MOLECULAR BREEDING: COTTON TRANSCRIPTOME ANALYSIS, CHARACTERISATION AND VALIDATION OF FIBRE STRENGTH GENES ASSISTIVE IN MARKER ASSISTED SELECTION.

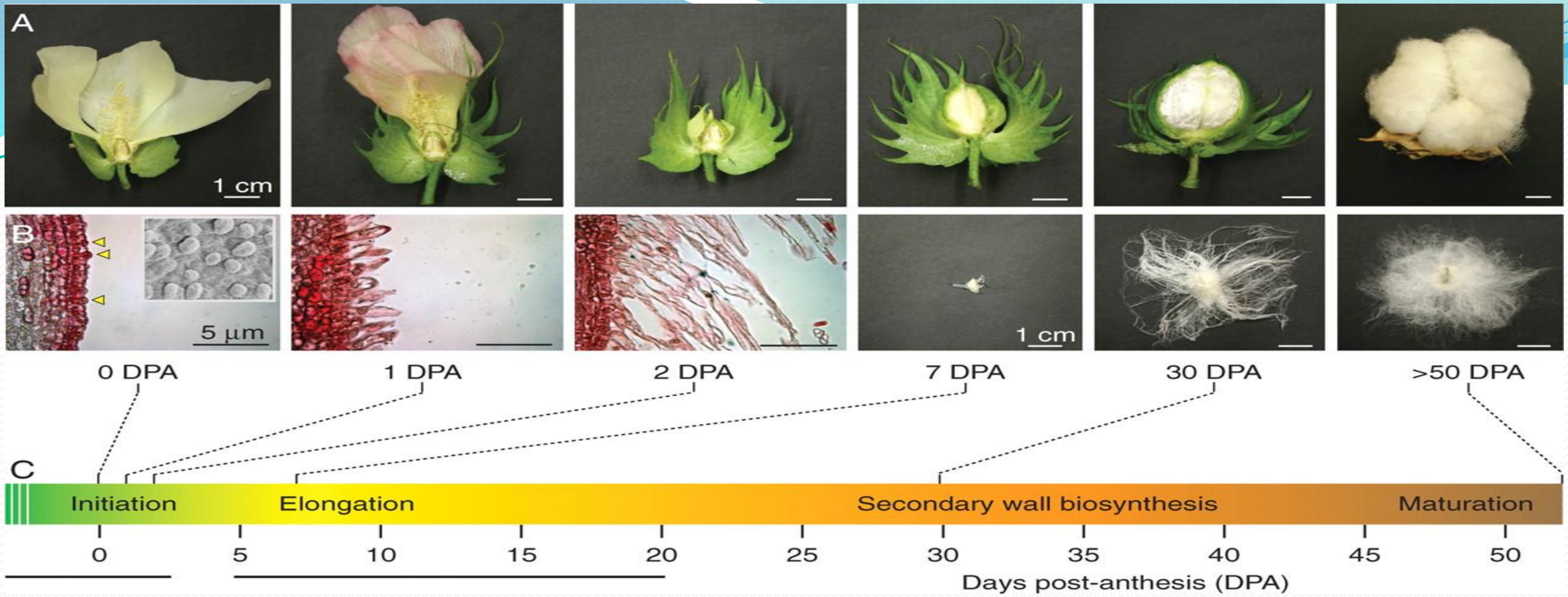
Dr Bhuvaneshwar.R.Patil
Professor, Genetics and Plant breeding
University of Agricultural Sciences,
Dharwad, India
Email: patilbhuvaneshwara@gmail.com

OBJECTIVES

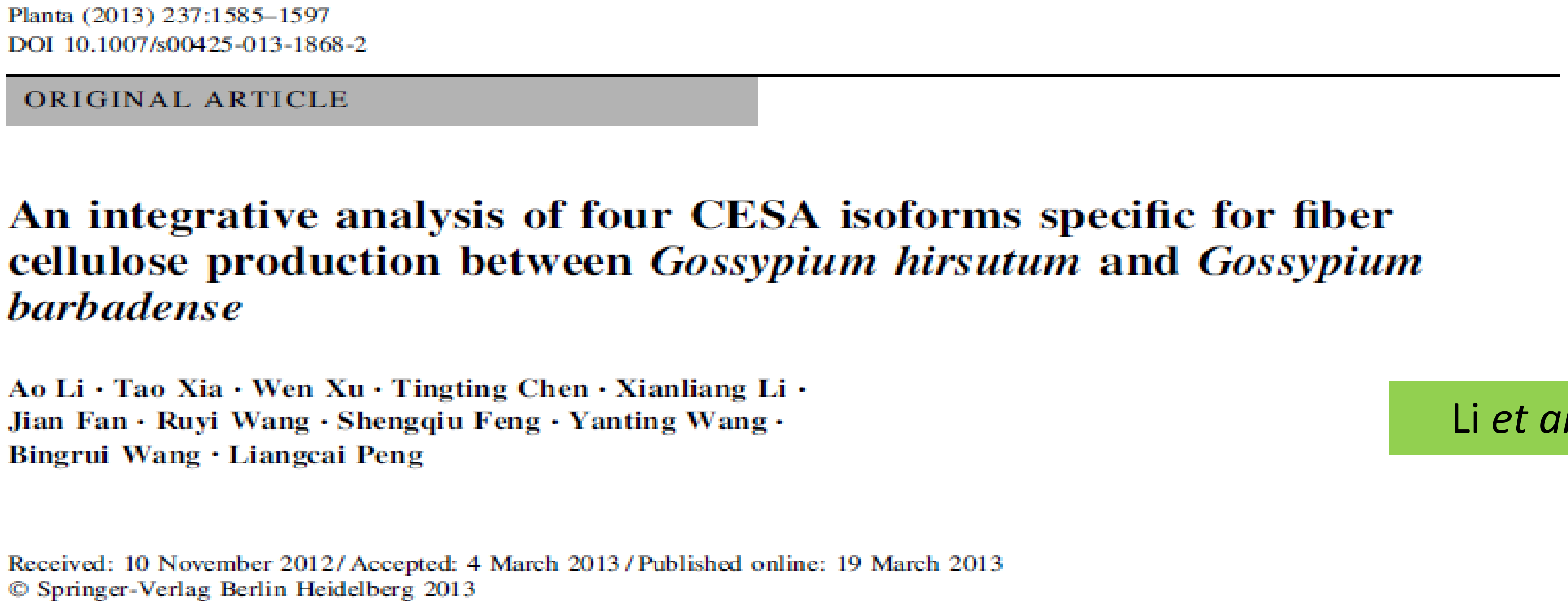
- To characterize **major genes that are responsible for high strength** taking advantage from already known information and then building the standard strategy for their **functional analysis**.(q PCR)
- Transcriptome analysis of the genotypes and identify SSR’s and SNP’s for Marker Assisted Selection
- Electron micrographs of the fibre (Validation)
- Biochemical analysis of cotton fibre (Cellulose)
- Microtome studies of fibre (Validation)

Sequence ID	Sequence description	Fold change	
		MD52ne and 16DPA	MD90ne 20DPA
U58283	Cellulose synthase (GhCesA1)	3.5	
TC66201.3	Cellulose synthase (GhCesA2)	2.55	
TC68655	Cellulose synthase (AtCesA7)	3.5	
TC59066	COBRA-LIKE4 (AtCOBL4)	2.26	1.98
Cotton12_06695_01	Arabinogalactan protein 4 (GhAGP4)	4.16	3.24
TC59194	*GhFLA2* Fasciclin-like arabinogalactan protein 3	2.61	4.52
Cotton12_06485_01	1,3-Glucanase-like protein	2.33	2.12
TC59405	Metallothionein-like protein (GhMT1)	3.18	8.4
Cotton12_15955_01	WAVE-DAMPENED2-LIKE1 (AtWDL1)		

(Hinchliffe *et al* 2010)



- Fiber development consists of four overlapping stages (initiation, elongation, secondary cell wall biosynthesis, and maturation),
- Fiber initiation is characterized by trichome protrusion and enlargement on the epidermal surface that occurs from 3 days before anthesis to 3 dpa.
- Only 25–30% of epidermal cells differentiate into the mature long-fiber cells, whereas others may develop into short fibers called fuzz (5–6 mm in length).
- During the most active elongation period (5–25 dpa), vigorous cell expansion with peak growth rates of >2 mm/day is observed in upland cotton, coupled with cell expansion and a specific set of metabolite syntheses.
- Cellulose synthesis dominates the period of secondary cell wall biosynthesis (20–45 dpa), which is followed by a dehydration period (45–50 dpa) to produce mature lint fibers.



Planta (2013) 237:1585–1597
DOI 10.1007/s00425-013-1868-2

ORIGINAL ARTICLE

An integrative analysis of four CESA isoforms specific for fiber cellulose production between *Gossypium hirsutum* and *Gossypium barbadense*

Ao Li · Tao Xia · Wen Xu · Tingting Chen · Xianliang Li ·
Jian Fan · Ruyi Wang · Shengqiu Feng · Yanting Wang ·
Bingrui Wang · Liangcai Peng

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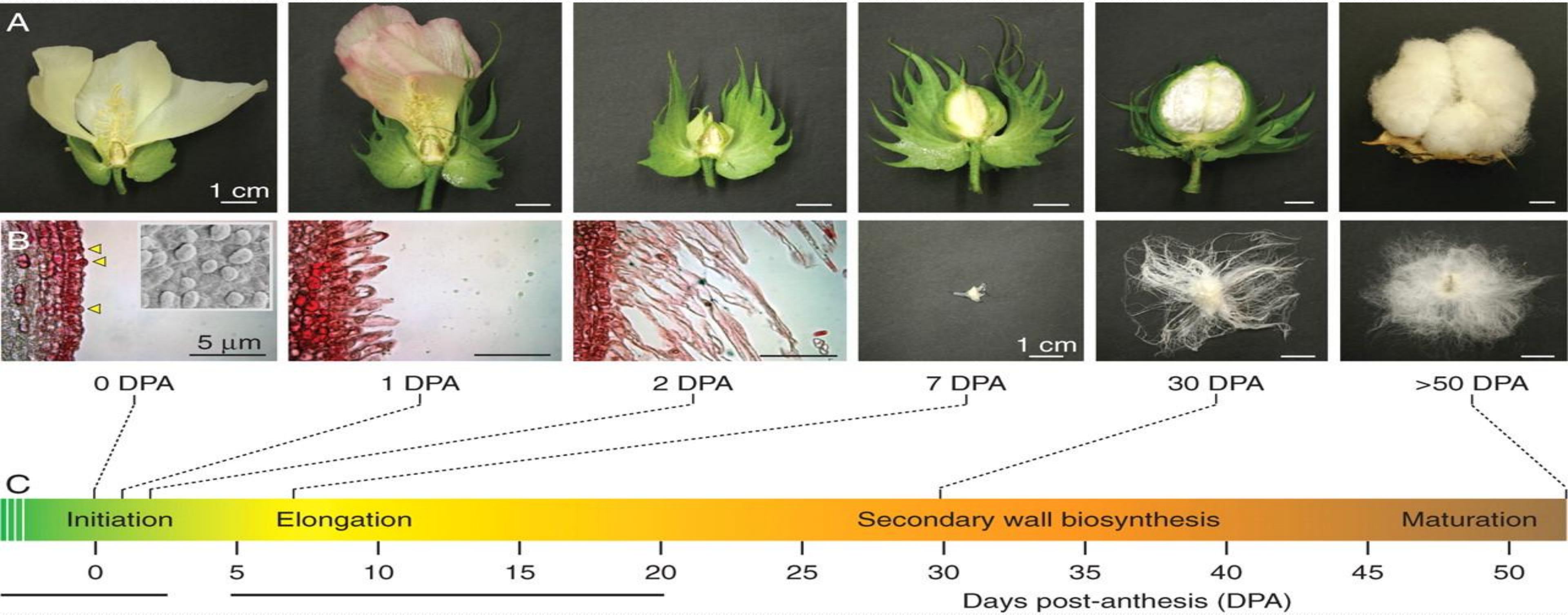
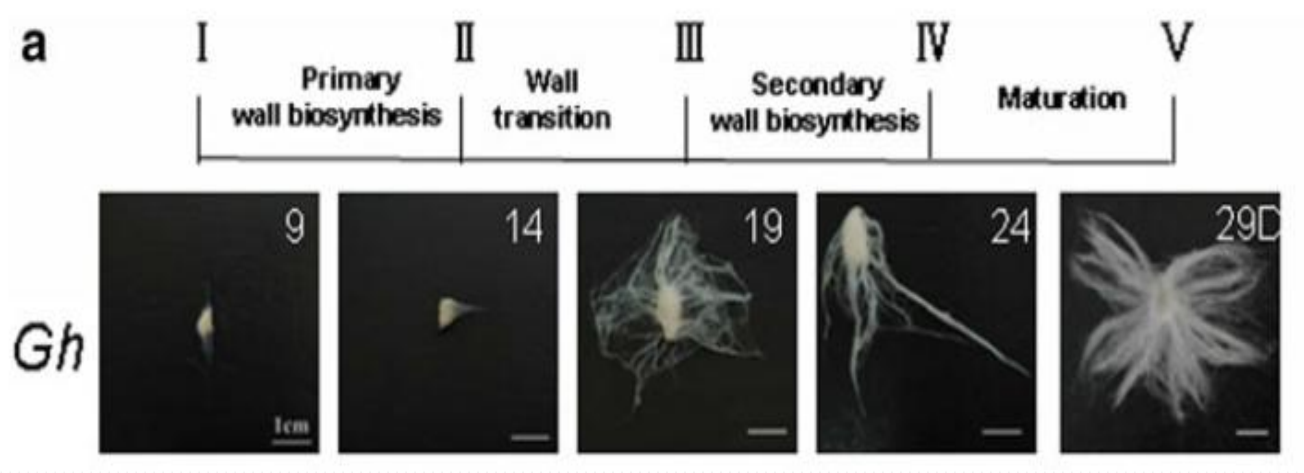
Li *et al* 2013

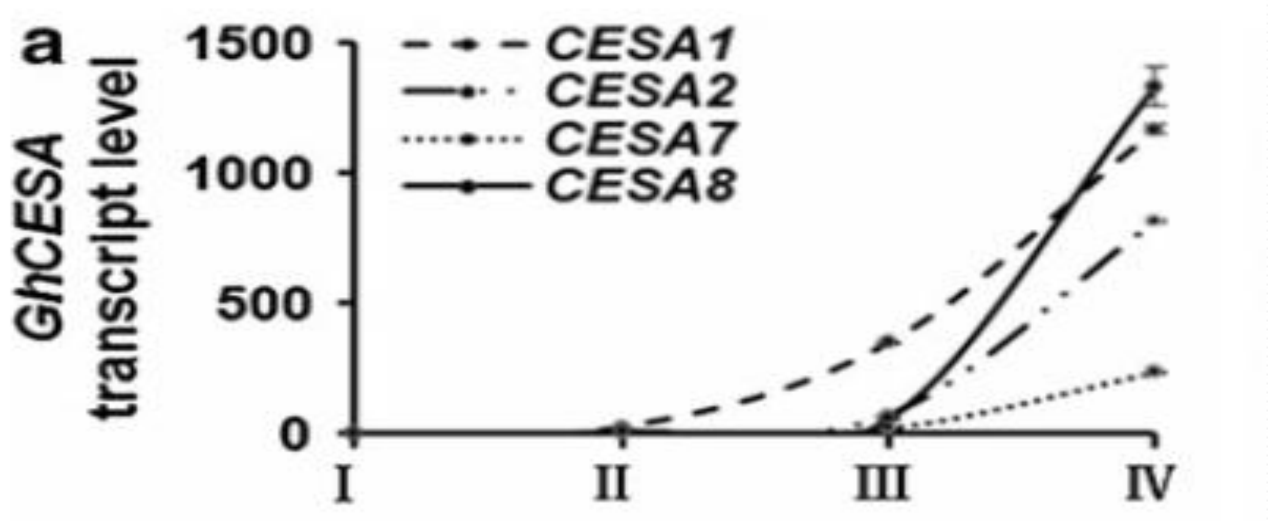
Genes associated with fiber developmental stages	Primary Cell wall synthesis	Secondary Cell wall synthesis
CesA genes	***CesA3, CesA5, CesA6, CesA9 and CesA10***	***CesA1, CesA2, CesA7 and CesA8***

CESA8 plays an enhancing role for rapid and massive cellulose accumulation in cotton fiber, where as ***CESA2*** displayed a major expression in non-fiber tissues and ***CESA1*** predominantly expressed in all tissues.

Reconfirmation of the gene expression of selected genes through qPCR (*GhcesA1, GhcesA2, GhcesA7, GhcesA8, Ghcobl4, Ghfla3 and GhMT1*) using RIL mapping population to establish correlation with fibre strength.







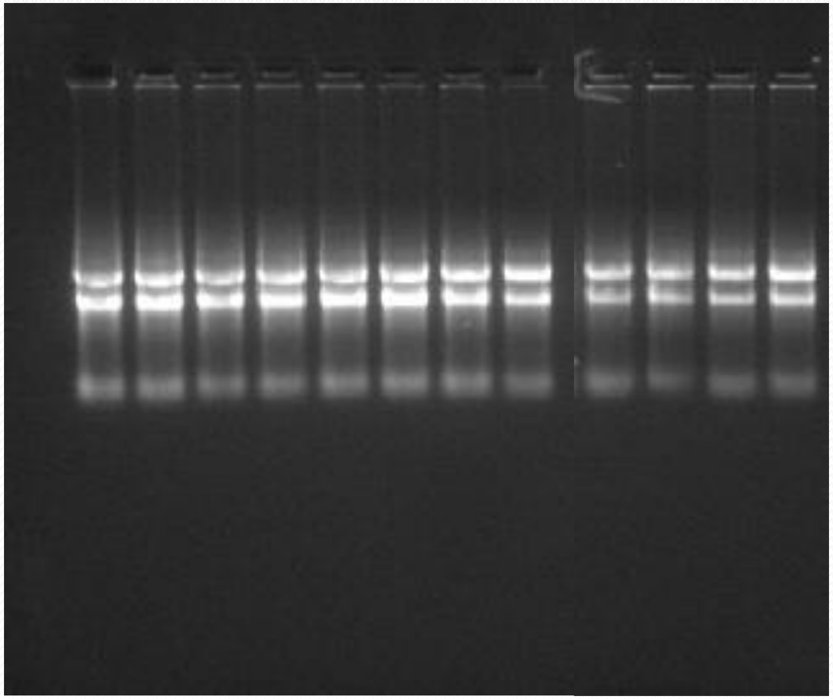
CESA1, 2, 7, 8 expression patterns by real-time PCR analysis. a CESA transcript level at cotton fibers.

Li *et al* 2013

RIL Mapping population UAS, Dharwad

Species	**RILs**	**Fibre strength (g/tex)**
G.hirsutum (High)	HBS 144	28.0
G.hirsutum (Low)	HBS 187	20.0
***G. arboreum* (High)**	**Arb 1**	**22.9**
***G. arboreum* (Low)**	**Arb 2**	**19.7**

RNA isolation from Mapping population



High strength line

12 DPA
18 DPA
24 DPA
30 DPA

Medium strength line

12 DPA
18 DPA
24 DPA
30 DPA

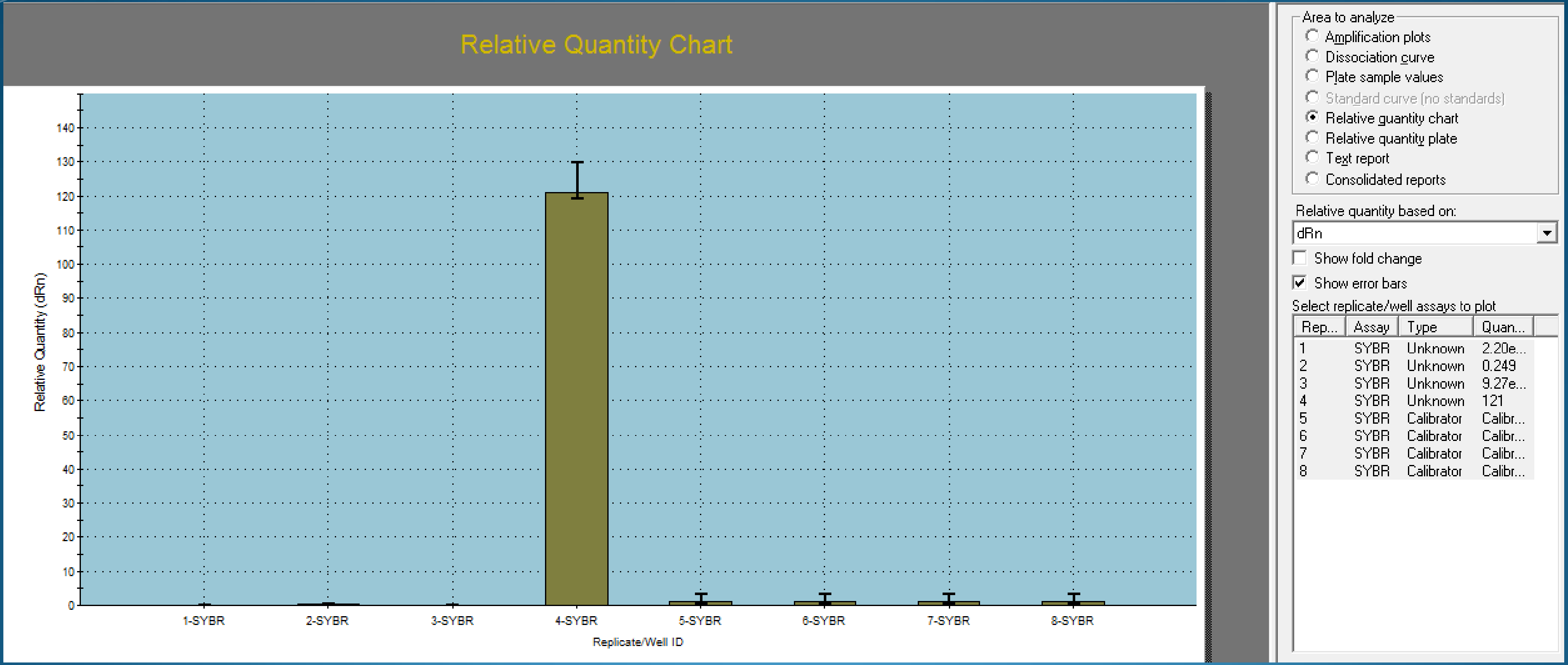
Low strength line

12 DPA
18 DPA
24 DPA
30 DPA

Real-Time PCR

UAS Dharwad

CesA1 gene : *Gossypium hirsutum*



Area to analyze
- ○ Amplification plots
- ○ Dissociation curve
- ○ Plate sample values
- ○ Standard curve (no standards)
- ● Relative quantity chart
- ○ Relative quantity plate
- ○ Text report
- ○ Consolidated reports

