Comparison of fiber genes expression in wild and lint-less mutant cotton

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Cotton

- ✓ Cotton accounts for >60 % of total **yarn** production worldwide, making it the most important **raw material** for the textile industr**y.**
- ✓ **Its cellulose fibers are valued for their strength and their ability to absorb moisture (water absorption capacity 65%), skin friendly and thermoprotection.**
- ✓ **Cotton fiber is nothing but elongated cells of the seed coat epidermis**
- ✓ **Pure cellulose: Primary cell wall 1ng/mm on set of secondary cell wall 130ng/mm**

• Cellulose	80-90%
• Hemi-cellulose & Pectins	4-6%
• Waxes and Fat	0.5 – 1.0%
• Proteins	0.0 – 1.5%
• Ash	1.0 – 1.8%

Fiber strength is..

Cotton fiber length/strength are the key factors in determining fiber quality in the textile industry throughout the world.

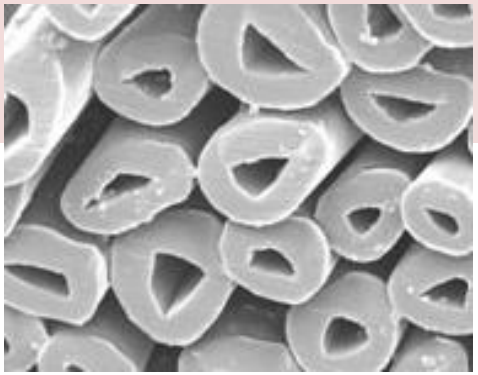
Fiber strength is more likely to withstand breakage during the manufacturing process.

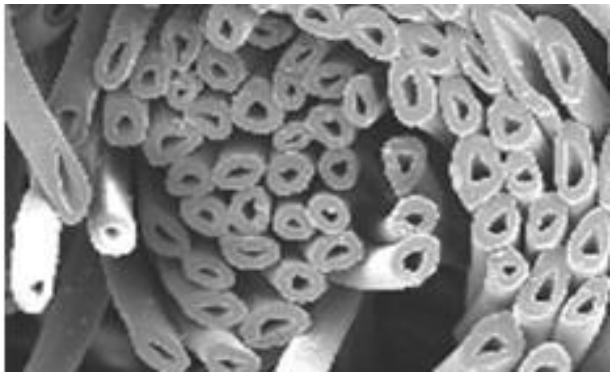
Strength measurements are documented in terms of grams per tex (g/tex)

A tex unit = weight in grams of 1000 meters of fiber.

g/tex = The force in grams required to break a tex unit

Degree of Strength	HVI Strength (grams per tex)
Very Strong	32 & above
Strong	29 -30
Average	26 – 28
? Intermediate	24 – 25
? Weak	23 & below

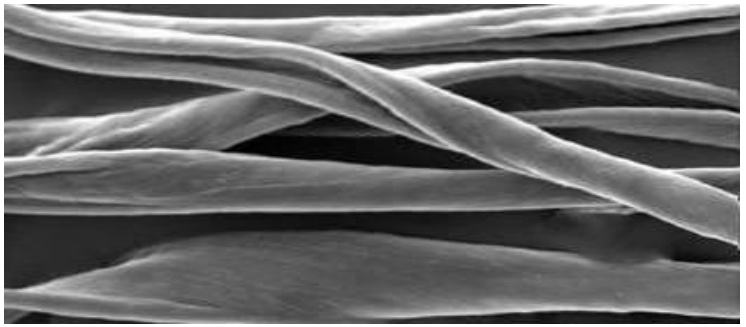


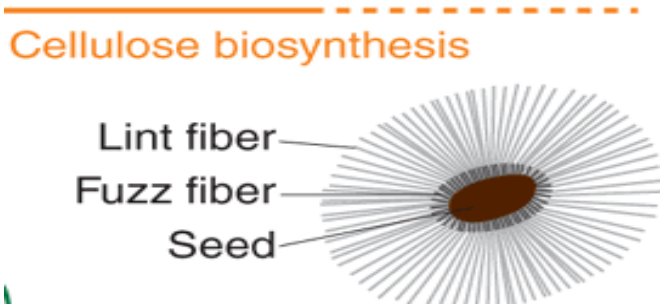


Requirement of textile industry !!

