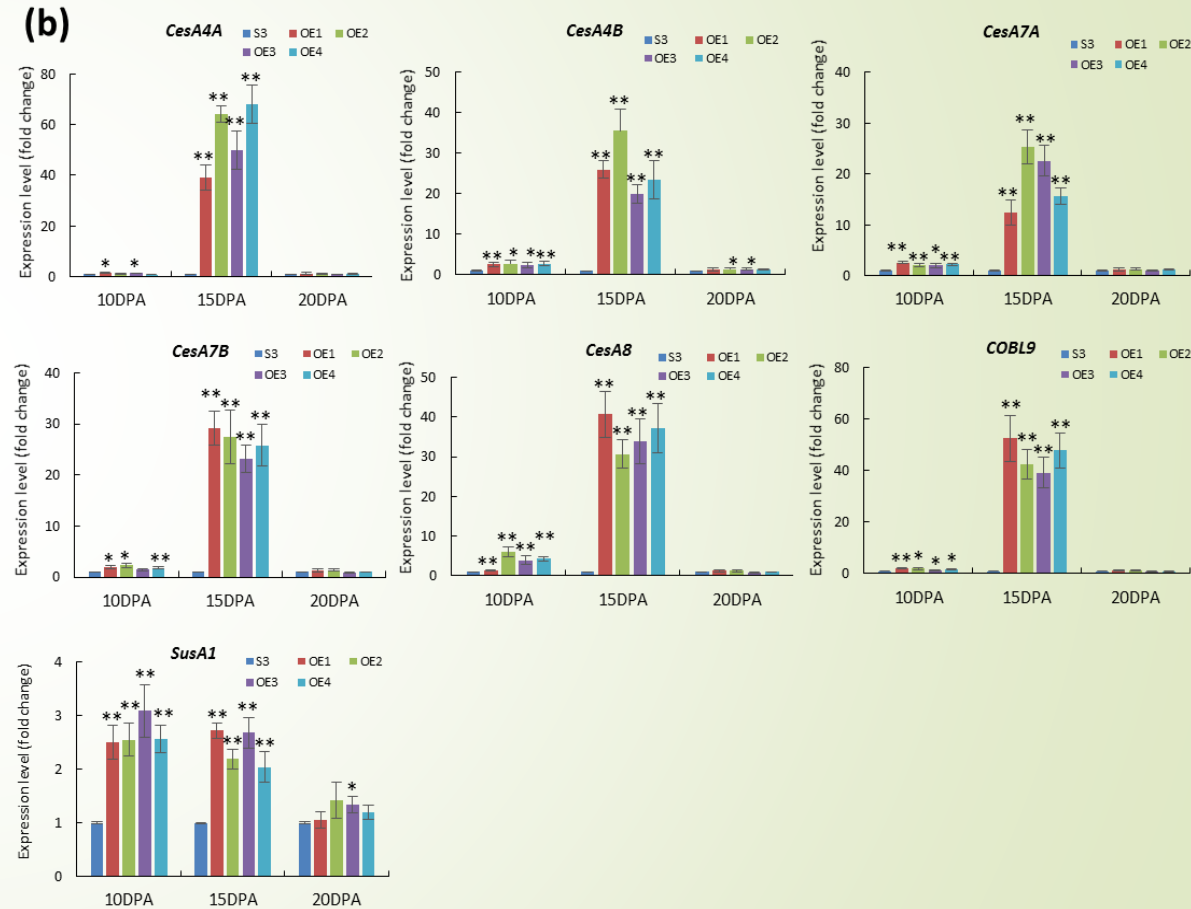


HK (Hexose Kinase);
PGM (PhosphoGluMuatse);
PGI (PhosphoGluIsomerase);
UDPGP (UDP-Glucose Pyrophosphorylase);
SPS (Sucrose Phosphate Synthase);
SPP (Sucrose Phosphate Phosphatase);
FrK (Fructose Kinase);
SuSy (Sucrose Synthase);
INV (Invertase)

(3) Expression of cellulose synthases involved in PCW and SCW cellulose deposition was up-regulated.

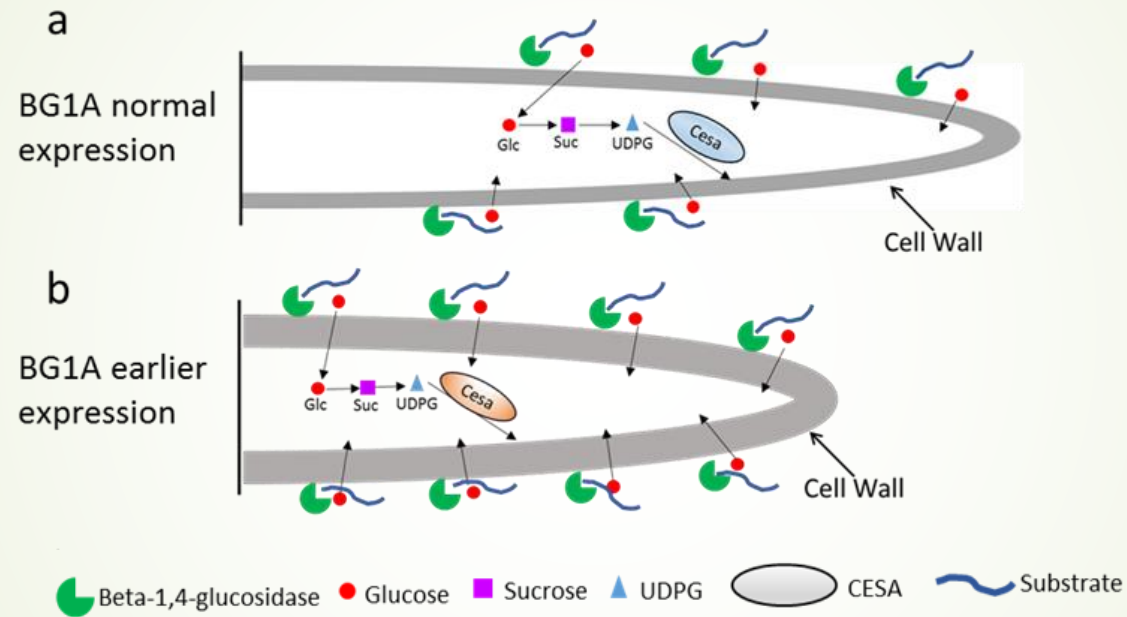


Expression pattern of *GhCesAs* involved in PCW deposition



Expression pattern of *GhCesAs* involved in SCW deposition

Schematic model of functions of *GhBG1A* in cotton fiber development



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