Relative quantity based on: dRn

☐ Show fold change

☑ Show error bars

Select replicate/well assays to plot

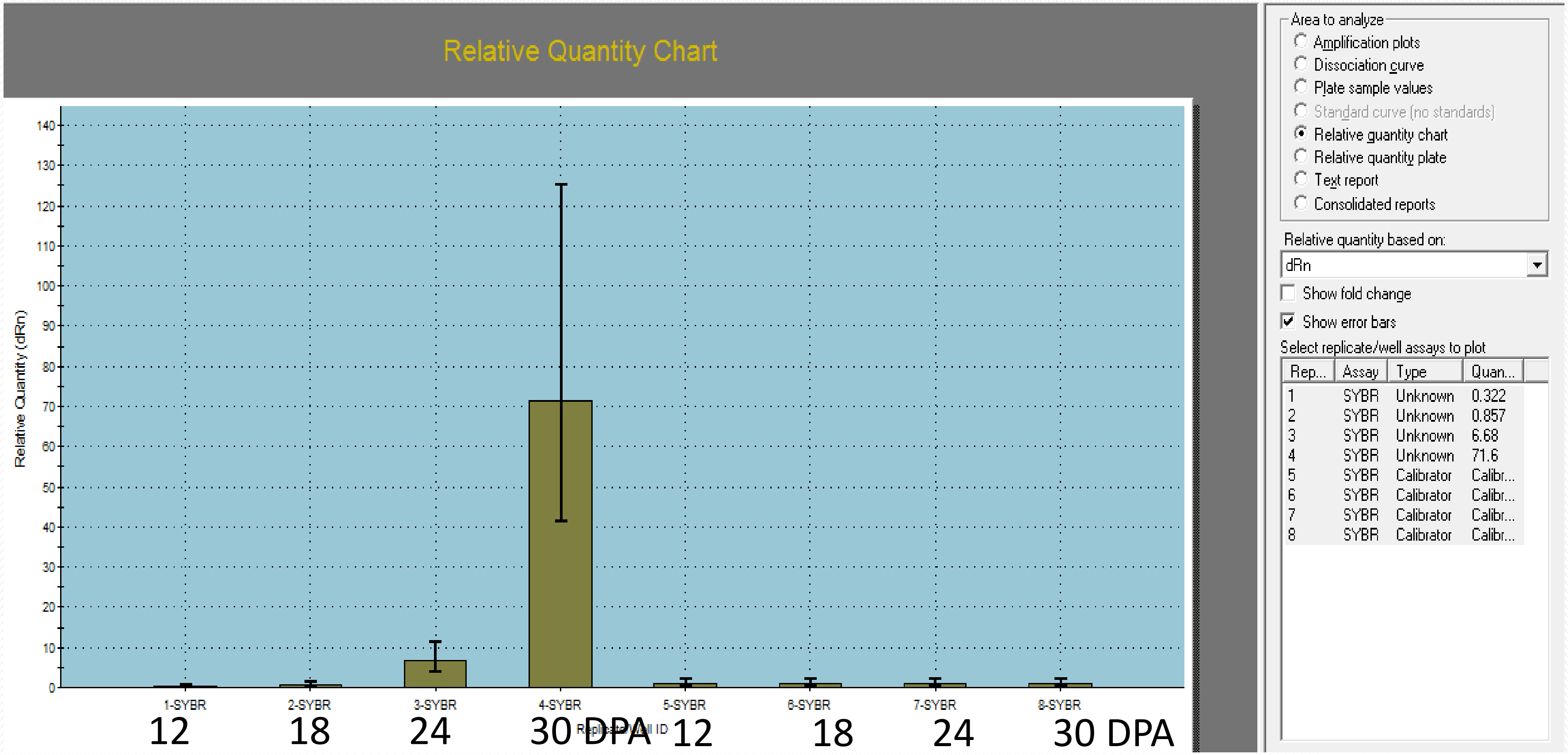
Rep...	Assay	Type	Quan...
1	SYBR	Unknown	2.20e...
2	SYBR	Unknown	0.249
3	SYBR	Unknown	9.27e...
4	SYBR	Unknown	121
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

UAS Dharwad

CesA1 gene : *Gossypium arboreum*



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● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on: dRn

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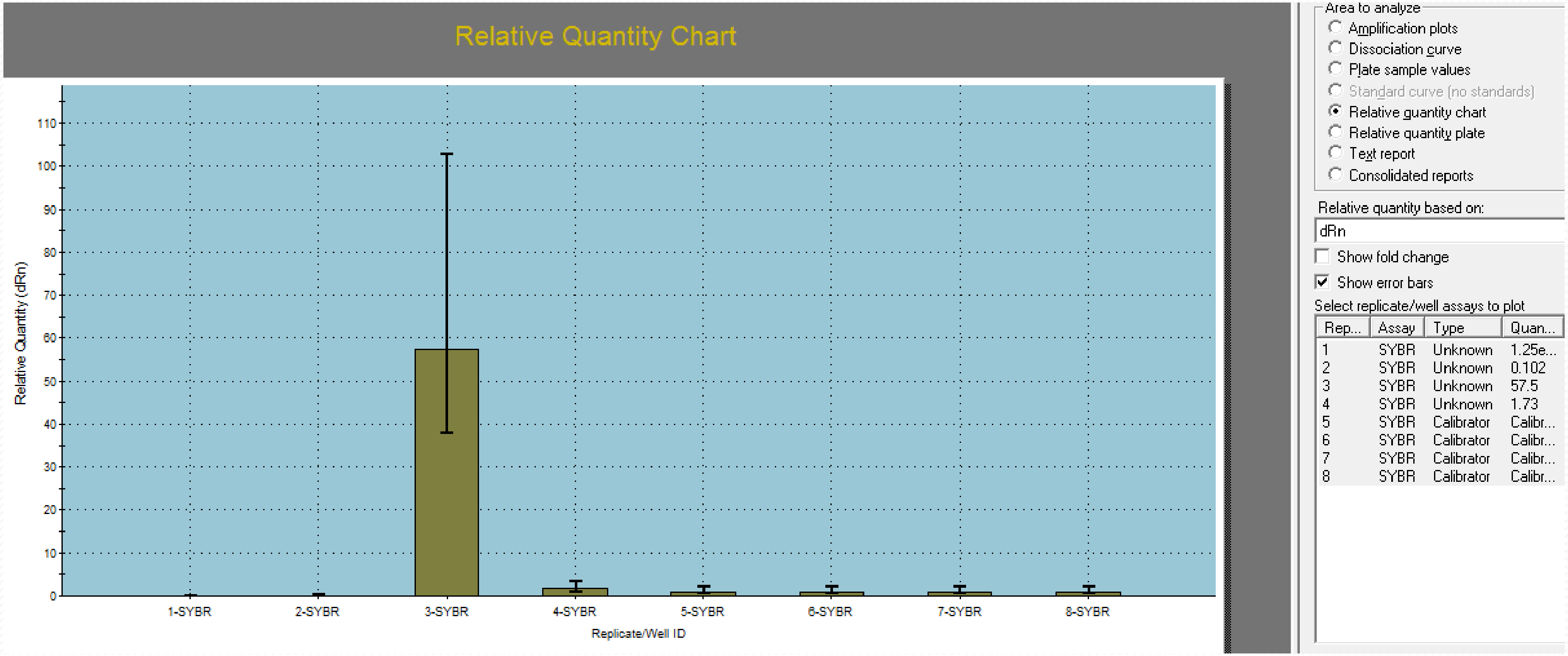
1	SYBR	Unknown	0.322
2	SYBR	Unknown	0.857
3	SYBR	Unknown	6.68
4	SYBR	Unknown	71.6
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

UAS Dharwad

CesA2 gene : *Gossypium hirsutum*



Area to analyze
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○ Dissociation curve
○ Plate sample values
○ Standard curve (no standards)
● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on:
dRn

☐ Show fold change
☑ Show error bars

Select replicate/well assays to plot

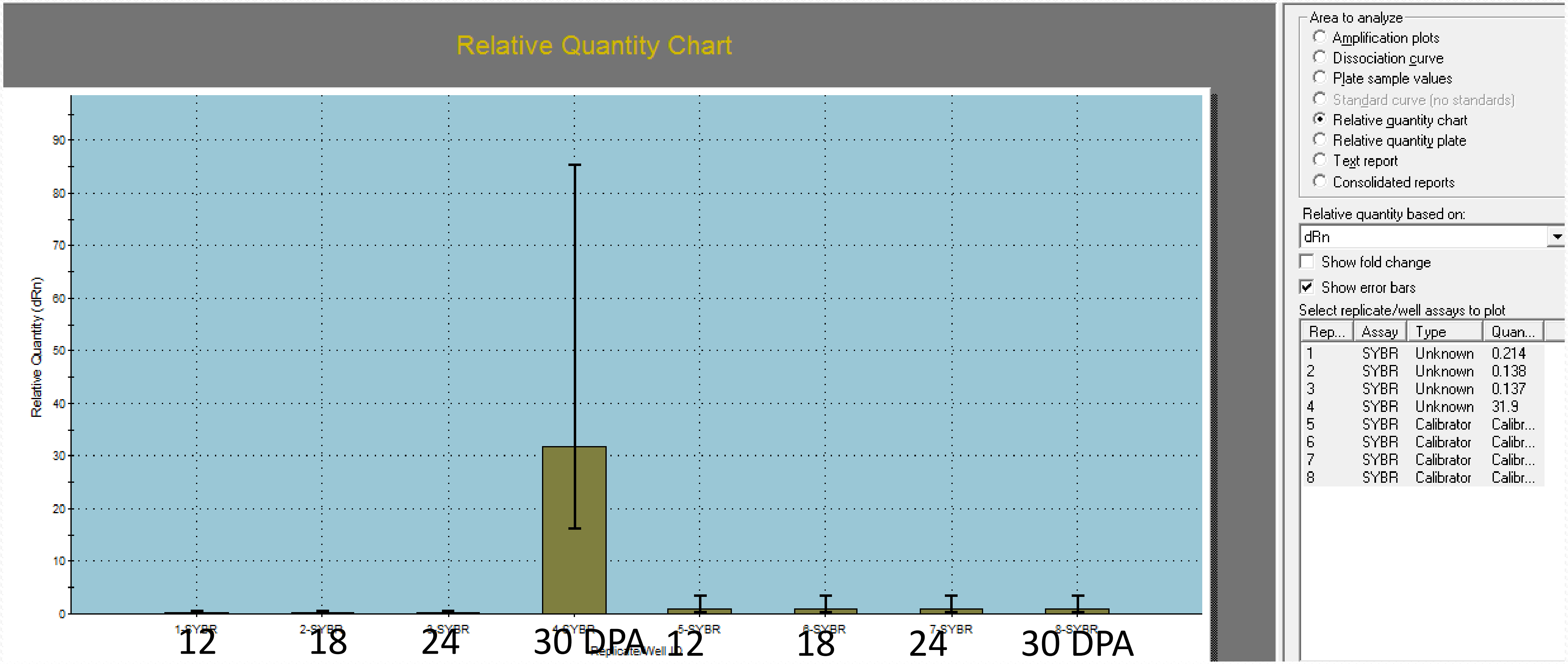
Rep...	Assay	Type	Quan...
1	SYBR	Unknown	1.25e...
2	SYBR	Unknown	0.102
3	SYBR	Unknown	57.5
4	SYBR	Unknown	1.73
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

UAS Dharwad

CesA2 gene : *Gossypium arboreum*



Area to analyze
○ Amplification plots
○ Dissociation curve
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○ Standard curve (no standards)
● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on: dRn

☐ Show fold change

☑ Show error bars

Select replicate/well assays to plot

Rep...	Assay	Type	Quan...
1	SYBR	Unknown	0.214
2	SYBR	Unknown	0.138
3	SYBR	Unknown	0.137
4	SYBR	Unknown	31.9
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

Characterization of GhCOBL4 , a fiber quality associated gene

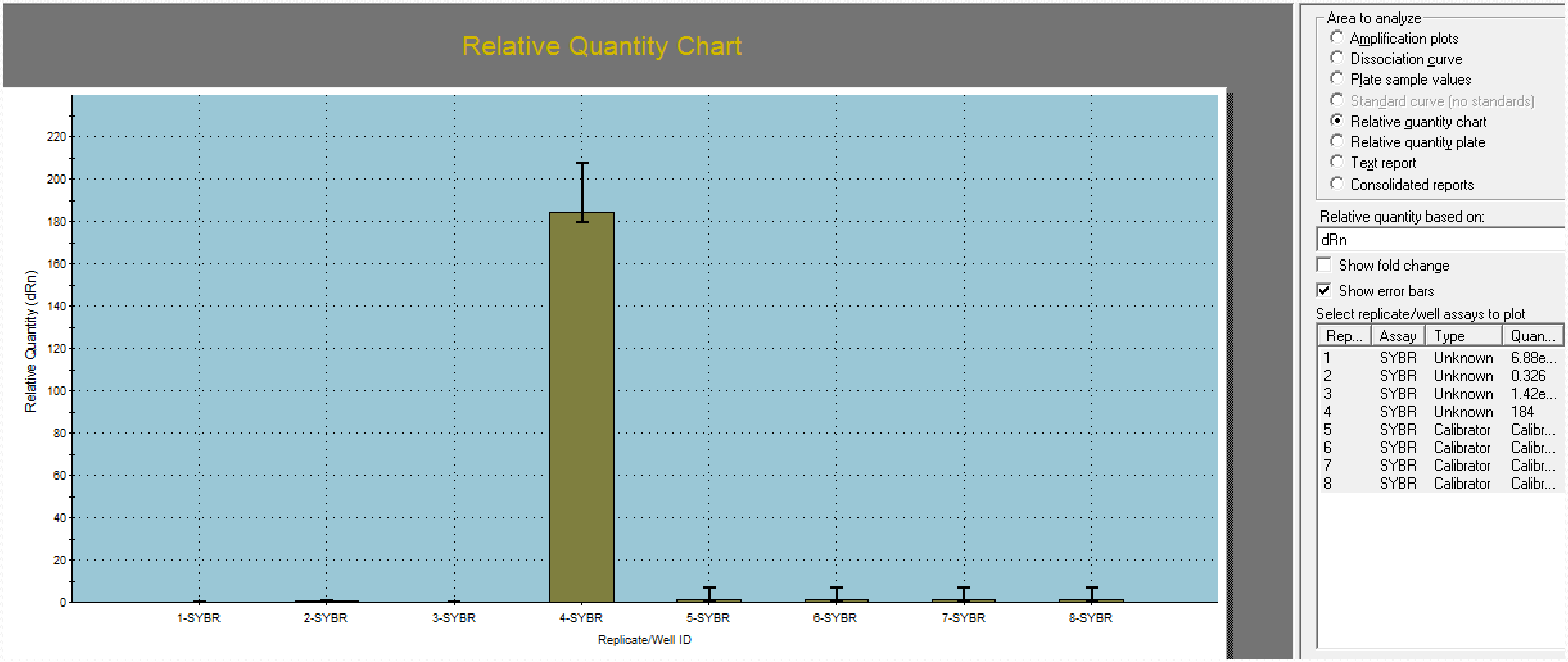
In addition to CesA and **microtubule-related genes**, several members of the COBRA gene family, which **encodeglycosyl phosphatidylinositol (GPI)-anchored proteins** have been identified as new players in the regulation of the orientation of cell expansion in the plant cell wall; these proteins appear to function at the plasma membrane–cell wall interface (Brady et al. 2007).

Mutations in COBRA were shown to affect the orientation of **cell expansion and reduce levels of crystalline cellulose microfibrils** of the root elongation zone, implying a role for COBRA in cell wall maintenance and/or biosynthesis in Arabidopsis (Roudier et al. 2002; Schindelman et al. 2001).

In addition, mutants exhibiting lowered cellulose content and weakened **mechanical strength of the stems in rice, maize, and Arabidopsis suggested a role for COBRA in secondary wall cellulose synthesis and/or deposition** (Brown et al. 2005;Li et al. 2003a, b; Sindhu et al. 2007). Together, these previous studies have demonstrated that the members of COBRA family play a conserved general role in cell wall biosynthesis of vascular plants.

UAS Dharwad

Cobl4 gene : *Gossypium hirsutum*



Area to analyze
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● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on: dRn

☐ Show fold change

☑ Show error bars

Select replicate/well assays to plot

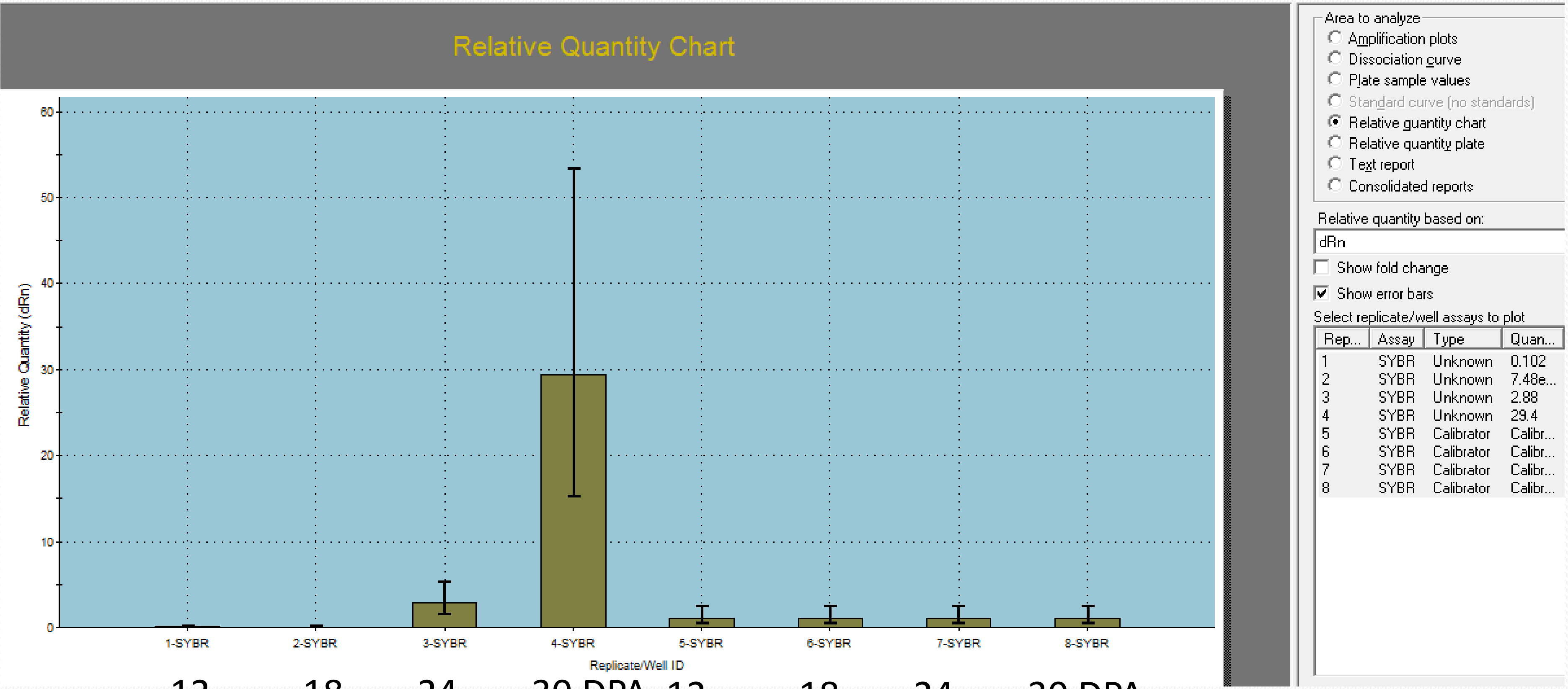
Rep...	Assay	Type	Quan...
1	SYBR	Unknown	6.88e...
2	SYBR	Unknown	0.326
3	SYBR	Unknown	1.42e...
4	SYBR	Unknown	184
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

UAS Dharwad

Cobl4 gene :*Gossypium arboreum*



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○ Dissociation curve
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○ Standard curve (no standards)
● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on:
dRn

☐ Show fold change
☑ Show error bars

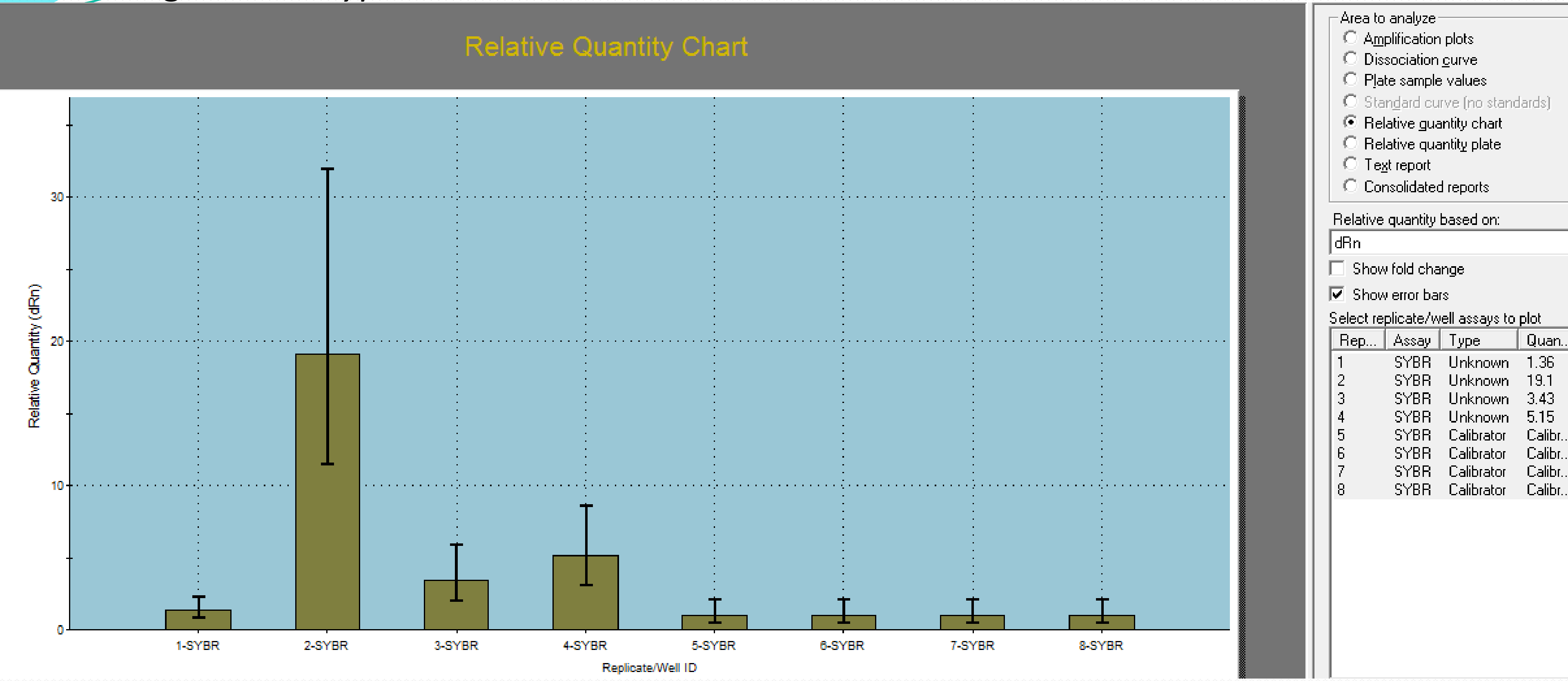
Select replicate/well assays to plot

Rep...	Assay	Type	Quan...
1	SYBR	Unknown	0.102
2	SYBR	Unknown	7.48e...
3	SYBR	Unknown	2.88
4	SYBR	Unknown	29.4
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line **Low strength line**