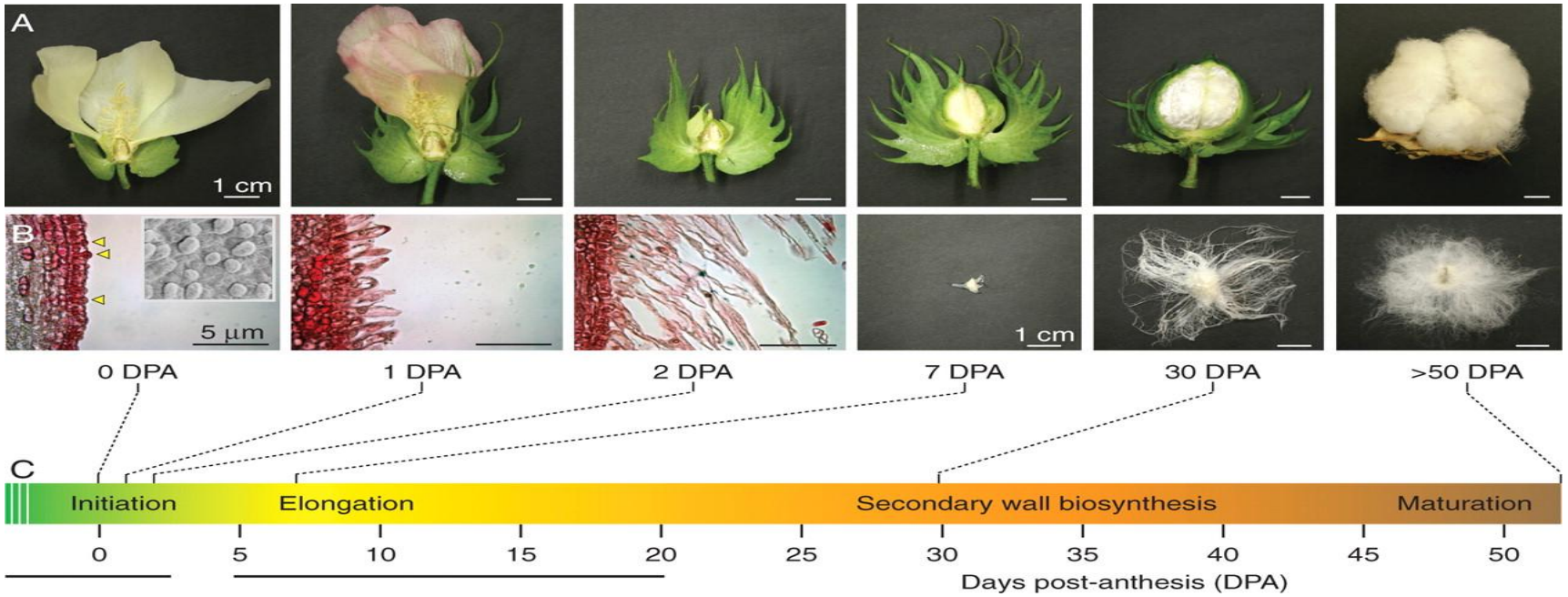
S. No.	Species	Available Fiber strength (g/tex)	Required (g/tex)
1.	*G. hirsutum*	18-25	30 - 35
2.	*G. arboreum*	14-20	25-30
3.	*G. barbadense*	45-52	Source

How to improve it…??
How to fill the gap…??





Molecular approach would help us to solve this perceived problem……



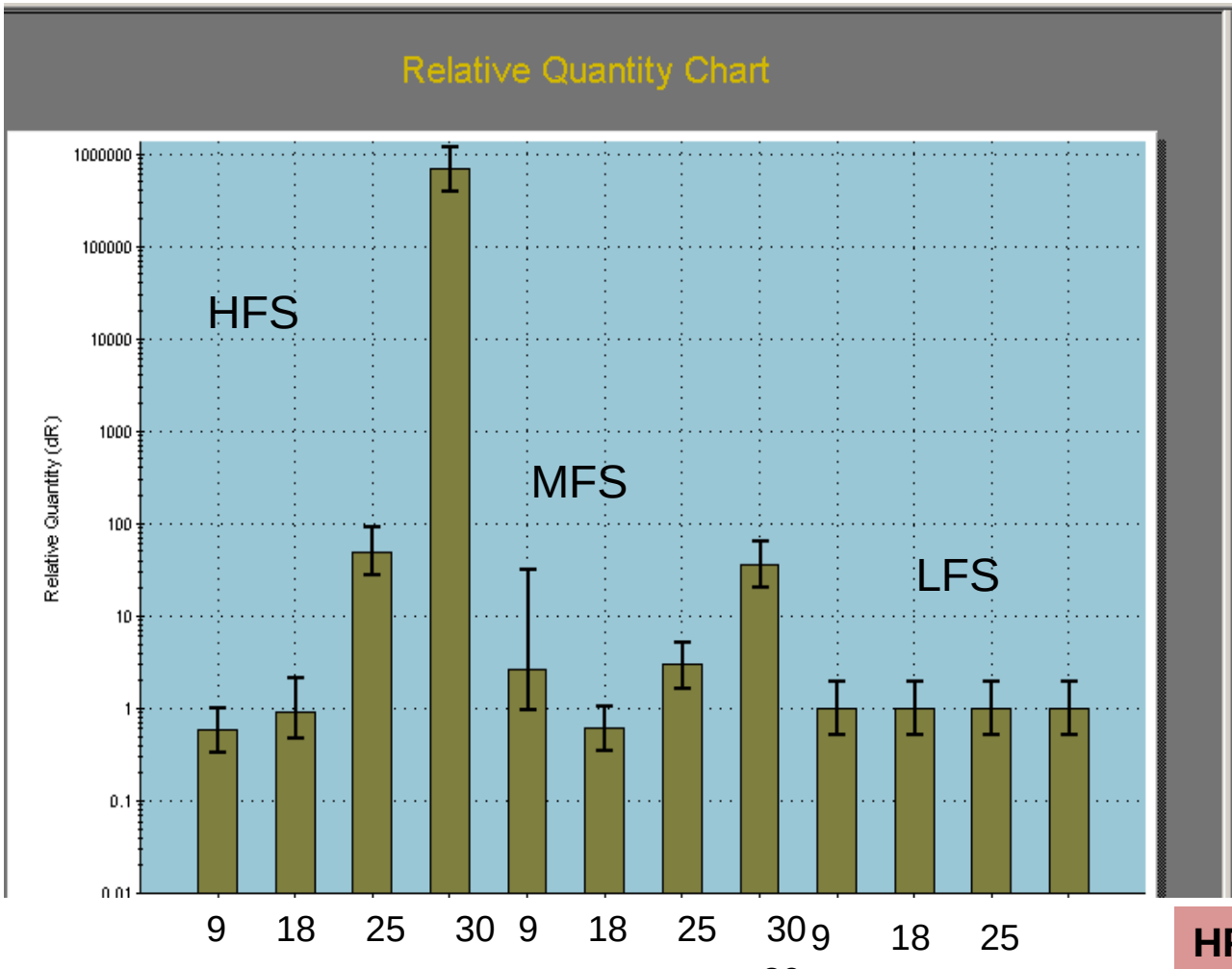
- **Fiber development consists of four overlapping stages (initiation, elongation, secondary cell wall biosynthesis, and maturation),**
- **Fiber initiation is characterized by trichome protrusion and enlargement on the epidermal surface that occurs from 3 days before anthesis to 3 dpa.**
- **Only 25–30% of epidermal cells differentiate into the mature long-fiber cells, whereas others may develop into short fibers called fuzz (5–6 mm in length).**

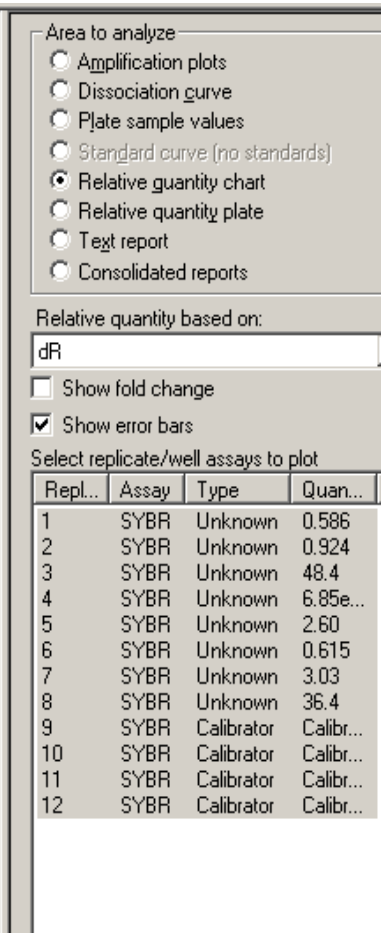
RIL mapping population
CICR, Nagpur

Gene expression analysis was carried out with selected genes through qPCR (*GhcesA1, GhcesA2, GhcesA7, GhcesA8, Ghcobl4, Ghfla3 and GhMT1*) using RIL mapping population to establish correlation with fibre strength.