MT1 gene : *Gossypium hirsutum*



Area to analyze
○ Amplification plots
○ Dissociation curve
○ Plate sample values
○ Standard curve (no standards)
● Relative quantity chart
○ Relative quantity plate
○ Text report
○ Consolidated reports

Relative quantity based on:
dRn

☐ Show fold change
☑ Show error bars

Select replicate/well assays to plot

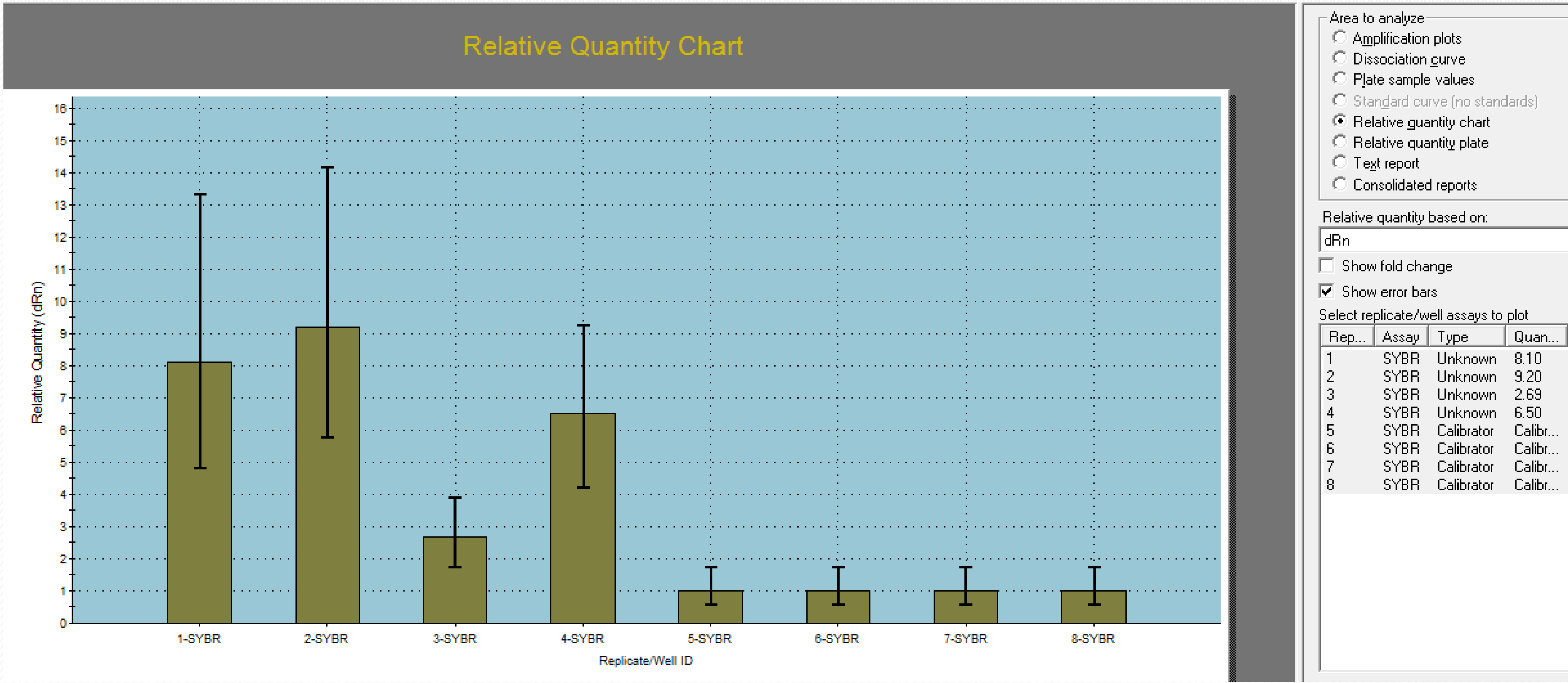
Rep...	Assay	Type	Quan...
1	SYBR	Unknown	1.36
2	SYBR	Unknown	19.1
3	SYBR	Unknown	3.43
4	SYBR	Unknown	5.15
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line

Low strength line

MT1 gene :*Gossypium arboreum*



Area to analyze
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- ○ Dissociation curve
- ○ Plate sample values
- ○ Standard curve (no standards)
- ● Relative quantity chart
- ○ Relative quantity plate
- ○ Text report
- ○ Consolidated reports

Relative quantity based on: dRn

☐ Show fold change

☑ Show error bars

Select replicate/well assays to plot

Rep...	Assay	Type	Quan...
1	SYBR	Unknown	8.10
2	SYBR	Unknown	9.20
3	SYBR	Unknown	2.69
4	SYBR	Unknown	6.50
5	SYBR	Calibrator	Calibr...
6	SYBR	Calibrator	Calibr...
7	SYBR	Calibrator	Calibr...
8	SYBR	Calibrator	Calibr...

12 18 24 30 DPA 12 18 24 30 DPA

High strength line Low strength line

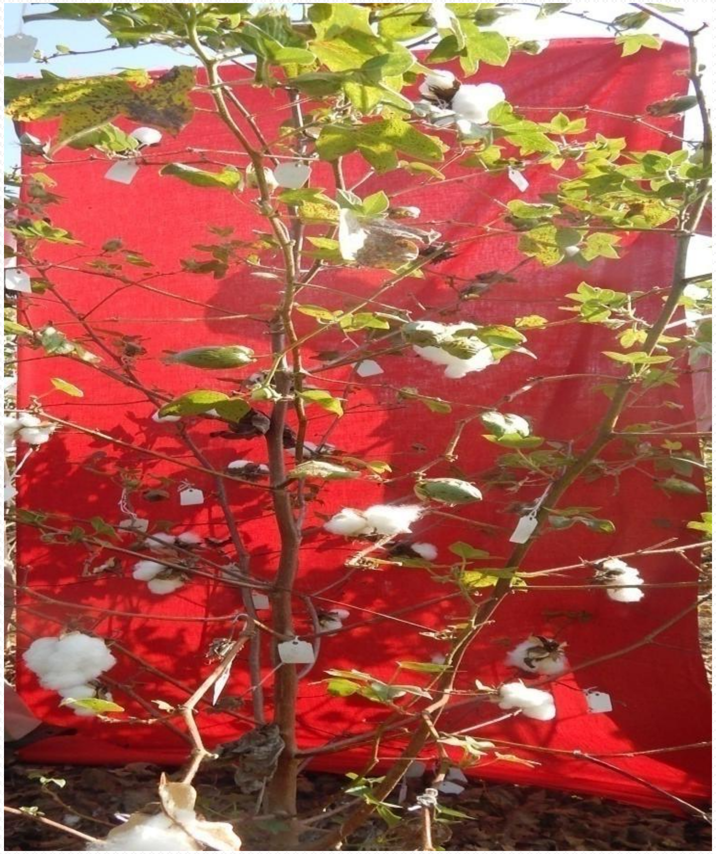
NCBI GenBank accession Nos.

S.No	Genes	Accession No
1	GhSusA1	KJ777133
2	GhCesA7	KJ777132
3	Ghfla11	KM457622
4	Ghfla12:	KM457623

Transcriptome analysis

Fibre quality parameters of *G hirsutum* cotton genotypes used in sequencing

HBS144 **HBS187**



Sl. No.	Character	HBS-187	HBS-144
1	Fibre strength (g/tex)	20.00	28.00
2	Micronaire index (µg/inch)	3.7	3.5
3	Uniformity ratio (%)	82.2	83.6

PRIMARY DATA ANALYSIS OF *G. HIRSUTUM* COTTON GENOTYPES HBS144 AND HBS187

Genotype	**HBS144 (28 g/ tex)**	**HBS187 (20 g/ tex)**
Total number of raw reads	74636658 (74.6 million)	53390446 (53.4 million)
Total number of processed reads	73463896 (73.4 million)	52432768 (52.4 million)
Contigs Generated	123189	98095
Per cent read assembled	88.71	87.09
Read length	101bp	101bp
Minimum contigs length	200bp	200bp
Maximum contigs length	27135bp	22511bp
Average Contig Length	1352.3bp	1341.3bp

Total Unigenes available	**140057**			
No. of unigenes in each category for 144 and 187 samples	**Total**	**Up**	**Down**	**Neutral**
No. of unigenes expressed in both samples	107700	14828	13468	79404
No. of uni genes expressed only in 144 sample	11328	NA	NA	NA
No. of uni genes expressed only in 187 sample	6866	NA	NA	NA
No. of P-significant unigenes	4953	2920	2033	0
No. of Q-significant unigenes	489	366	123	0

TRANSCRIPT ANNOTATION IN G. *HIRSUTUM* COTTON GENOTYPES

Genotypes	**HBS 144 (28 g/ tex)**	**HBS 187 (20g/ tex)**
Total transcripts	123189	980095
Total transcripts (COG/ cluster)	63131	49482
Total annotated transcripts	51133	41310
Total unannotated transcripts	11998	8172

List of annotated and non annotated genes derived from transcriptome analysis

Sl. No.	Gene id	Forward primer (5’-3’)	Reverse primer(3’-5’)
1	42183	GGGTGGTGGATAATCTGTTGT	GTGAAGCTGAGGAAATGTTTGG
2	55398	CCAAGTCCCGTTTCTGGTATT	TCTTGACTGGGCTCTTCTCT
3	43908	TATGGAACAAGGGATGTCGATG	GCTGGAGACAAACTGGAACT
4	32671	GGATCACACCTGTTGGTCATTA	GTGCTGTCAAGCTCTCTCTATG
5	55808	GGACAAGGCTCAGGCTATAATG	ACGGCAAGTCCATGATTCTAC
6	41929	GATAGGGATAGGGCGAGAGAA	GGAGCGTTTGCCATCAAATC
7	60537	GCTCTTCTTCCGGCTCTAATCGC	GCTAAGCACTTTGGCACATAC
8	24332	TGGGTTCGCCTTTGACTATC	CTTTGATGTCTGCCCTTCTCT
9	47473	GGCCATTCTGATGCAAAGATAC	GGCCCACAACCTAAACCTATT
10	12657	CCATGCTCTAGTCTCACTTCTTT	GCACTTCACTCCCACGTATT
11	59335	GACTTTGGCTTCGGTCTTATACT	CCCTGGTCCCTCATATCATTTC
12	27890	TGTCATTCGGGAGCAGAAAC	GAGCGACCCATCTGACATTATT
13	1272	GGGAAACTTAAGCTGGTAGACA	GGTGAAGTTGGAGATTCCTGAT
14	53503p	GGCCAATTCAATACCAAGCAATA	GACGGAATGTCACCTCCTATTT
15	32670	GGATCACACCTGTTGGTCATTA	GTGCTGTCAAGCTCTCTCTATG
16	52736	CAGGTCATCCTCTGAAGCTAAGG	CCTACTGTCAACCCAATCT
17	44381	CCGTGATTTCCTACGTCTACTG	TCCTCCTTACGCGTGATTTG
18	27890	TGTCATTCGGGAGCAGAAAC	GAGCGACCCATCTGACATTATT
19	1272	GGGAAACTTAAGCTGGTAGACA	GGTGAAGTTGGAGATTCCTGAT
20	53503	GGCCAATTCAATACCAAGCAATA	GACGGAATGTCACCTCCTATTT
21	32670	GGATCACACCTGTTGGTCATTA	GTGCTGTCAAGCTCTCTCTATG
22	52736	CAGGTCATCCTCTGAAGCTAAG	GCCTACTGTCAACCCAATCT
23	44381	CCGTGATTTCCTACGTCTACTG	TCCTCCTTACGCGTGATTTG
24	36742	CCATTTATGCTTGTGCTCCTTG	TTGGTGGGTGCTTAGGTTTAG
2 5	28264	GGAAGTCCTTGGTGTCGATTAT	AAGGAGGTGCTGAAGAAGATG

Sl. No.	Gene id	Forward primer (5’-3’)	Reverse primer(3’-5’)
26	32589	GAGGCTCTGTCTGCATTTCT	CGATCTAAACTCTCCACCACTATC
27	41930	GATAGGGATAGGGCGAGAGAA	GGAGCGTTTGCCATCAAATC
28	47930	CCAATGTGGGTTCGGATTTATTG	CCTAGGGTTTCCTTGTCTGTTT
29	56045	TAAACGGCGGCCCAAATAA	GGGCCTGTGGACTATTGTAATC
30	46575	CTCTGCTTCTTCCCACTCTTTC	CGGTTACTGTGGTTATGGAGATT
31	37409	GTTACTACCTGGAGCGTCTTTC	CAGGGTAGACATGAAAGGAGTG
32	13434	ACCTTCGAACCTTTGACAGATAA	GAAAGCGGTGGAGATGATAGAG
33	28266	GGAAGTCCTTGGTGTCGAATTAT	AAGGAGGTGCTGAAGAAGATG
34	59335	GACTTTGGCTTCGGTCTTACT	CCCTGGTCCCTCATATCATTTC
35	40900	ATCCGTTGGGAATGGAGAAC	AACGACACGGTGGGTATTG
36	44553	AACCGGGTTTAGGACTGTATTT	CCTAATTTGCTCGGCCCTTA
37	59335	GACTTTGGCTTCGGTCTTACT	CCCTGGTCCCTCATATCATTTC
38	61265	CTGTGAATCGAGGGAGAAGATG	TCATTCCCGTGTGAAGTAAA
39	57982	GATGCAGTCGCCTCTTCTAAA	AACTTCGACACGGCCTATAATC
40	58405	GGGAGTCGTAAAGGACATCATC	CTCCTTGCGTGTCTTGAACT
41	23512	TGGCGAGGAGGATTGAATTAG	CAGTCGCTTAGTCCTGGTATG
42	123411	TGGCTCCAAGAAGGAACAAATA	CACCAACTCCACCGTAAACA
43	35652	ATGTAATTGCCTGACCCTACC	TCCTCCTCCTAGACGAAGTAAG
44	33381	GTGCTTGTGATCCTGAAATTGG	TCACCATAAGGGTCTAGGGAATA
45	25956	CGCTTTCTCCGATACTCCTATTC	GACACCAAAGGCTGCATAAAC
46	12656	TGTGAAGAGCTGAATGTGGTAA	TGGCGAAAGGTGTCTGTATC
47	25865	GGCTATAAAGCCTACGGATTCT	ACCACCTTCTCTTCGTCTATTAC
48	41605	GGAATGCCTCCTAGAGATCAATAC	CCTCCTGTTCAGTGCGTTTA
49	39730	ATCGAGTGGCTCATGAACTTT	CCTTTGCTGGTGGTTTCATTAC
50	53103	GGACTCATCAATGGCCCATAG	CTTGCCCTTCAACTGTCTCA

The list of genes validated along with the oligo’s used for analysis of *G. arboreum* cotton genotypes

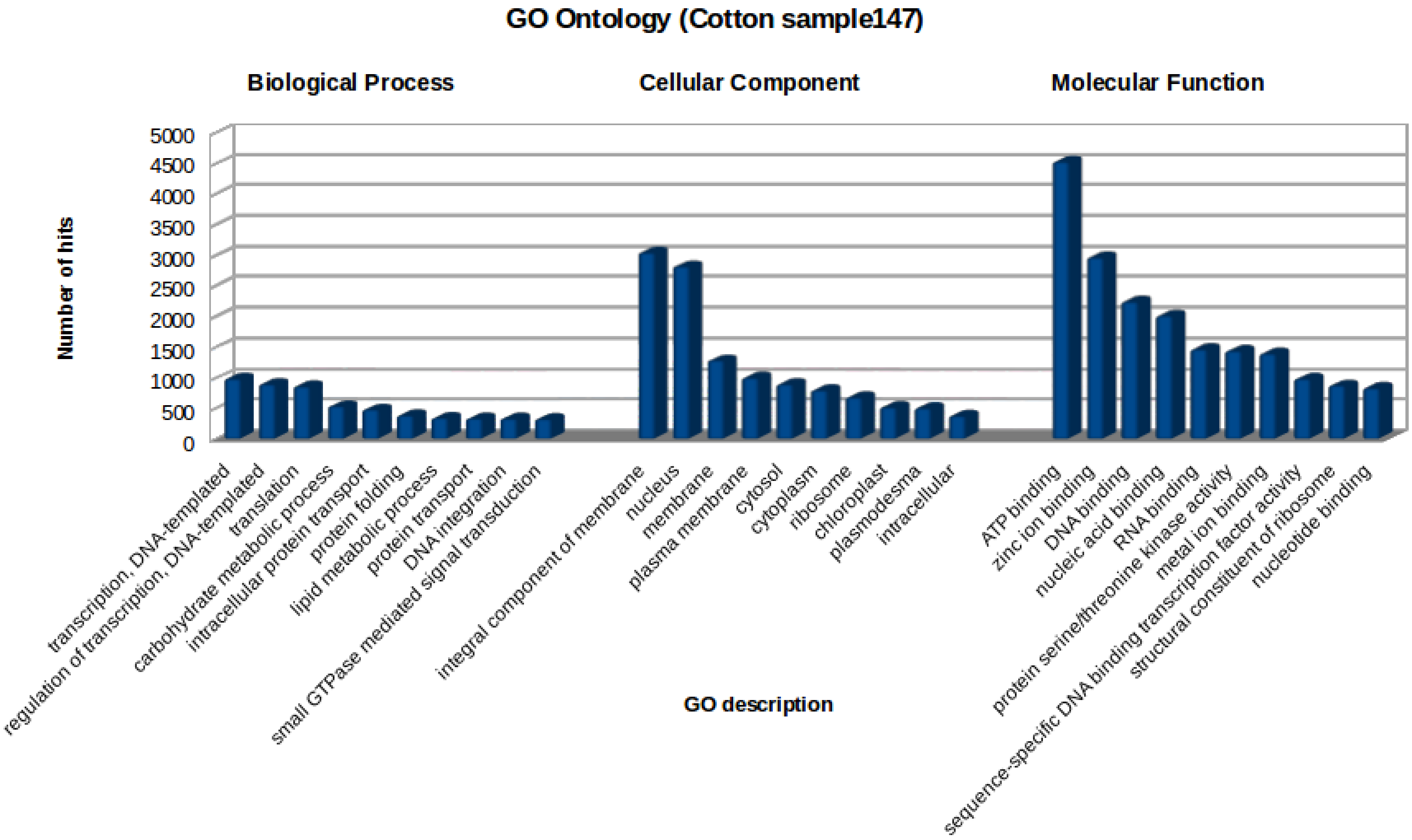
Gene	Forward primer (5'-3')	Reverse primer (5'-3')
CesA 8	TGGACTACCCGCTGTGGATAAGGT	CTCTTCTTGCAAAGTCGGCTGTT
1272	GGGAAACTTAAGCTGGTAGA	GGTGAAGTTGGAGATCCTGAT
32671	GGATCACACCTGTTGGTCATTA	GTGCTGTCAAGCTCTCTCTATG
47473	GGCCATTCTGATGCAAAGATAC	GGCCCACAACCTAAACCTATT
43908	TATGGAACAAGGGATGTCGATG	GCTGGAGACAAACTGGAACT
GhMT1	CTGACAAGTGTGGCAACTGCGAT	TGCTGATGTAGCTCTCCTCAGTC

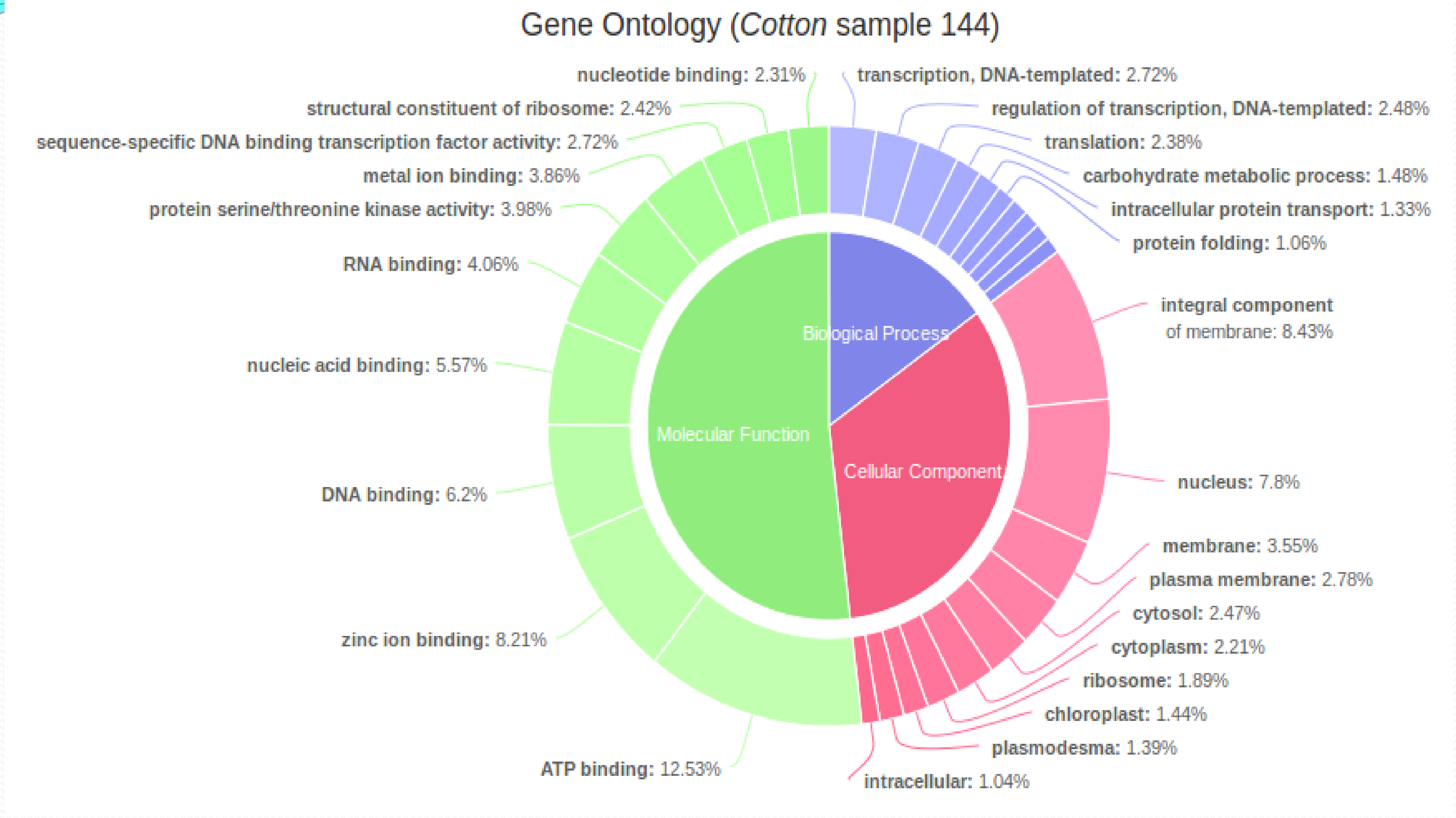
SSR Type	Set of Repeating Bases	Repetition number for the set	Example	Total Length
Mono nucleotide Repeats (p1)	1	>= 10 bases	AAAAAAAAAAAA	>=10 tp Any length
Di nucleotide Repeats (p2)	2	>= 6 Pairs	CACACACACACACA	>=12 to Any length
Tri nucleotide Repeats (p3)	3	>= 5 Sets	ATGATGATGATG	>=15 to Any length
Tetra nucleotide Repeats (p4)	4	>= 3 Sets	TGAGTGAGTGAG	>=20 to Any length
Penta nucleotide Repeats (p5)	5	>= 5 Sets	TGAGTGAGTGAGTGAGTGAG	>=25 to Any length
Hexa nucleotide Repeats (p6)	6	>= 5 Sets	CATATACATATACATATACATATACATATA	>=30 to Any length

SSR’sz	144	187
Total number of sequences examined:	123189	98095
Total size of examined sequences (bp):	166584897	131571739
Total number of identified SSRs:	29868	21680
Number of SSR containing sequences:	24965	18224
Number of sequences containing more than 1 SSR	4086	2882
Number of compound SSRs(i.e c)	2037	1586
p1	16350	11618
p2	4325	3019
p3	6309	4876
p4	385	275
p5	109	103
p6	353	203

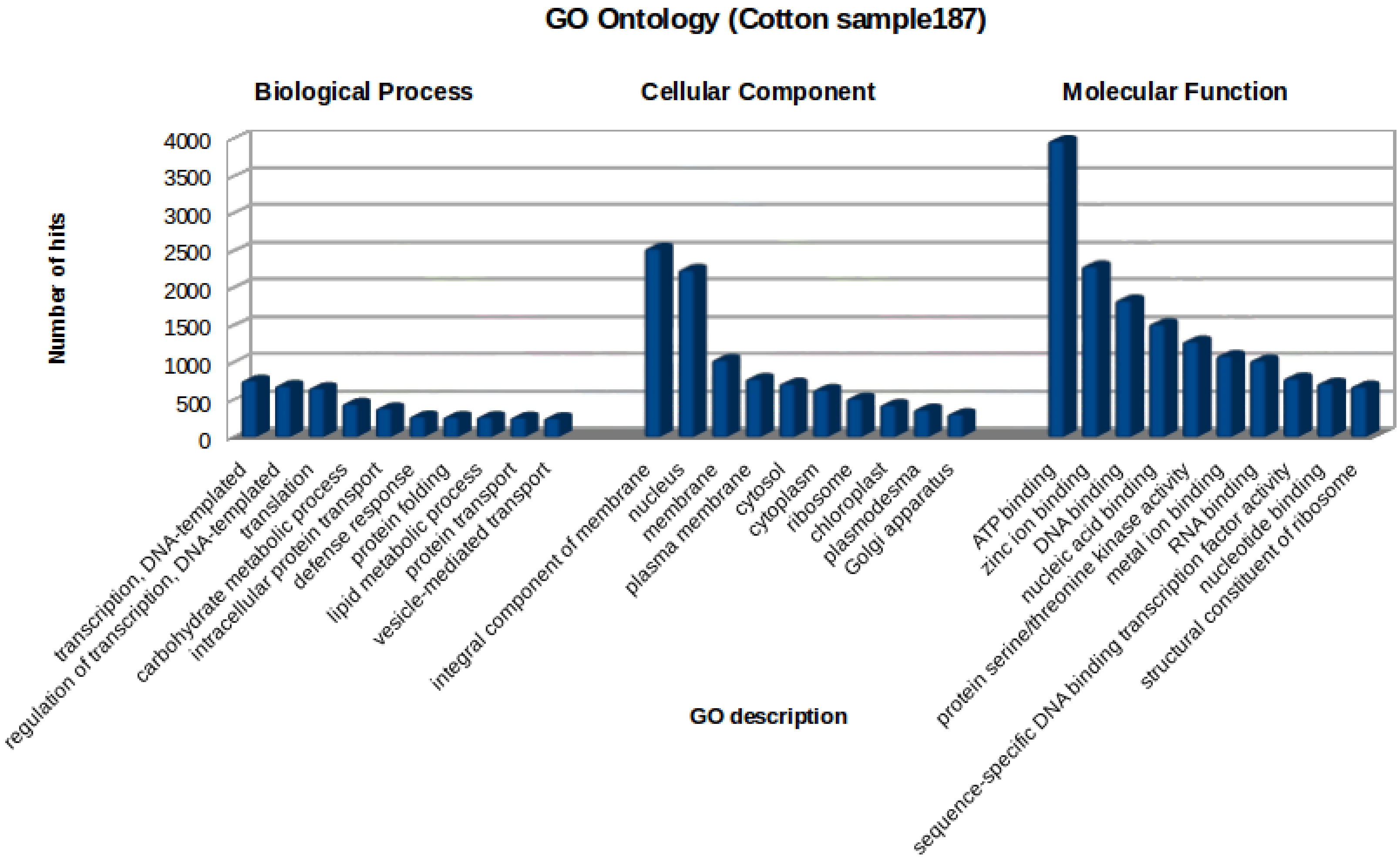
SINGLE NUCLEOTIDE POLYMORPHISM

SNP	HBS 144	HBS 187
Total number of variants	90857	74161
Total number of SNP’s	83120	68463
Total number of In-dels	7737	5698

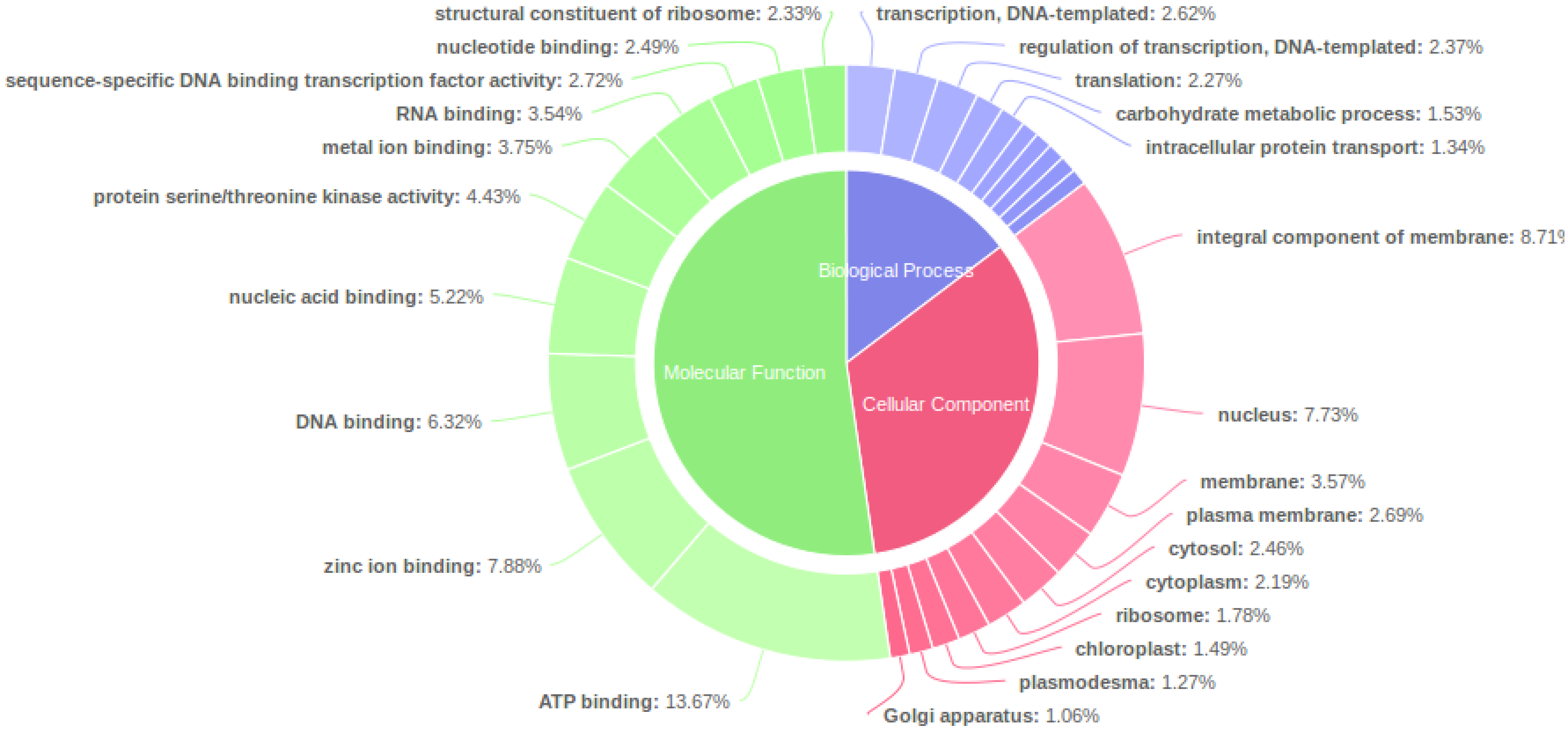

GO Ontology (Cotton sample147)
Biological Process
Cellular Component
Molecular Function
Number of hits
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
transcription, DNA-templated
regulation of transcription, DNA-templated
translation
carbohydrate metabolic process
intracellular protein transport
protein folding
lipid metabolic process
protein transport
DNA integration
small GTPase mediated signal transduction
integral component of membrane
nucleus
membrane
plasma membrane
cytosol
cytoplasm
ribosome
chloroplast
plasmodesma
intracellular
ATP binding
zinc ion binding
DNA binding
nucleic acid binding
RNA binding
protein serine/threonine kinase activity
metal ion binding
sequence-specific DNA binding transcription factor activity
structural constituent of ribosome
nucleotide binding
GO description



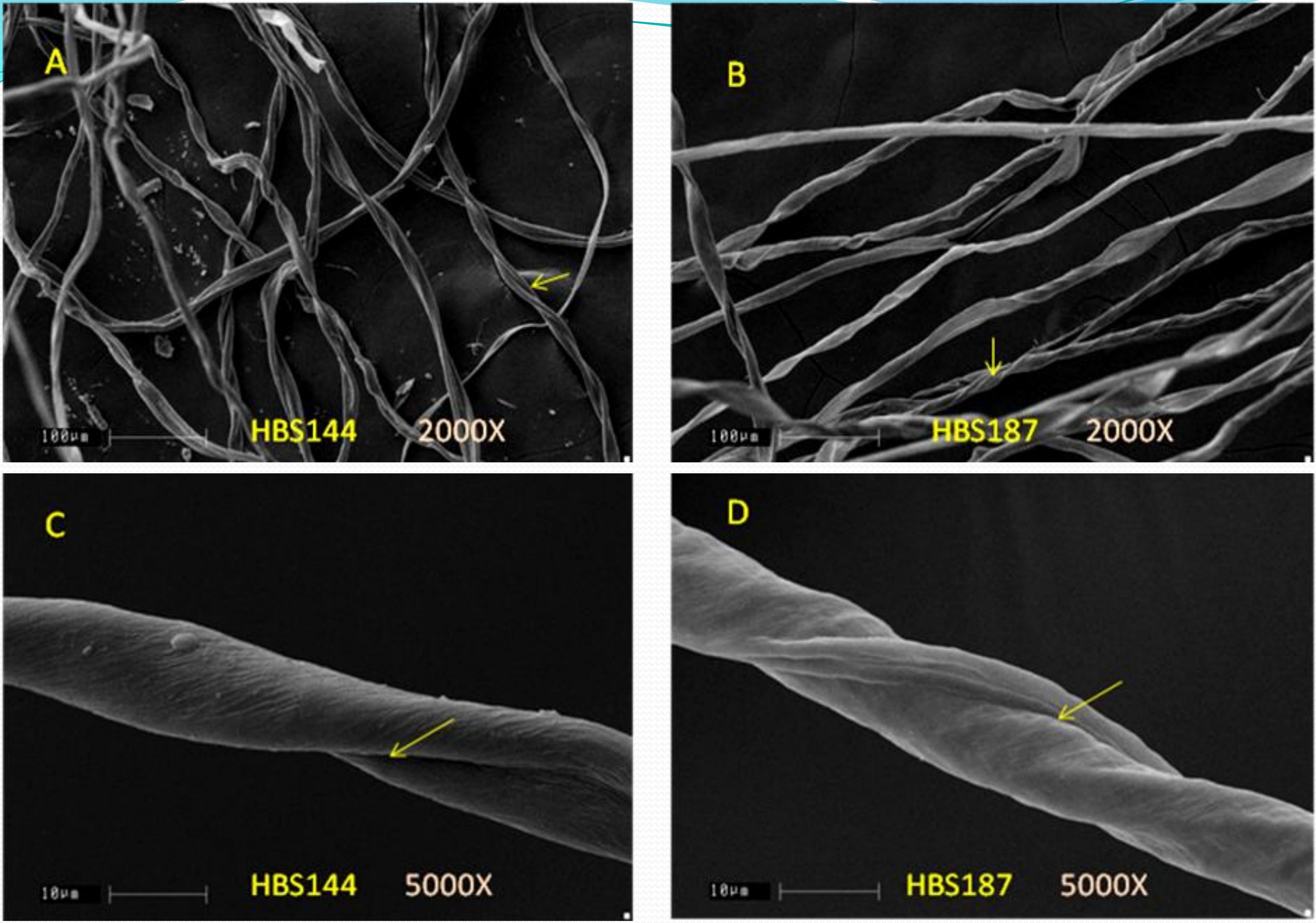
Gene Ontology (*Cotton* sample 144)


GO Ontology (Cotton sample187)
Biological Process
Cellular Component
Molecular Function
Number of hits
4000
3500
3000
2500
2000
1500
1000
500
0
transcription, DNA-templated
regulation of transcription, DNA-templated
translation
carbohydrate metabolic process
intracellular protein transport
defense response
protein folding
lipid metabolic process
protein transport
vesicle-mediated transport
integral component of membrane
nucleus
membrane
plasma membrane
cytosol
cytoplasm
ribosome
chloroplast
plasmodesma
Golgi apparatus
ATP binding
zinc ion binding
DNA binding
nucleic acid binding
protein serine/threonine kinase activity
metal ion binding
RNA binding
sequence-specific DNA binding transcription factor activity
nucleotide binding
structural constituent of ribosome
GO description

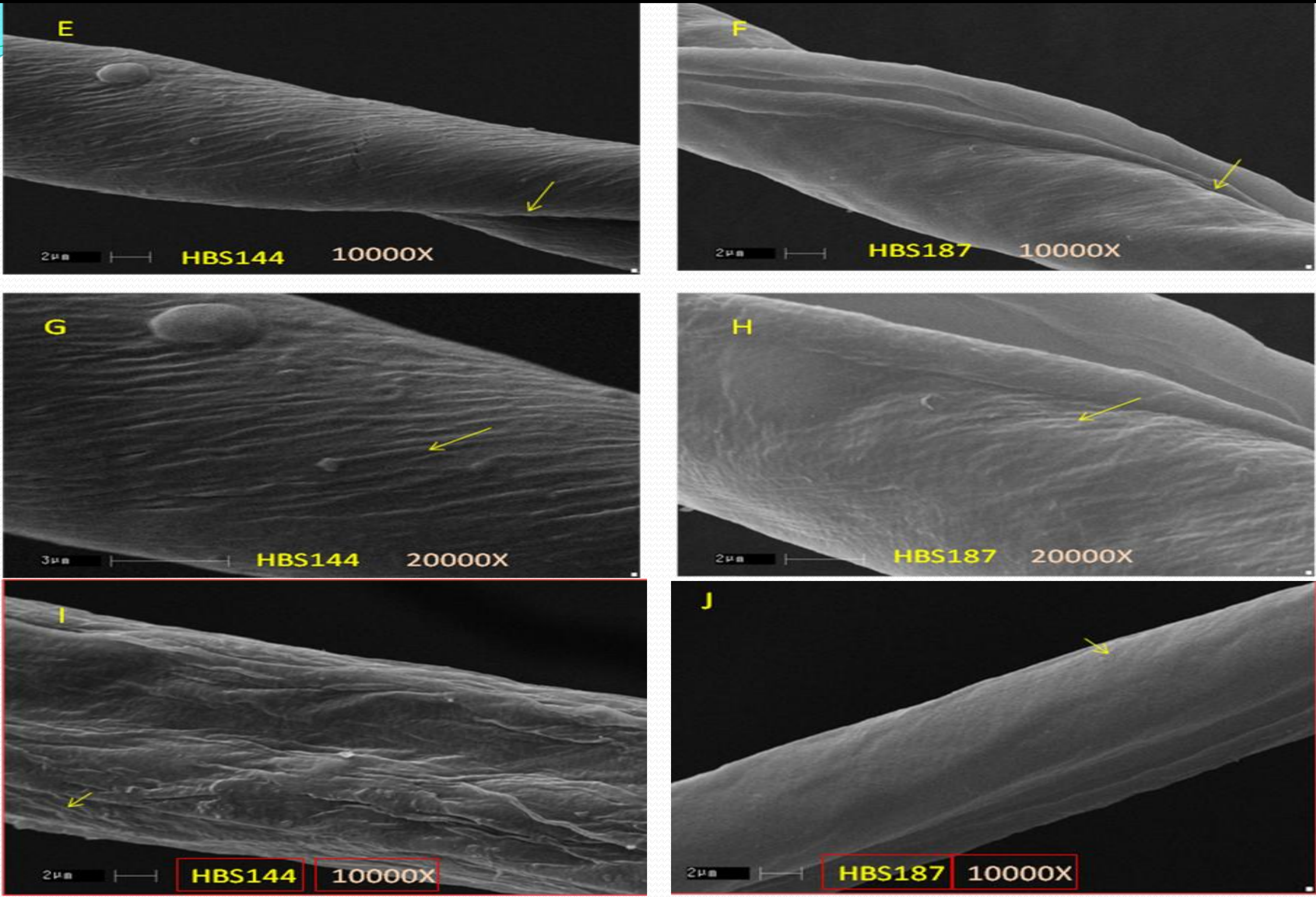
Gene Ontology (*Cotton* sample 187)



Images of Cotton fibre under Scanning Electron Microscope



Images of Cotton fibre under Scanning Electron Microscope



Thickness of the cotton fibre

Sl. No.	Genotype	Fibre Diameter (µm)	fibril Diameter (µm)
1	HBS 144	17	0.2
2	HBS 187	10	0.1

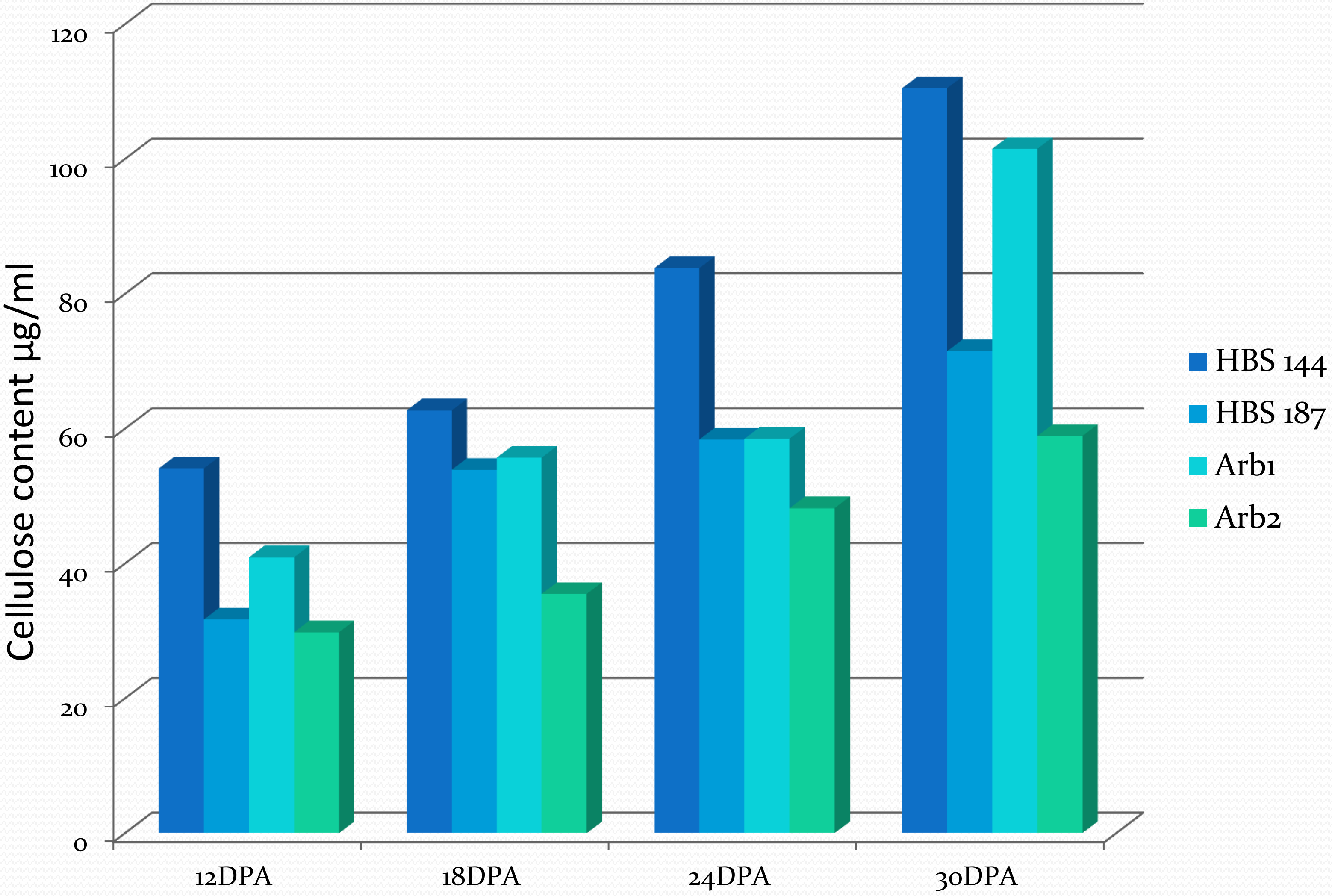
These fibres contain rough thick fibrillar coils and formed the folds on fibre surfaces and their inclination is related to the mechanism of cellulose deposition as spiral structures.

Cellulose Estimation: Updegroff DM (1969) protocol.