Among them *GhcesA1, GhcesA2, Ghfla3 and Ghcobl4* were showed strong association and higher gene expression during secondary wall synthesis.

Quantitative RT- PCR for expression analysis of gene *GhCesA1* at different stages in different fiber strength using *Actin* as endogenous control





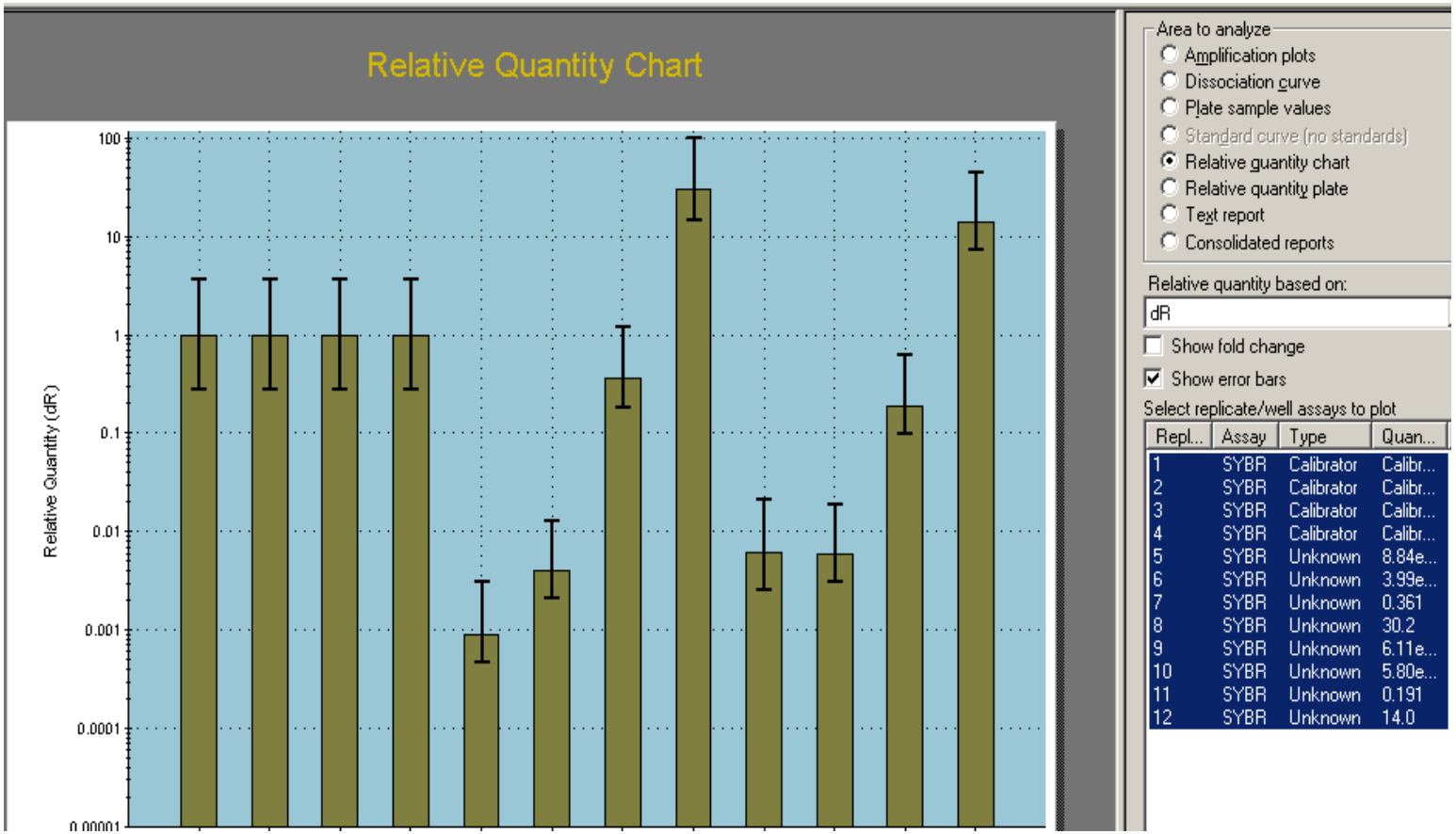
High strength line

medium strength line

Low strength line

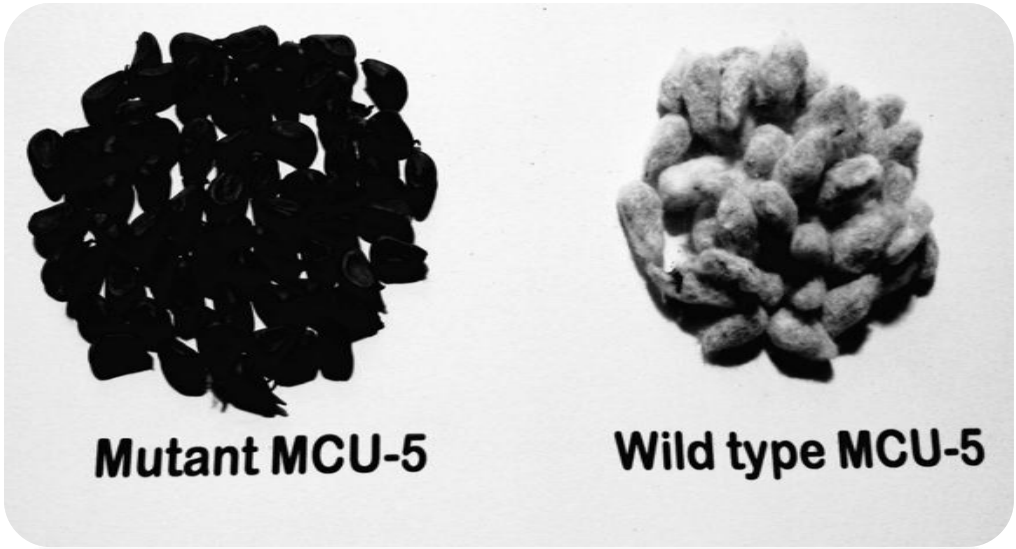
HFS: High Fiber Strength
MFS: Medium Fiber Strength