Sl. No.	Genotypes	Cellulose content (µg/ml)			
		12DPA	18DPA	24DPA	30DPA
1	HBS 144	54.1	62.7	83.8	110.5
2	HBS 187	31.7	53.9	58.4	71.5
3	Arb1	40.9	55.7	58.5	101.5
4	Arb2	29.8	35.5	48.2	58.9

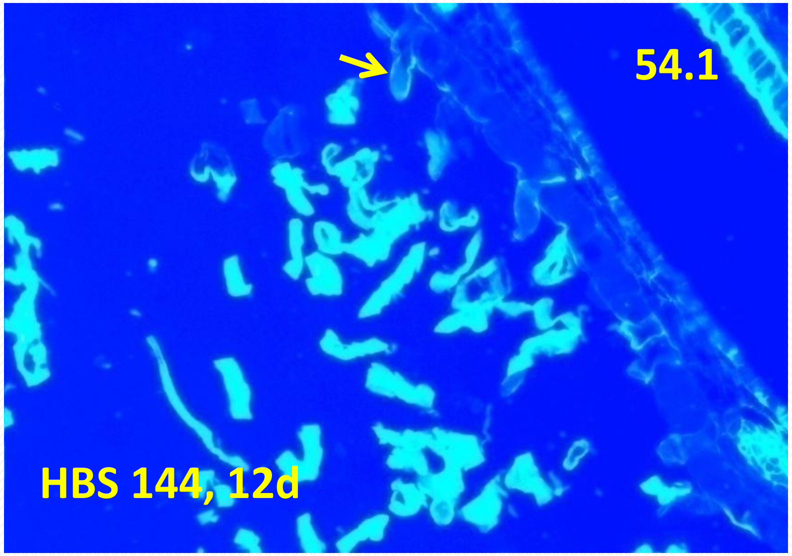
Eppendorf Biospectrometer (Kinetic)

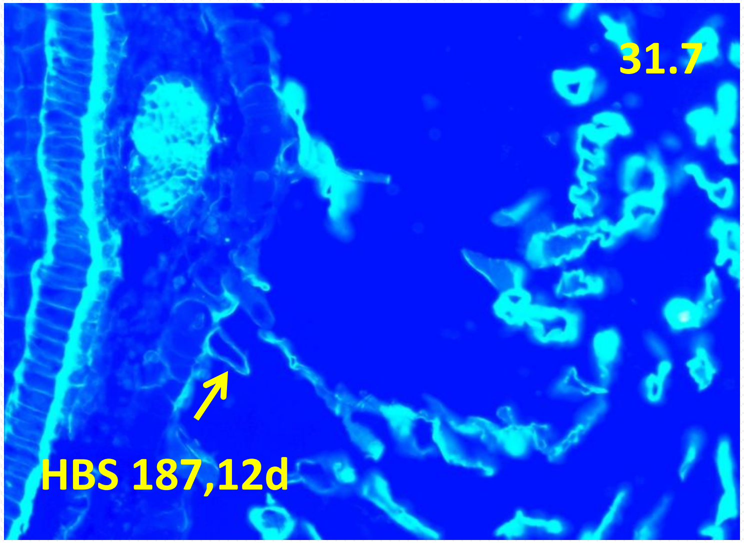
CELLULOSE CONTENT OF COTTON FIBERS

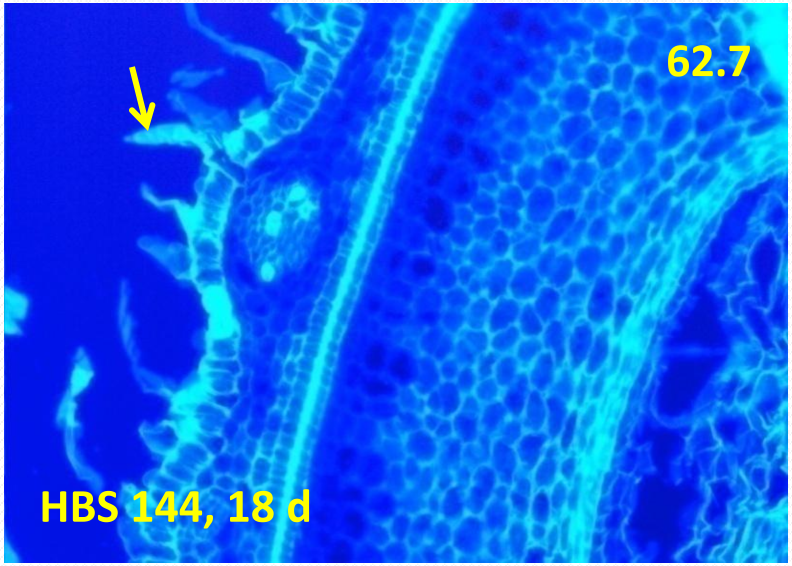


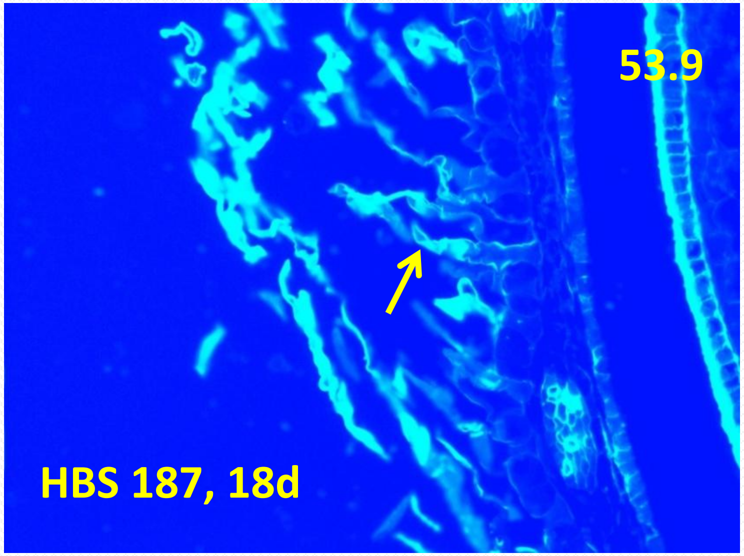
HBS 144:28,HBS 187:20,Arb 1:22.9,Arb 2:19.7

HBS 144 and HBS 187 - The seed sections stained with Calcofluor white stain

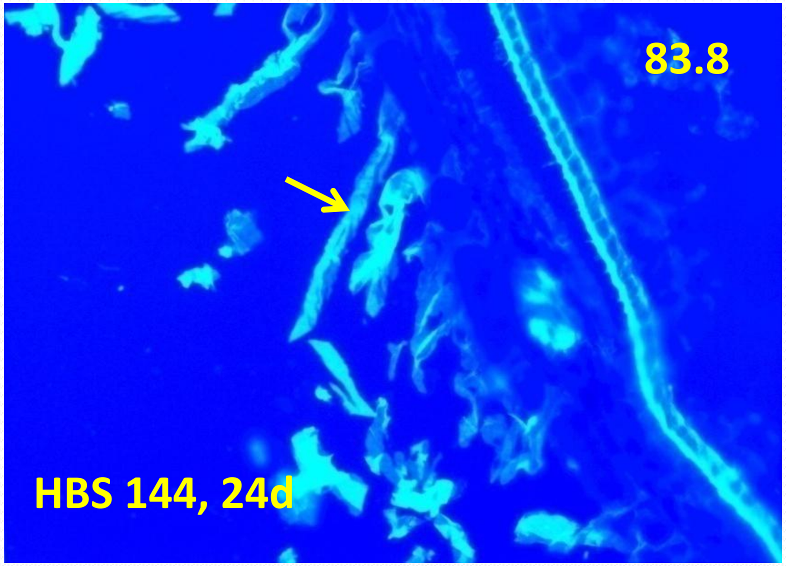


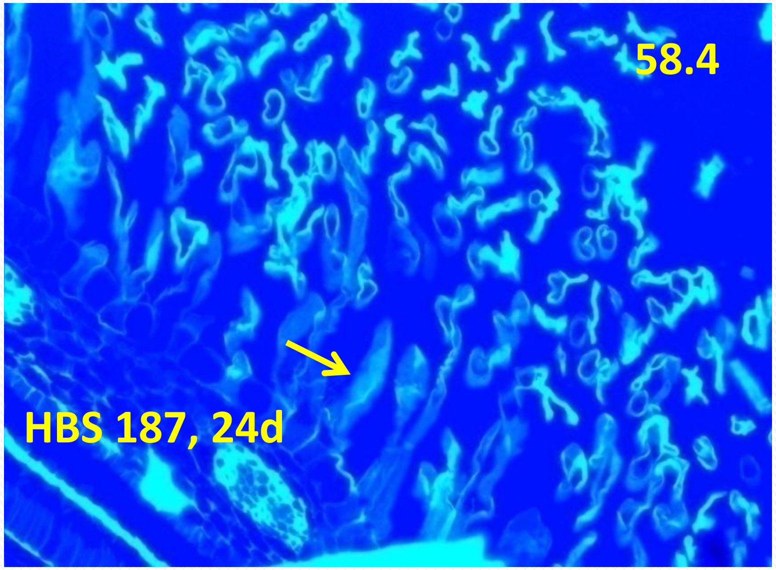


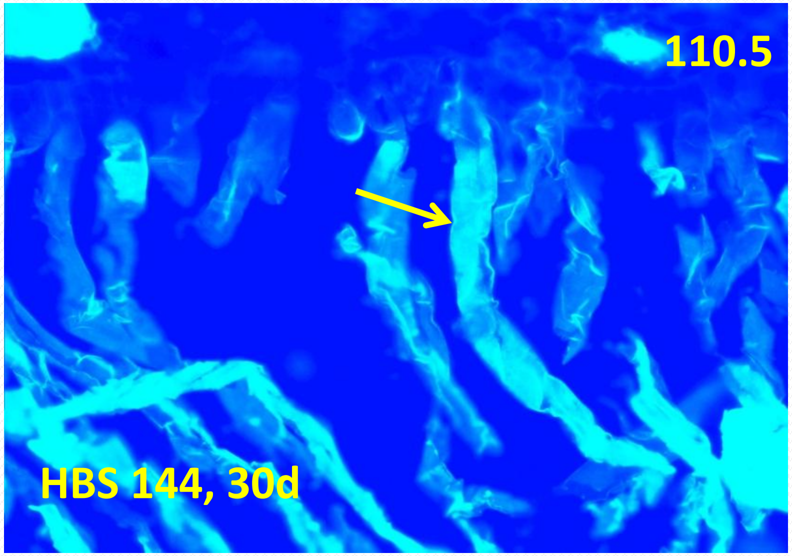


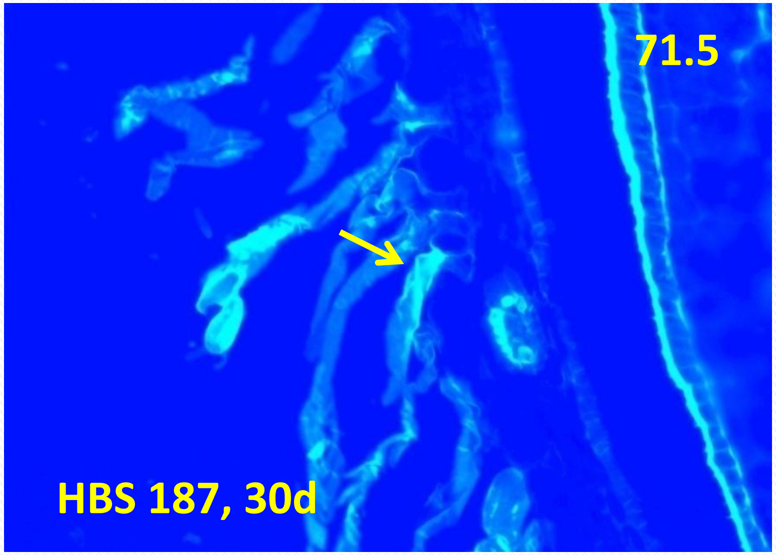


HBS 144 and HBS 187 - The seed sections stained with Calcofluor white stain

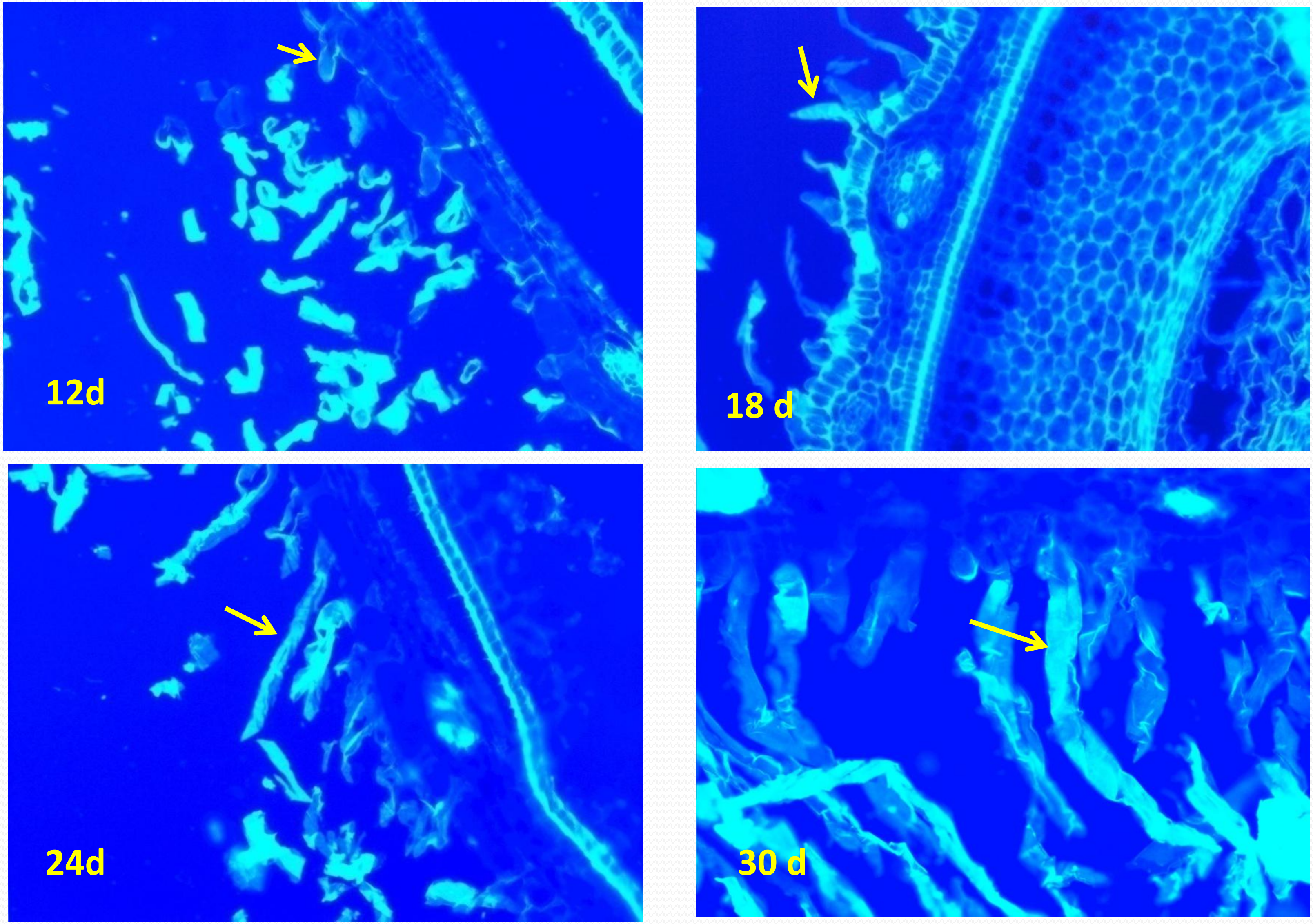




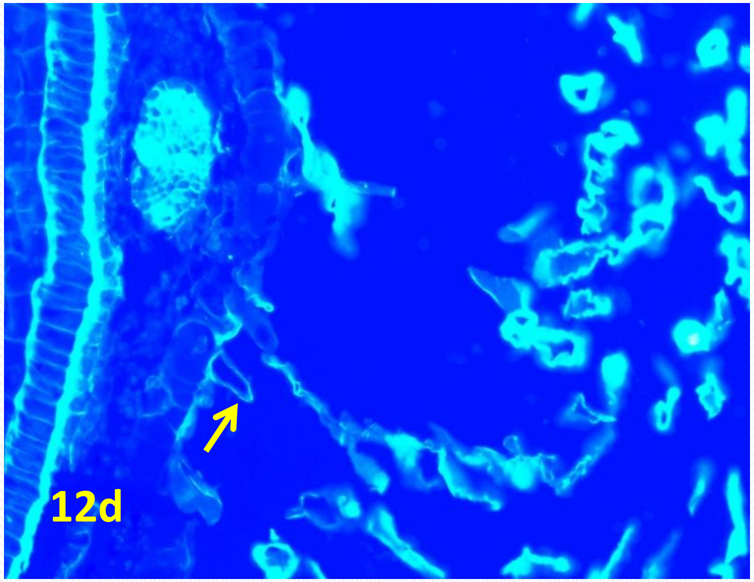


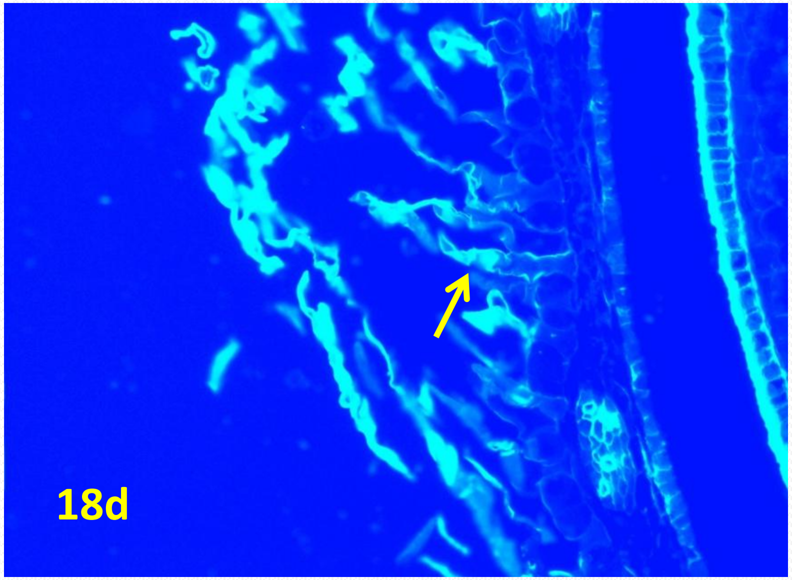


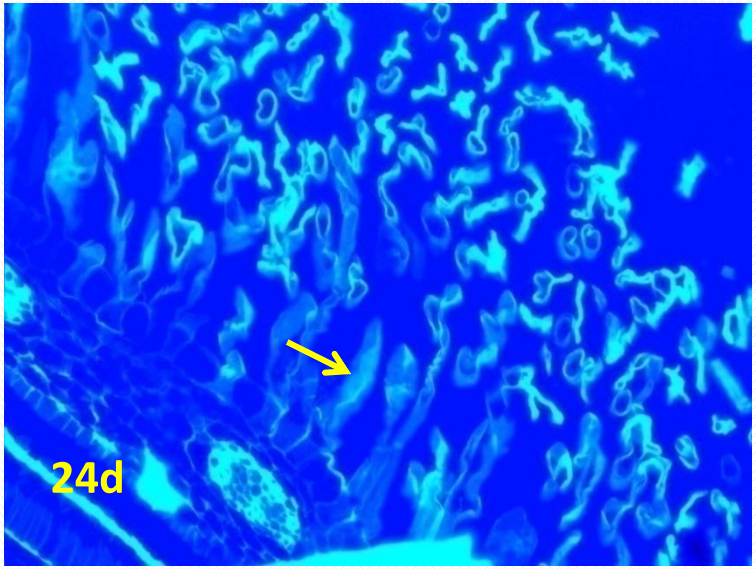
HBS 144- The seed sections stained with Calcofluor white stain

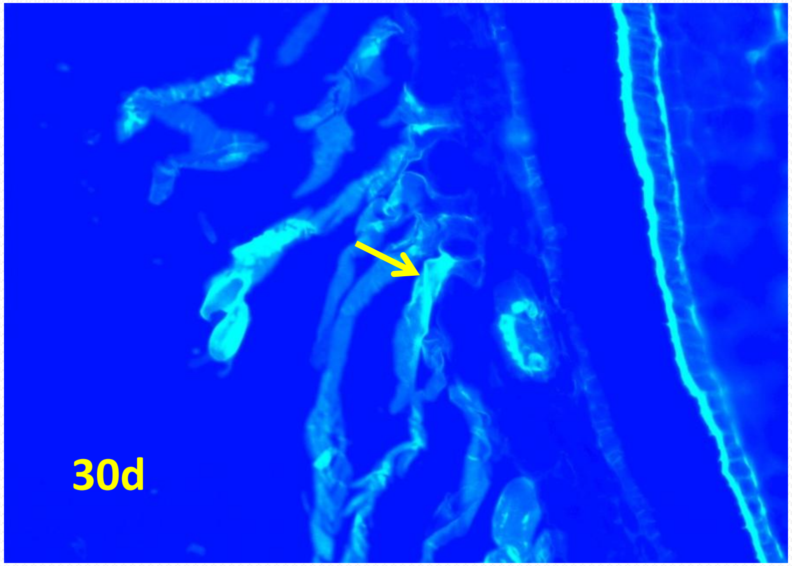


HBS 187- The seed sections stained with Calcofluor white stain

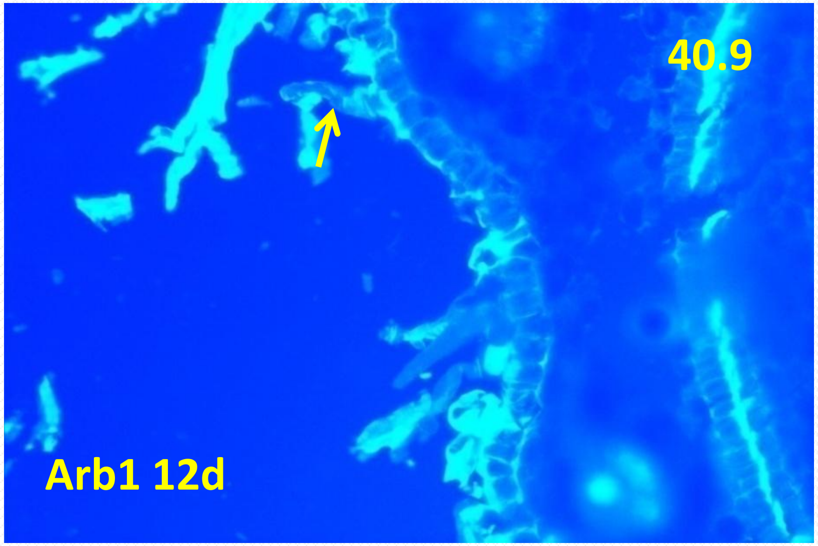

12d

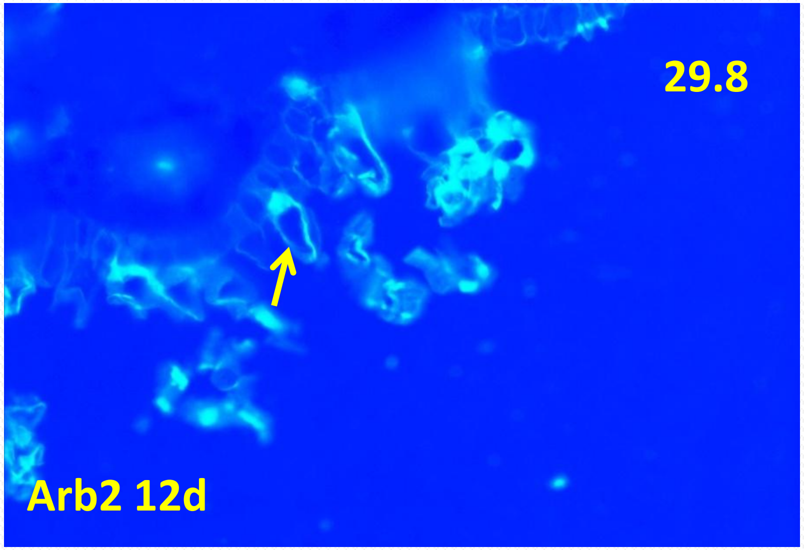

18d

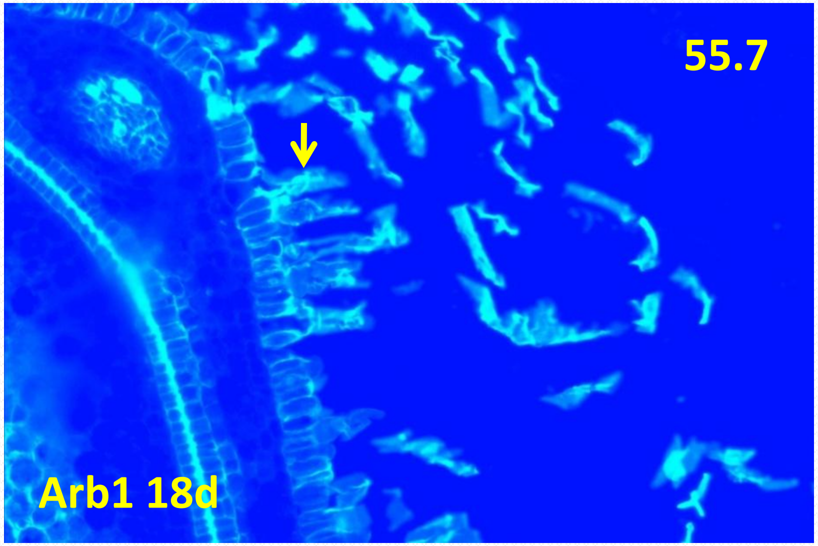

24d

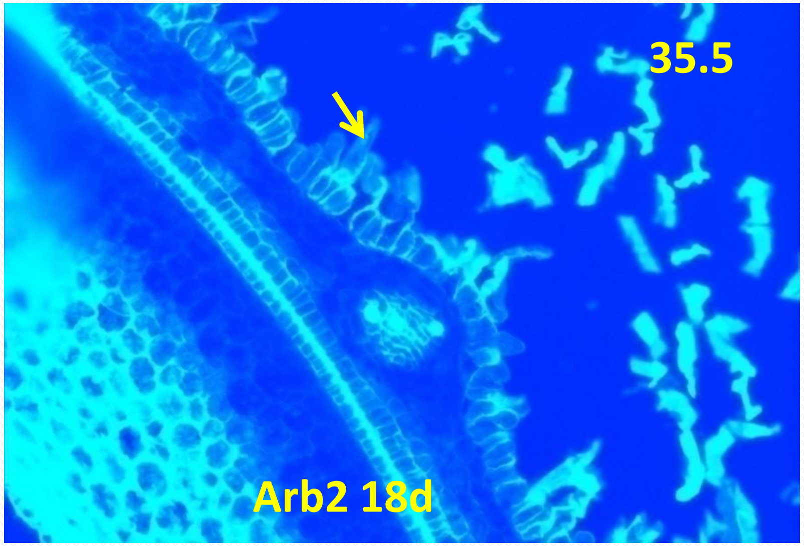

30d

Arb1 and Arb2 - The seed sections stained with Calcofluor white stain

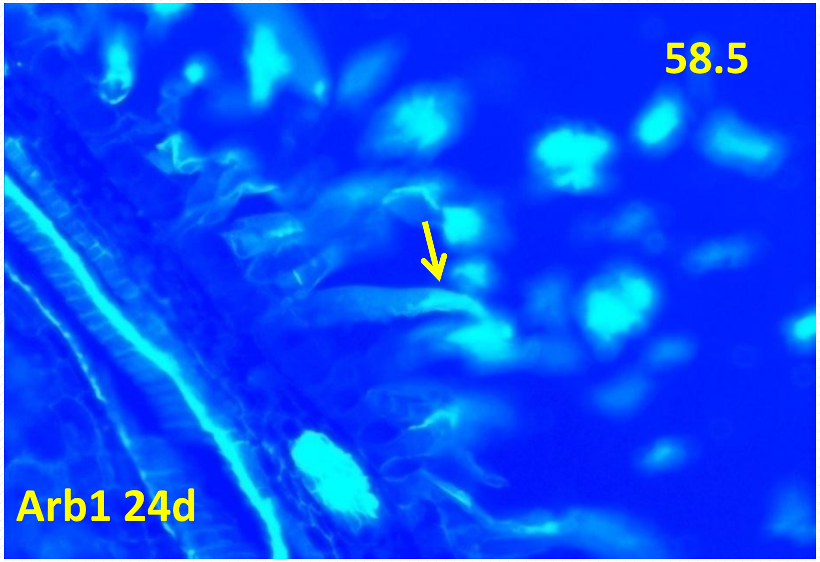


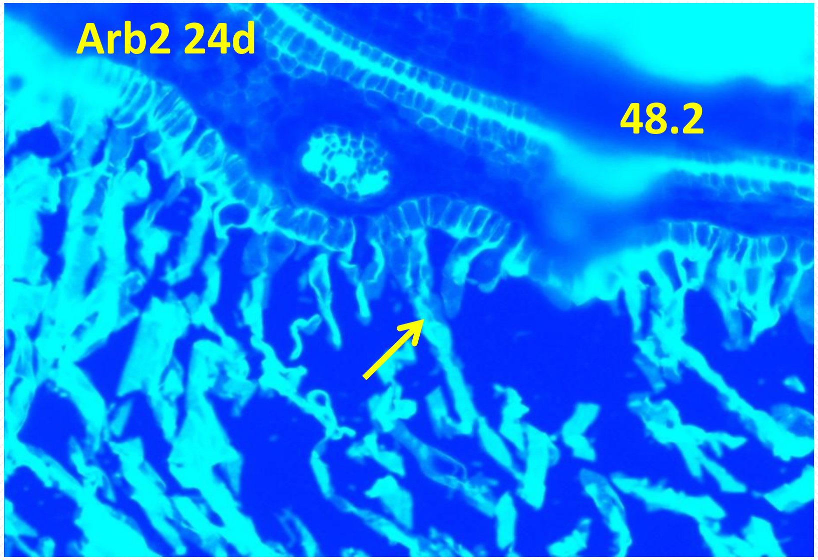


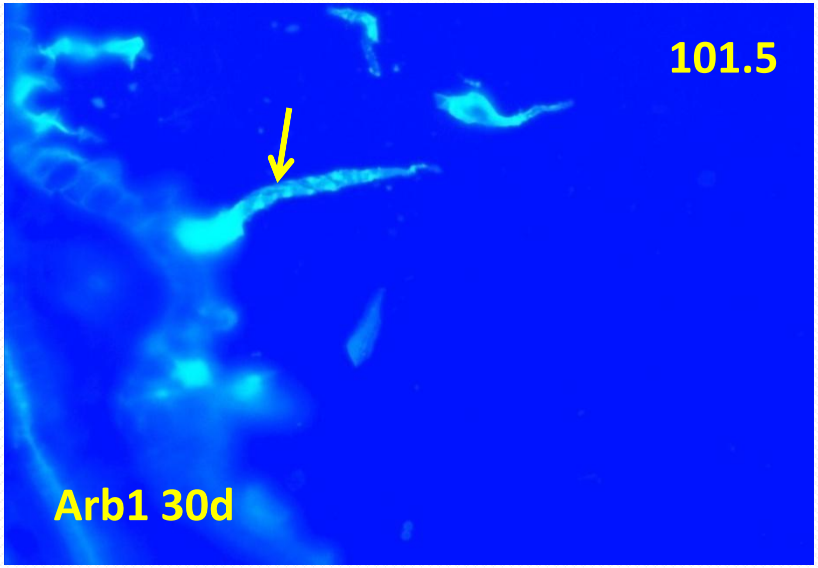


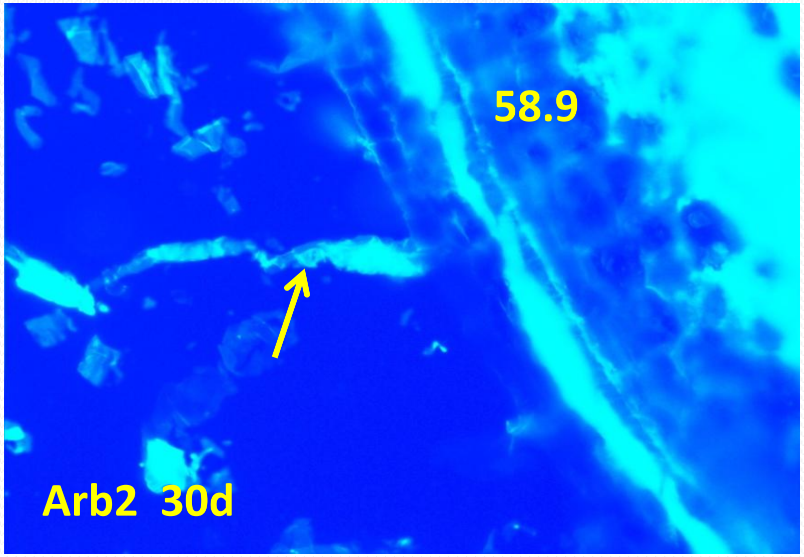


Arb1 and Arb2 - The seed sections stained with Calcofluor white stain

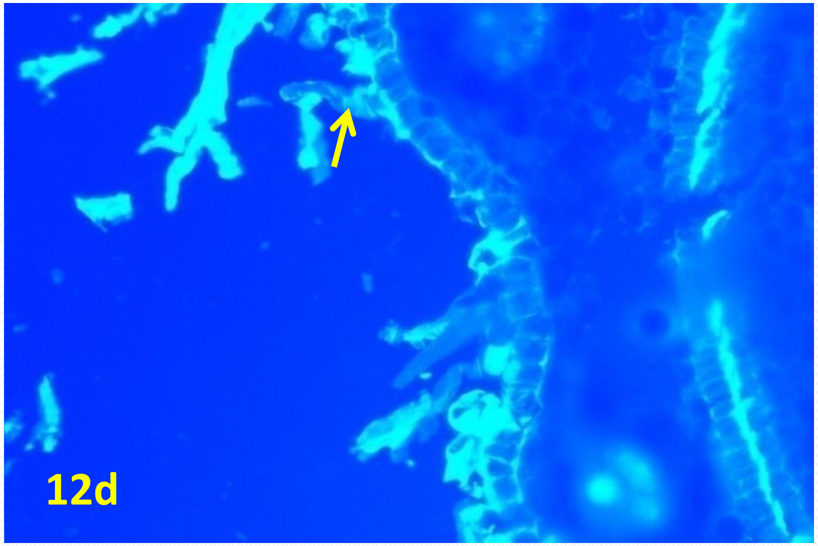


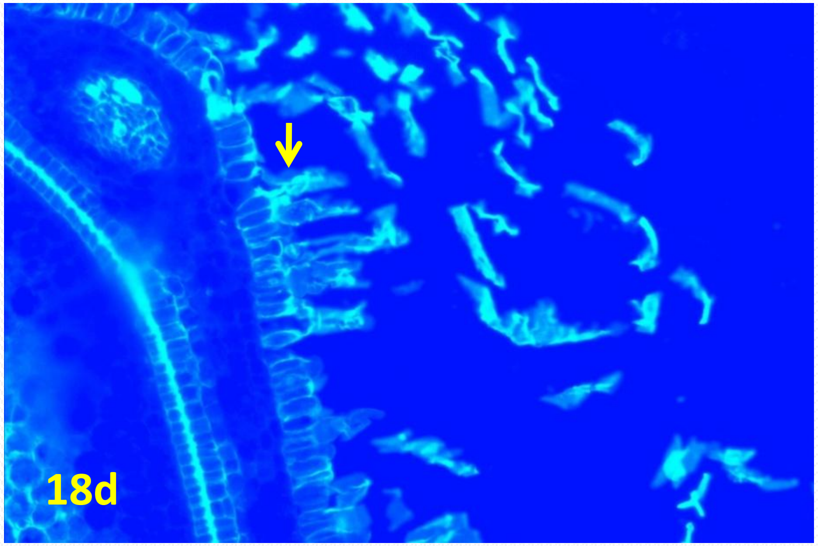


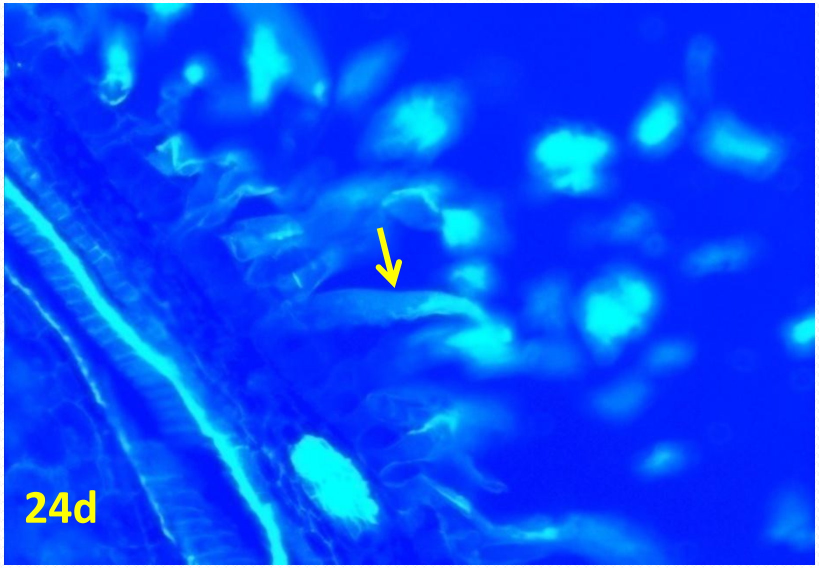


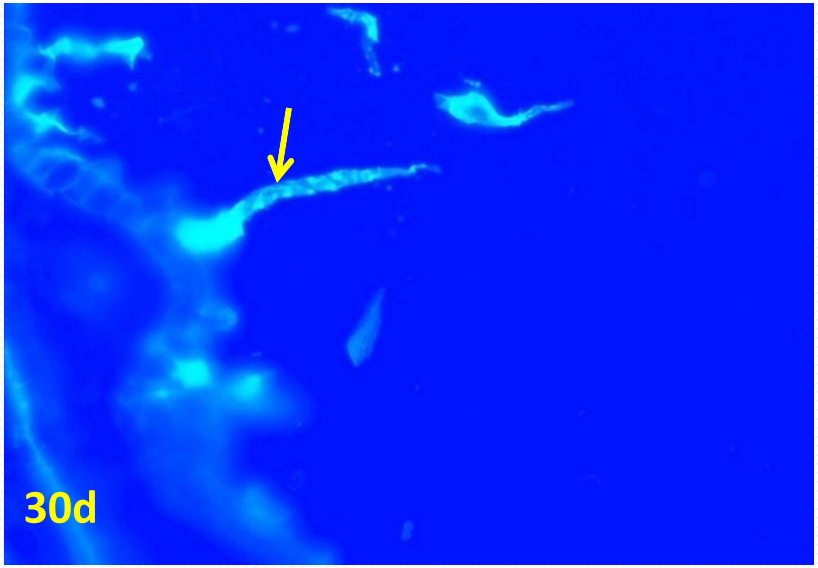


Arb1- The seed sections stained with Calcofluor white stain

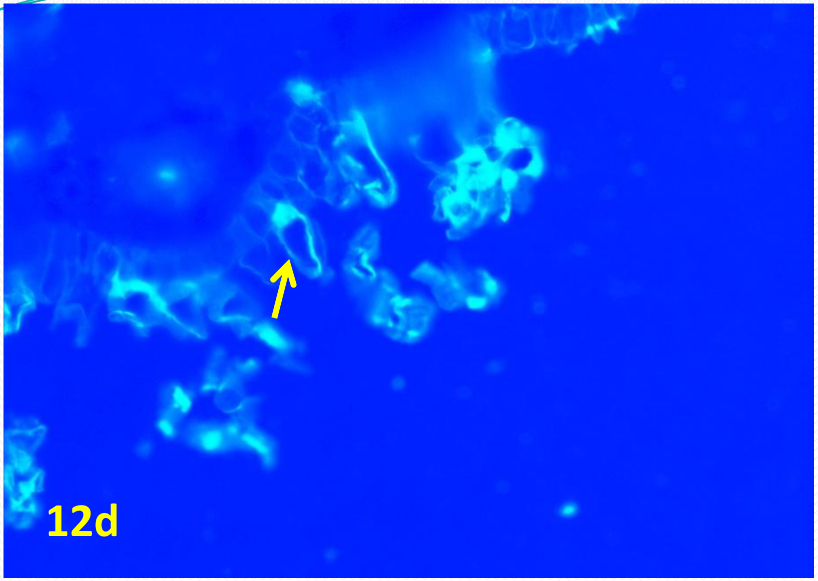


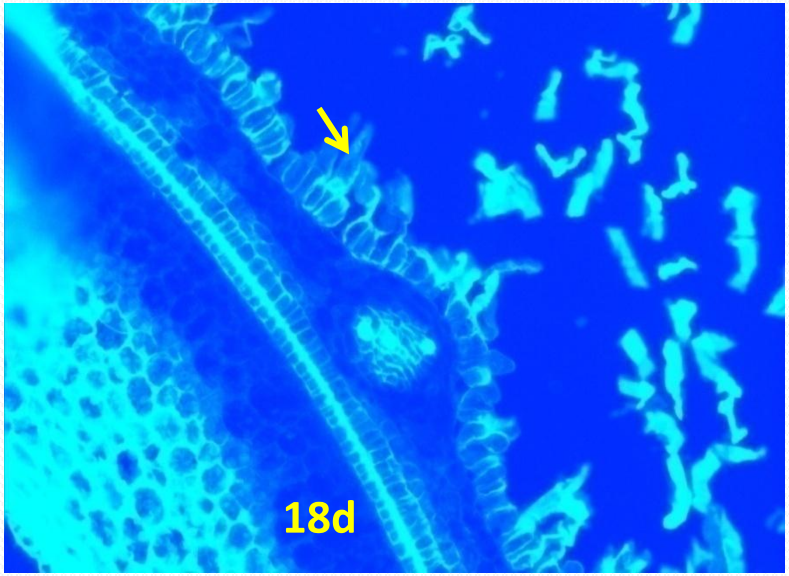


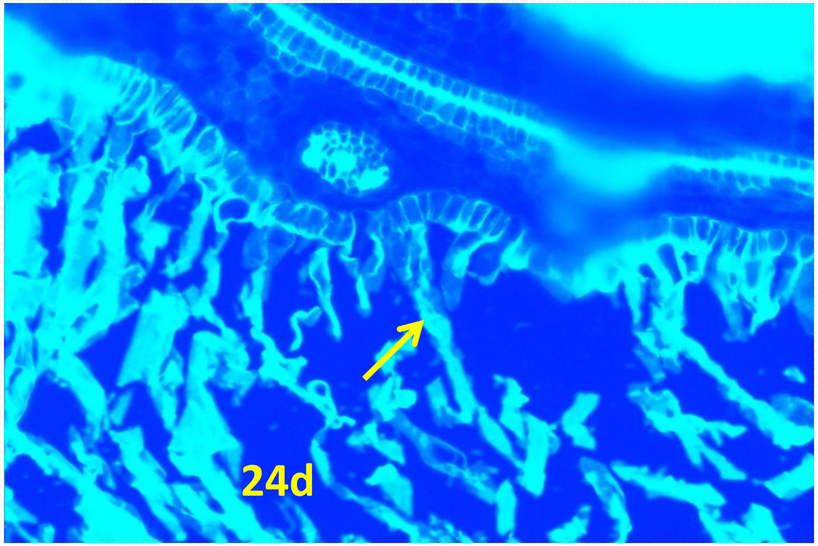


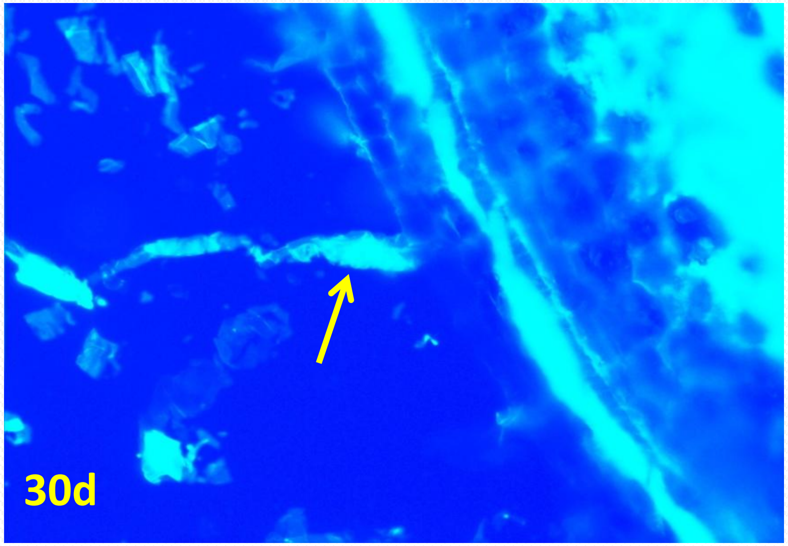


Arb2- The seed sections stained with Calcofluor white stain

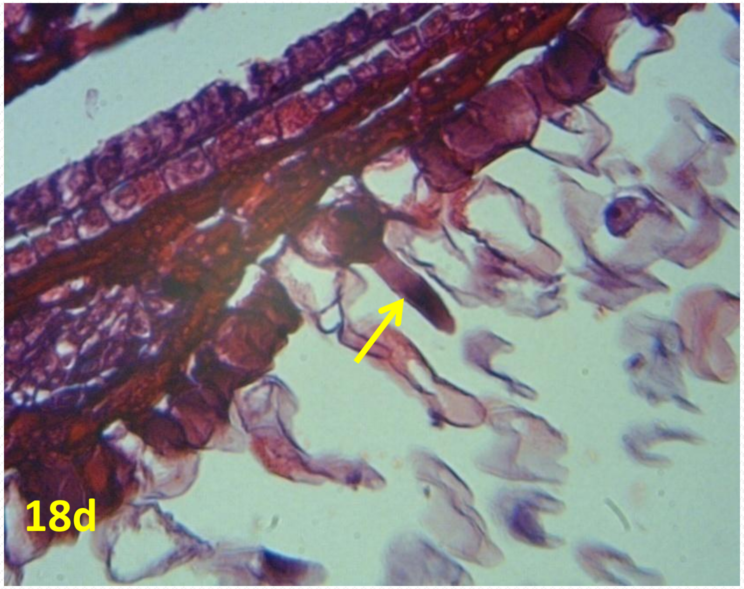


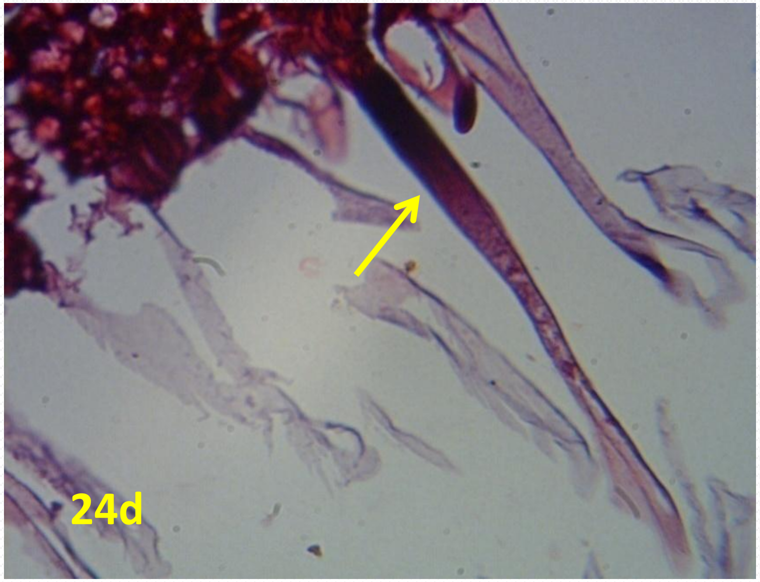


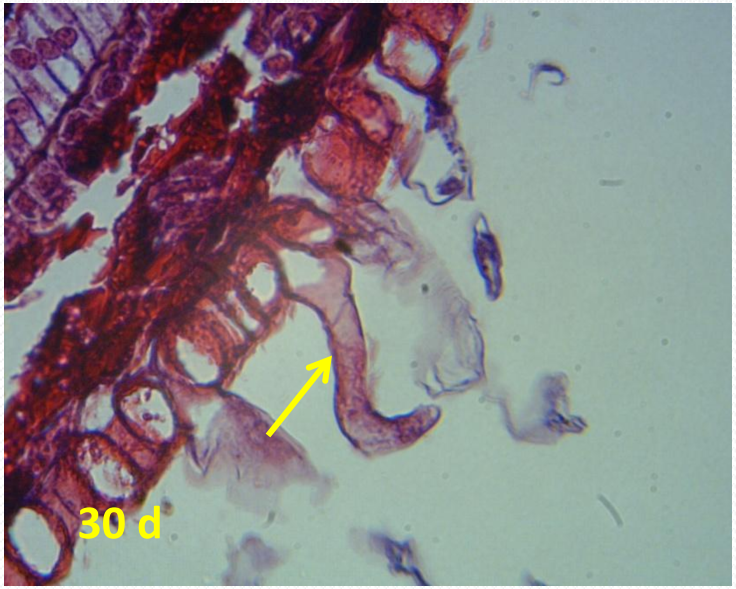




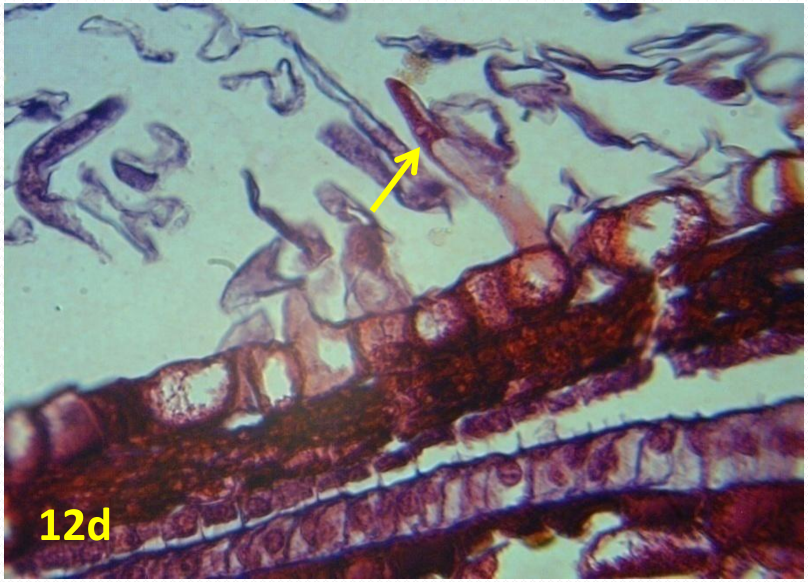
HBS 144- The seed sections stained with safranin

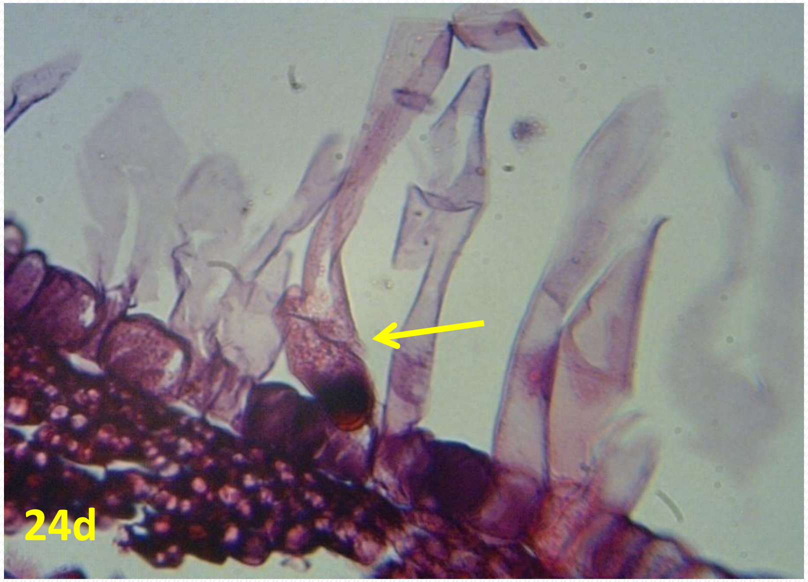




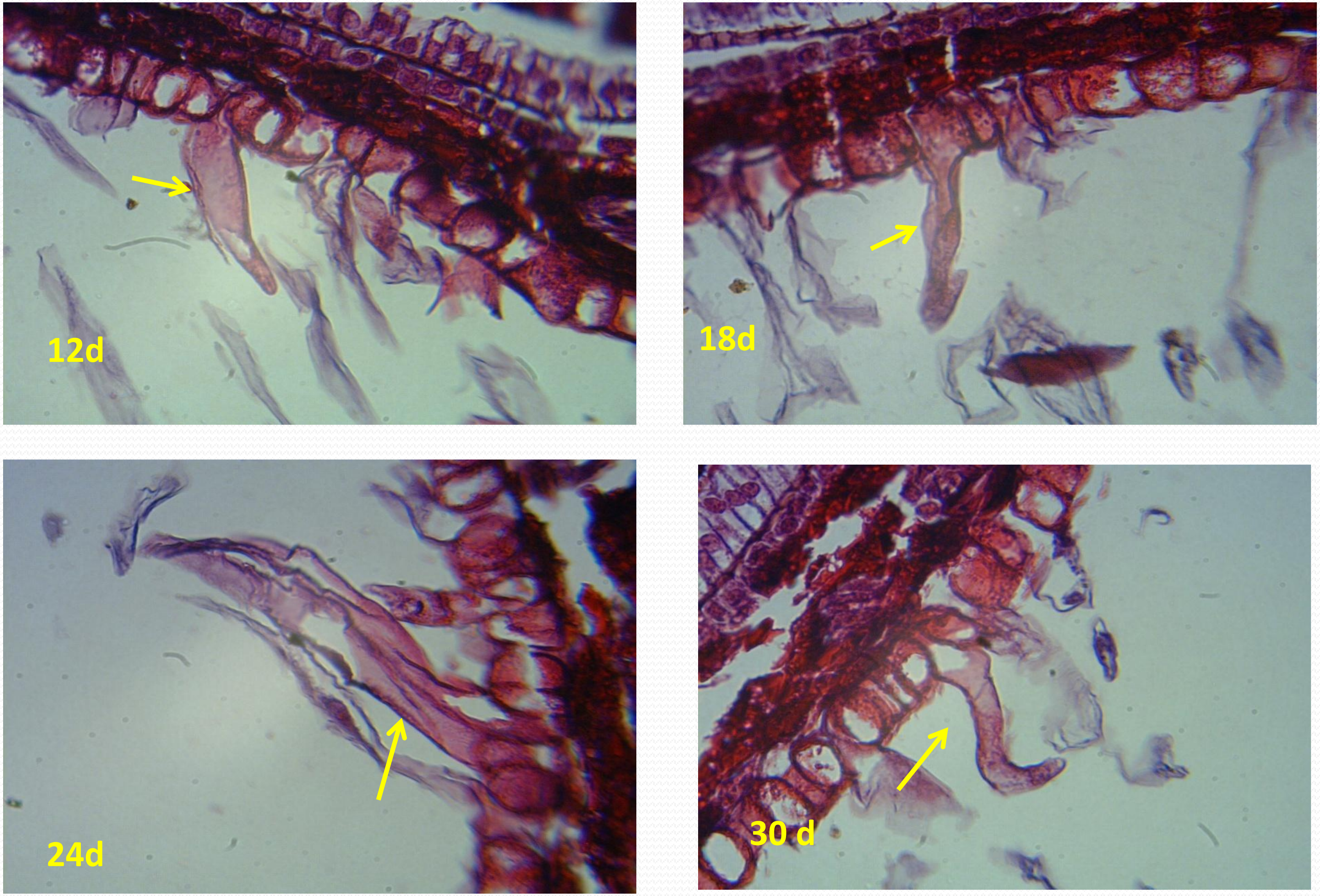


HBS 187- The seed sections stained with safranin

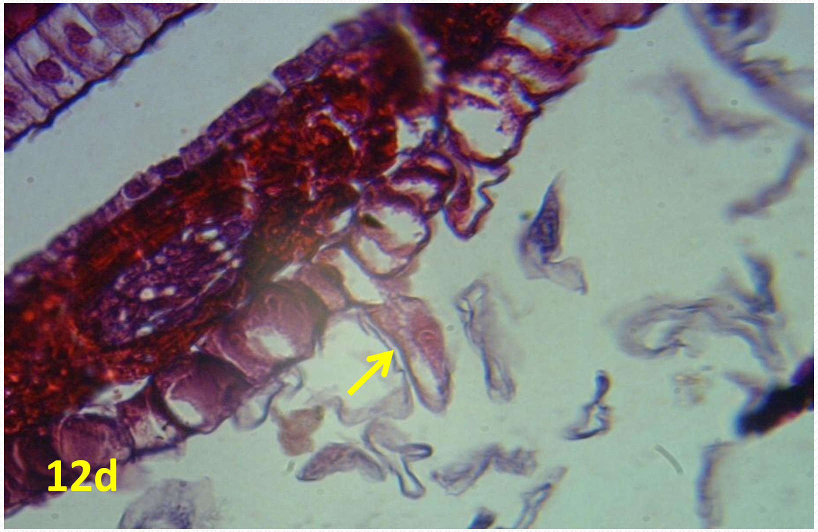


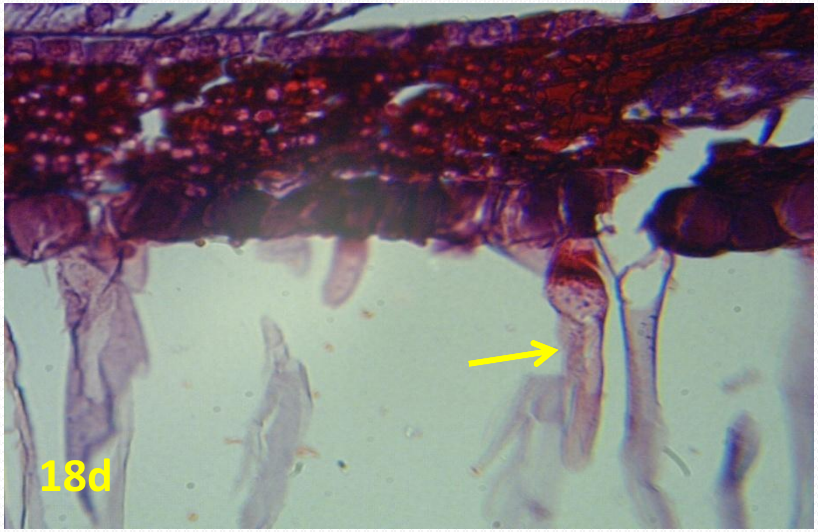


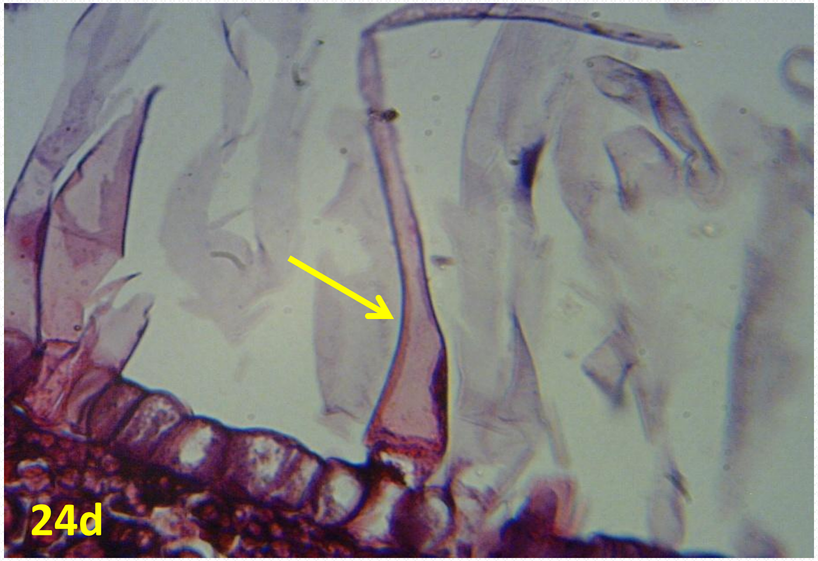
Arb1- The seed sections stained with Safranin

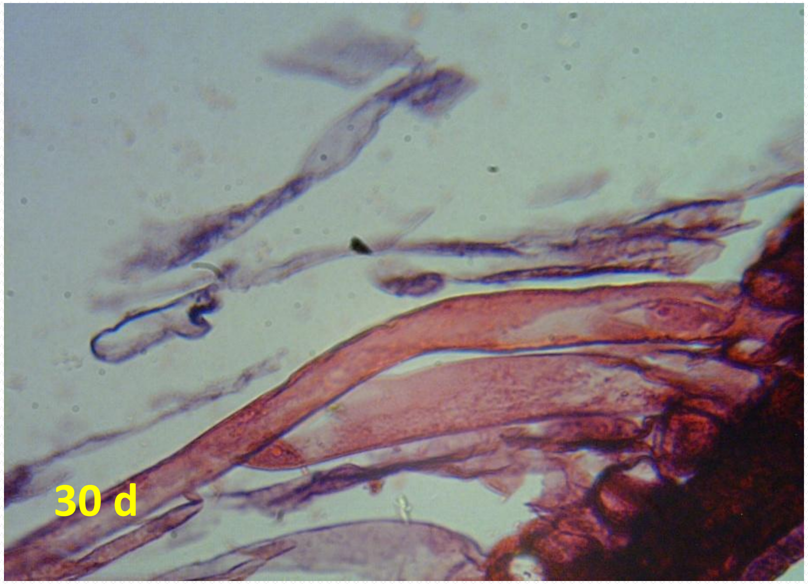


Arb2- The seed sections stained with Safranin









CONCLUSIONS AND FUTURE RECOMMENDATIONS

I.TRANSGENIC

- **Broad assessment of expression of the *Mt1, FLA,SusA1 and Ces A* genes**

- ***Ces A8* and *SusA1* genes have unique and mutual expression patterns-Genes may be involved in strengthening fiber (**Silencing of the genes**)**

- **Study on cloning, expression, and creation of transgenic plant -Role in fiber strength**

II.Marker Assisted Selection

- Design SSR primers from the cDNA library & employ them in MAS
- SNP variants
- QTL’s

The unigene expression in *G. hirsutum* cotton genotypes HBS144 and HBS187

Genotypes	HBS 144 (20 g/)	HBS 187 (28 g/)
No. of unigenes expressed	11328	6866
Up regulation of unigenes	14828	14828
Down regulation of unigenes	13468	13468
Number of P-significant unigenes	4956	4956
Number of P-significant for up regulation of unigenes	2033	2033
Number of Q-significant unigenes	489	489
Number of Q-significant for up regulation of unigenes	366	366
Number of Q-significant for down regulation of unigenes	123	123

Histology Procedure

Anatomical studies were carried out in seed fibers, following the procedures given by Khasim (2002) and Bhandari (1997) with some modifications.

1. Khasim S.M. (2002). Botanical microtechnique: Principles and practice. Capital Publishing Company, New Delhi, India.

2. Bhandari N.N. (1997). Staining techniques: a manual. Ultimate Printers, New Delhi, India

Staining

Calcofluor white (Fluka, Sigma-Aldrich, USA) is a non specific fluorochrom that binds to cellulose and chitin in cell walls. The excitation occurs at 355 nm.

Cotton seed section slides were observed under fluorescent microscope (Carl Zeiss, AxioImager.M2, Germany) by using DAPI reflector/filter (Range 340 – 400 nm).

The cellulose in the seed section fluoresce bluish white with blue back ground when we used DAPI filter (The florescence colour is depends on the type of filters which we are using).

Safranin- which indicates the brilliant red colour of fibres showing the lignin deposition (Khasim, 2002).

1.Khasim S.M. (2002). Botanical microtechnique: Principles and practice. Capital Publishing Company, New Delhi, India pp 73-74.

Plant Biotechnology Journal

aab Association of Applied Biologists

SEB Society for Experimental Biology

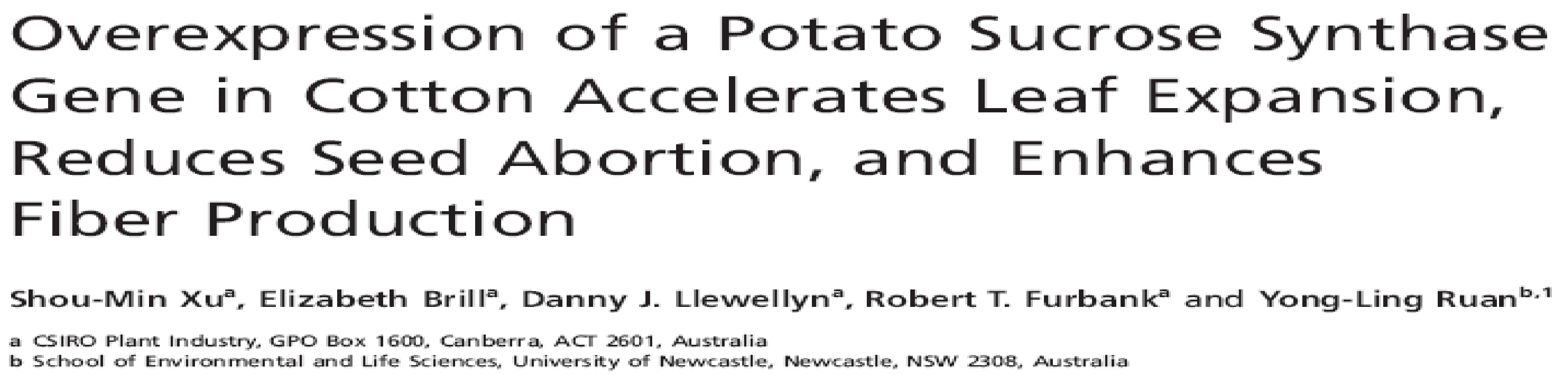
Plant Biotechnology Journal (2012) **10**, pp. 301–312

doi: 10.1111/j.1467-7652.2011.00662.x

Overexpression of *GhSusA1* increases plant biomass and improves cotton fiber yield and quality

Yanjie Jiang[1], Wangzhen Guo[1], Huayu Zhu[1], Yong-Ling Ruan[2,3] and Tianzhen Zhang[1,*]

[1]*National Key Laboratory of Crop Genetics and Germplasm Enhancement, Cotton Research Institute, Nanjing Agricultural University, Nanjing, China*
[2]*School of Environmental and Life Sciences, the University of Newcastle, Callaghan, NSW, Australia*
[3]*Australia-China Research Centre for Crop Improvement, the University of Newcastle, Callaghan, NSW, Australia*



Overexpression of a Potato Sucrose Synthase Gene in Cotton Accelerates Leaf Expansion, Reduces Seed Abortion, and Enhances Fiber Production

Shou-Min Xu[a], Elizabeth Brill[a], Danny J. Llewellyn[a], Robert T. Furbank[a] and Yong-Ling Ruan[b,1]

a CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601, Australia
b School of Environmental and Life Sciences, University of Newcastle, Newcastle, NSW 2308, Australia

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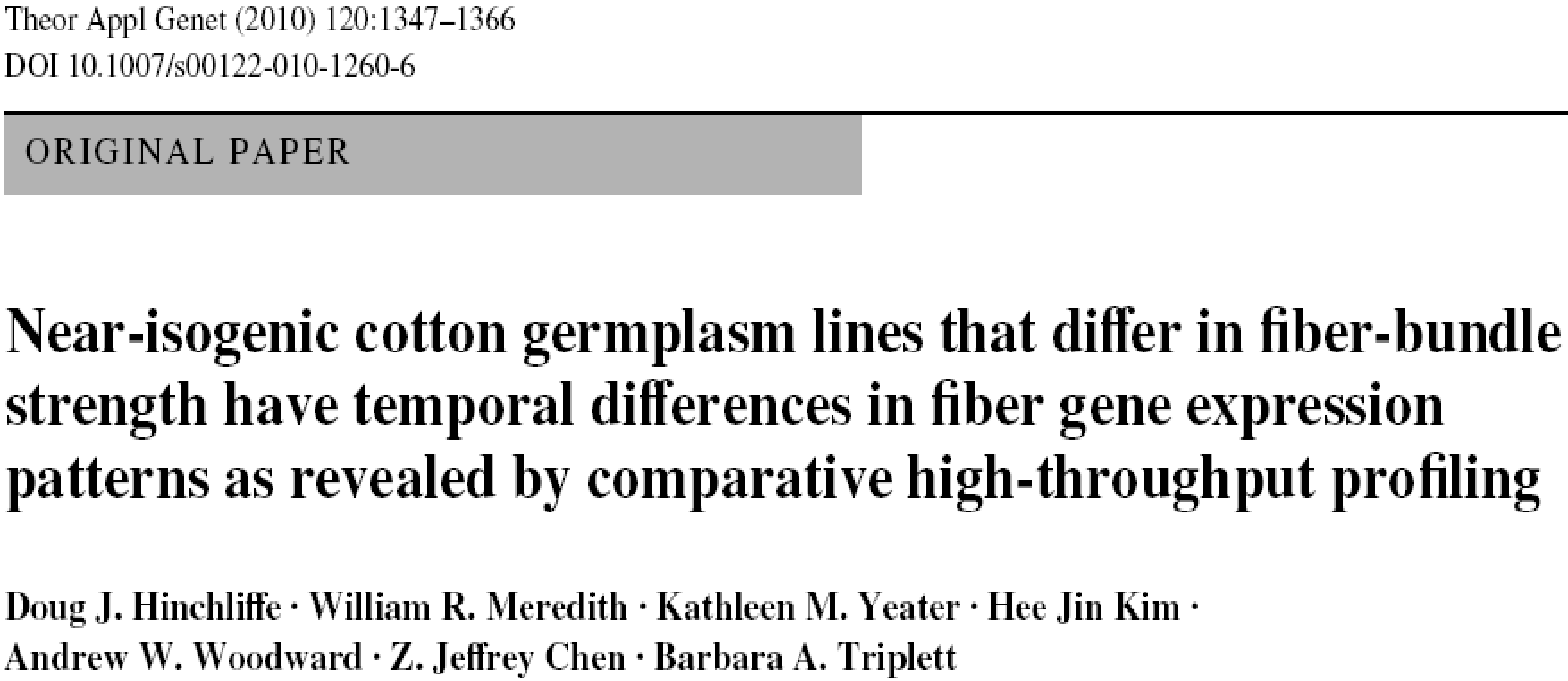
Journal of Integrative Plant Biology 2013, **55** (7): 572–575

Invited Expert Review

Boosting Seed Development as a New Strategy to Increase Cotton Fiber Yield and Quality[F]

Yong-Ling Ruan[1,2*]



Theor Appl Genet (2010) 120:1347–1366
DOI 10.1007/s00122-010-1260-6

ORIGINAL PAPER

Near-isogenic cotton germplasm lines that differ in fiber-bundle strength have temporal differences in fiber gene expression patterns as revealed by comparative high-throughput profiling

Doug J. Hinchliffe · William R. Meredith · Kathleen M. Yeater · Hee Jin Kim ·
Andrew W. Woodward · Z. Jeffrey Chen · Barbara A. Triplett

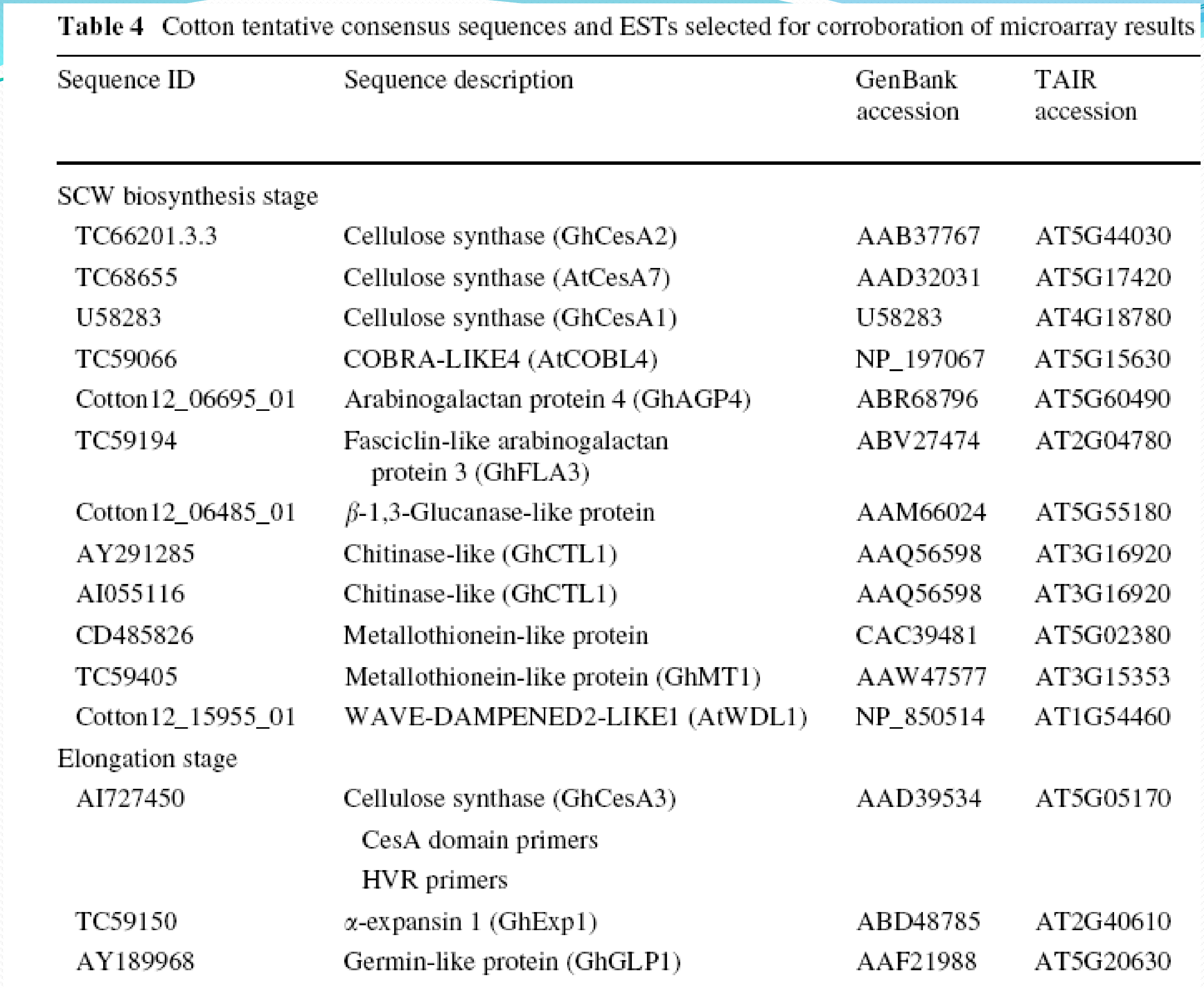
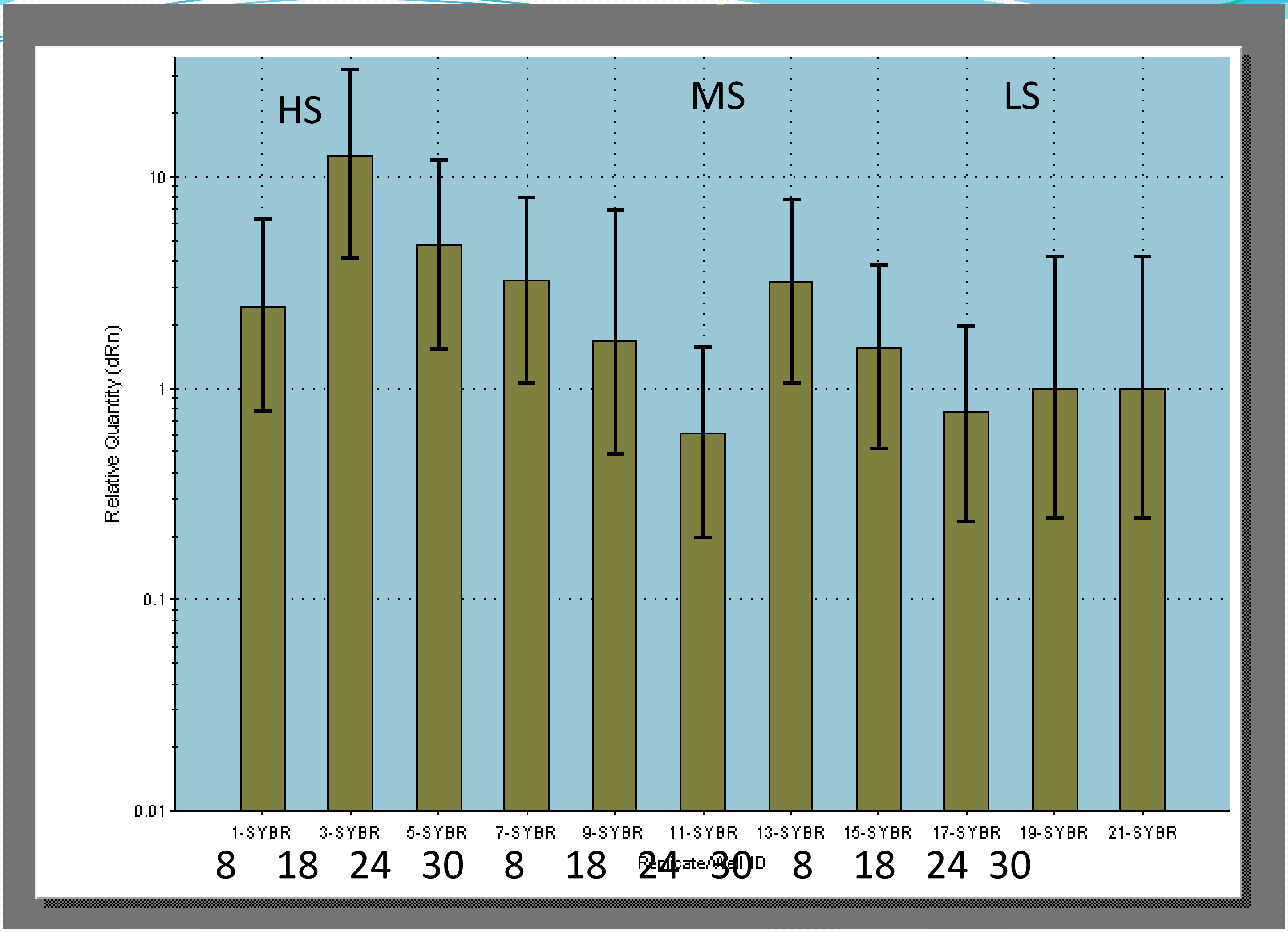


Table 4 Cotton tentative consensus sequences and ESTs selected for corroboration of microarray results

Sequence ID	Sequence description	GenBank accession	TAIR accession
SCW biosynthesis stage			
TC66201.3.3	Cellulose synthase (GhCesA2)	AAB37767	AT5G44030
TC68655	Cellulose synthase (AtCesA7)	AAD32031	AT5G17420
U58283	Cellulose synthase (GhCesA1)	U58283	AT4G18780
TC59066	COBRA-LIKE4 (AtCOBL4)	NP_197067	AT5G15630
Cotton12_06695_01	Arabinogalactan protein 4 (GhAGP4)	ABR68796	AT5G60490
TC59194	Fasciclin-like arabinogalactan protein 3 (GhFLA3)	ABV27474	AT2G04780
Cotton12_06485_01	β-1,3-Glucanase-like protein	AAM66024	AT5G55180
AY291285	Chitinase-like (GhCTL1)	AAQ56598	AT3G16920
AI055116	Chitinase-like (GhCTL1)	AAQ56598	AT3G16920
CD485826	Metallothionein-like protein	CAC39481	AT5G02380
TC59405	Metallothionein-like protein (GhMT1)	AAW47577	AT3G15353
Cotton12_15955_01	WAVE-DAMPENED2-LIKE1 (AtWDL1)	NP_850514	AT1G54460
Elongation stage			
AI727450	Cellulose synthase (GhCesA3)	AAD39534	AT5G05170
	CesA domain primers		
	HVR primers		
TC59150	α-expansin 1 (GhExp1)	ABD48785	AT2G40610
AY189968	Germin-like protein (GhGLP1)	AAF21988	AT5G20630

Important genes involved in cotton fibre strength

Sr. No.	Name of gene	Function	Reference
2	*FLA*	Cell-to-cell communication during cotton fiber elongation and secondary cell wall development	Liu et al., 2008
3	*AGP*	Cytoskeleton network and the cellulose deposition of fiber cells	Li et al., 2009
4	*CesA4*	cellulose biosynthesis during cotton fiber development	Kim et al., 2010
5	*Ces A 7*	participate in secondary cell wall synthesis (Li et al., 2013)	Li et al., 2013
6	*Ces A 8*	An enhancer specific for massive cellulose production in cotton fibers	Li et al., 2013
7	*MT1*	Mettaloprotein, detoxify excess metals, limiting oxidative damage	NCBI
1	*CobL*	Maintain the functionality of the cellulose synthase complex	Sato et al., 2010



Quantitative RT-PCR expression for *MT1* gene in cotton fiber, comparison between high, medium and low fiber strength

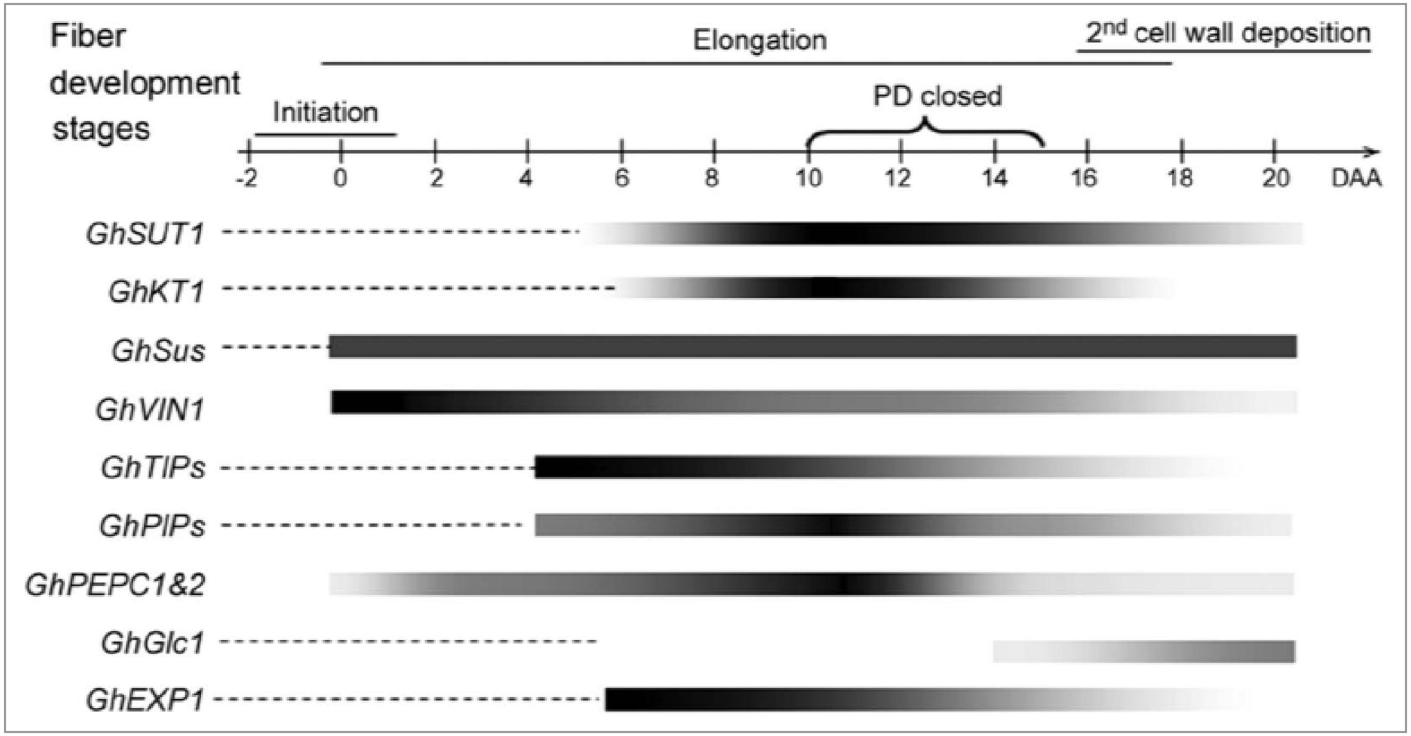
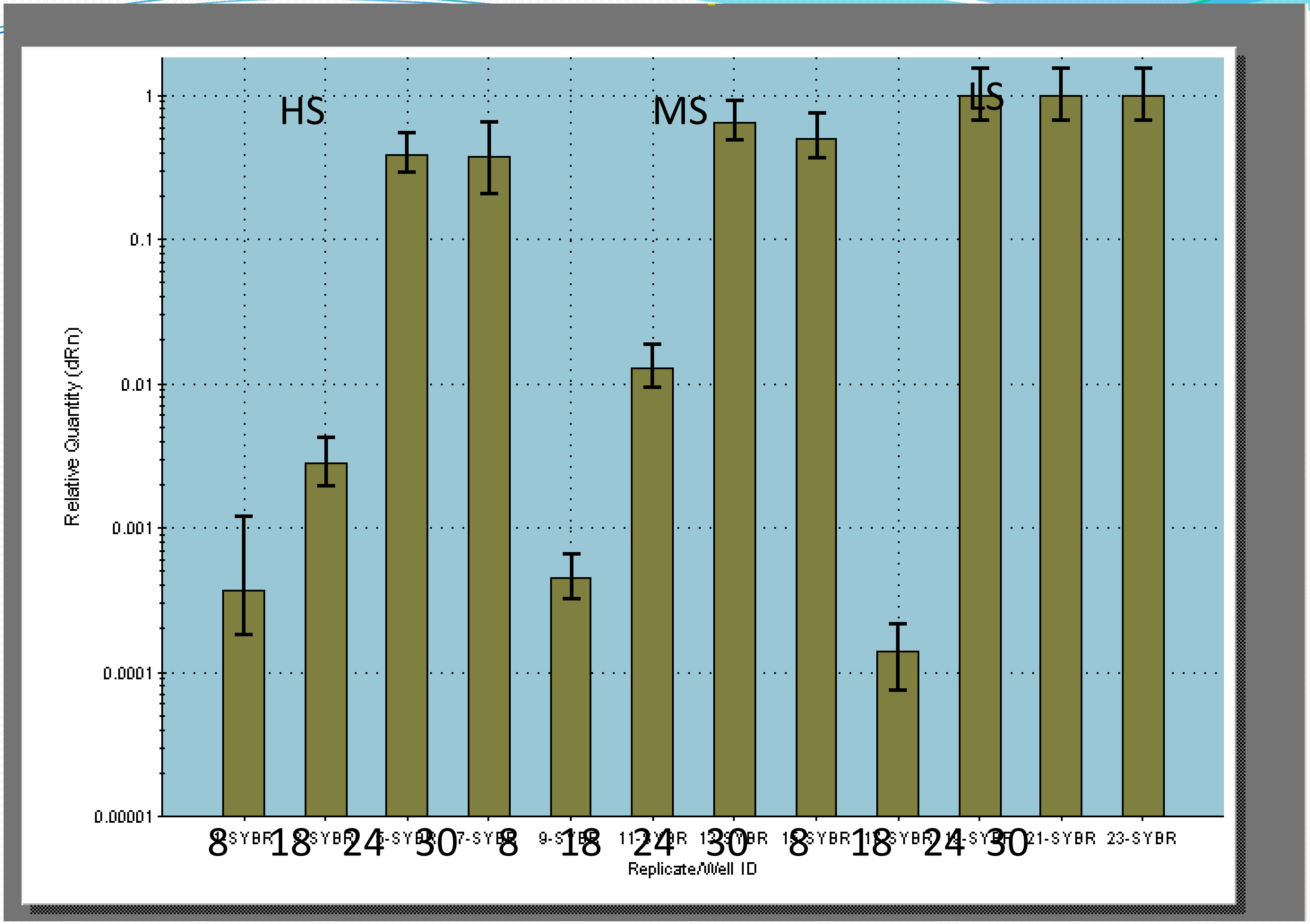


Figure 1. A schematic diagram of temporal expression pattern of turgor-related genes in cotton fiber of *Gossypium hirsutum*. The degree of darkness in a given bar represents level of gene expression as such that the darker the color the higher the gene expression level. Dash lines indicate the period when no expression data are available. *GhSUT1, GhKT1, GhEXP1* are genes encoding sucrose and K^+ transporter and expansin, respectively;[5] *GhSus*: sucrose synthase genes;[10,19] *GhVIN1:* vacuolar invertase gene;[12] *GhPIPs and TIPs* encode plasma membrane and tonoplast intrinsic proteins, respectively (Ashley J, Patrick JW, Ruan YL, unpublished data); *GhPEPC 1* and *2*, and *GhGluc1* are genes encoding phospho*enol*pyruvate carboxylase[11] and β1, 3-glucanase,[9] respectively. PD: plasmodesmata.[5,9]

Ruan Y L 2010



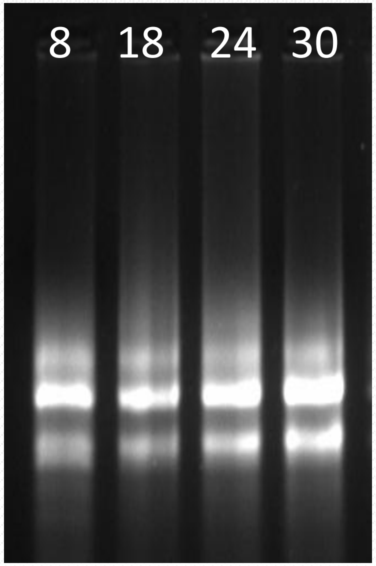
Quantitative RT-PCR expression for *FLA* gene in cotton fiber, comparison between high, medium and low fiber strength

Target Genes& Primers, RNA extraction,cDNA synthesis, qPCR

- RNA extraction by Sigma **kit,cDNA synthesis by Affinity Agilent, qPCR by Agilent**.

Target Genes & Primers: ***CesA8, CesA4, CesA7, FLA1,*** **MT1**

RNA Extraction and Determining Quality of Isolated Total RNA Using Agarose Gel Electrophoresis



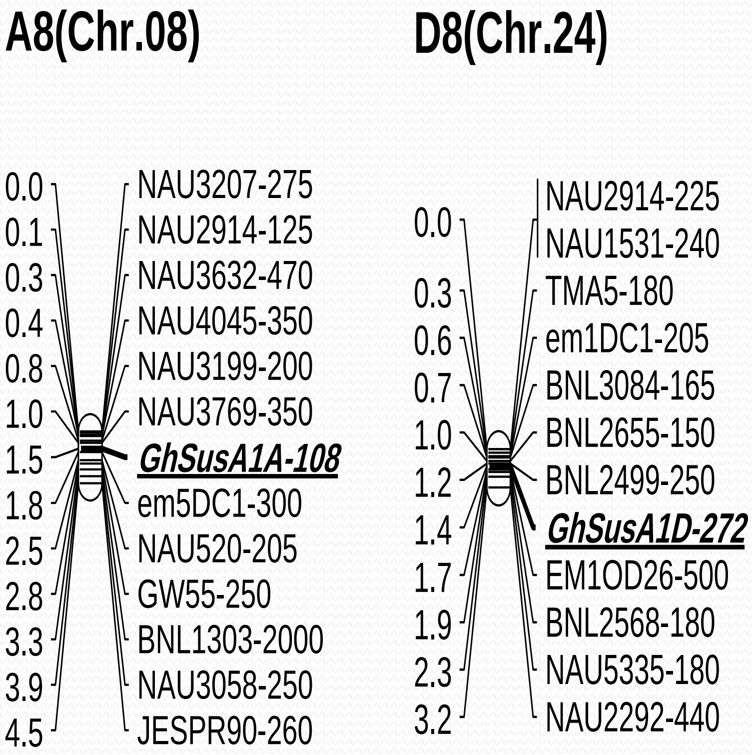
cDNA

qPCR

* Numbers are Indicating
Total RNA of Respective DPA

Association analysis and genome localization of GhSusA1

(b)



GhSusA1 was mapped on homeologous chromosomes A8 (chro.8) and D8 (chro.24). which are densely populated with markers and quantitative trait loci (QTLs) associated with fiber strength, elongation and finenessfrom different groups of diverse cotton lines cross (Shen et al., 2005, 2007; Qin et al., 2008).

Biological process

	HBS 144	HBS 187
Transcription DNA template	2.72	2.62
Regulation of transcription , DNA template	2.48	2.37
Translation	2.38	2.27
Carbohydrate metabolic process	1.48	1.53
Intra cellular protien transport	1.33	1.34
Protein folding	1.06	<1.00
Total	11.45	10.13

Cellular component

	HBS 144	HBS 187
Integral component of membrane	8.43	8.71
Nucleus	7.8	7.73
Membrane	3.55	3.57
Plasma membrane	2.78	2.69
Cytosol	2.47	2.46
Cytoplasm	2.21	2.19
Ribosome	1.89	1.78
Chloroplast	1.44	1.49
Plasmadesma	1.39	1.27
Intra cellular	1.04	1.06
Total	33	32.95

Molecular function

	HBS 144	HBS 187
Nucleotide binding	2.31	2.49
Structural constituent of ribosome	2.42	2.33
Sequence specific DNA binding transcription factor activity	2.72	2.72
Metal ion binding	3.86	3.75
Protein serine therionin- Kinase activity	3.98	4.43
RNA binding	4.06	3.54
Nucleic acid binding	5.57	5.22
DNA binding	6.2	6.32
Zinc ion binding	8.21	7.88
ATP binding	12.53	13.67
Total	51.86	52.35