LFS: Low Fiber Strength

Quantitative RT- PCR for expression analysis of gene *GhCesA2* at different stages in different fiber strength length



Low strength line High strength line Medium strength line

Gene expression validation in mutant MCU-5 and wild type MCU-5 genotype



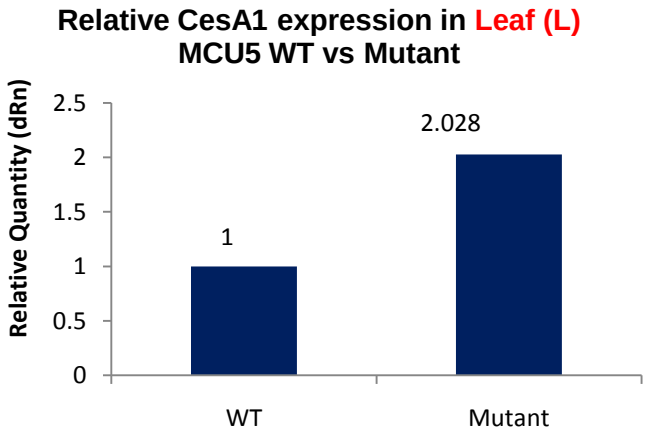
Plant material: Leaf, Square, ovule from 10dpa and ovule from 20 dpa – mutant and wild type MCU-5 genotype

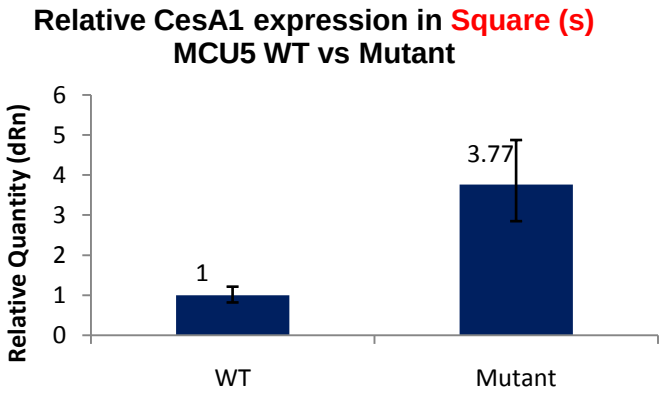
Genes for RT PCR:

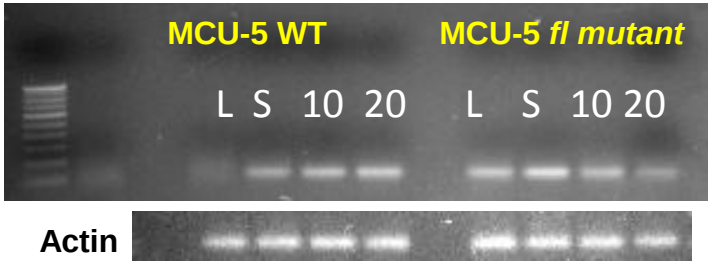
- *GhcesA1, GhcesA2, GhcesA7, Ghfla3 , Ghcobl4,*

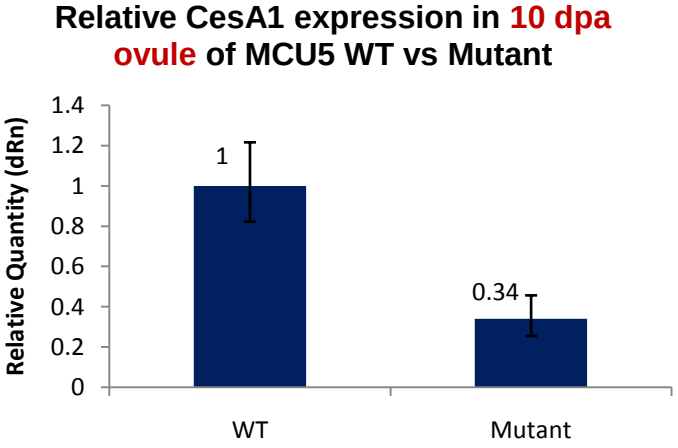
- This mutant provides an excellent model system to study the molecular mechanisms of cotton fiber elongation.
- Quantitative real time PCR (qRT-PCR) were used to evaluate differentially expressed genes (DEGs) in the lint-less mutant compared to the wild-type.

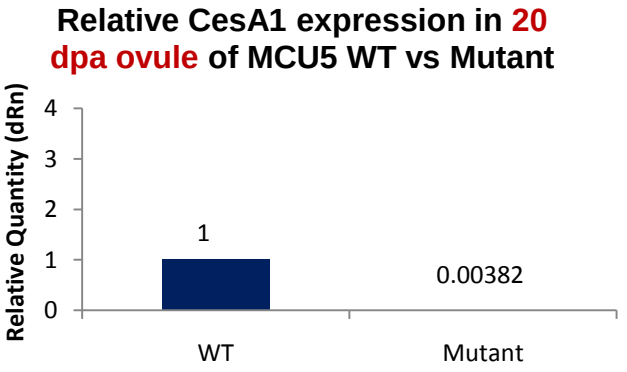
RT-PCR and qPCR analysis GhcesA1 in mutant and wild type MCU-5 genotype



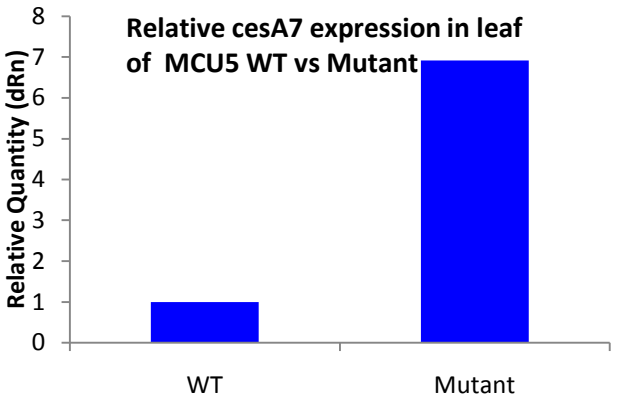


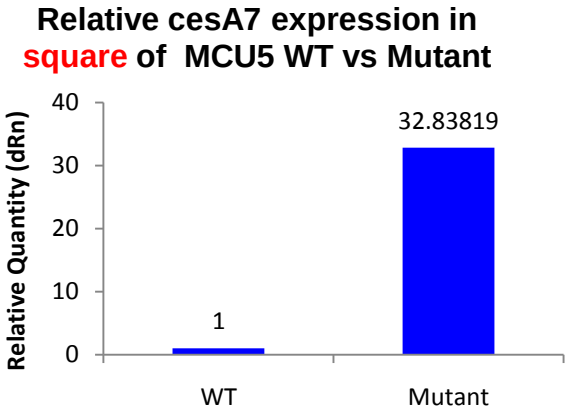




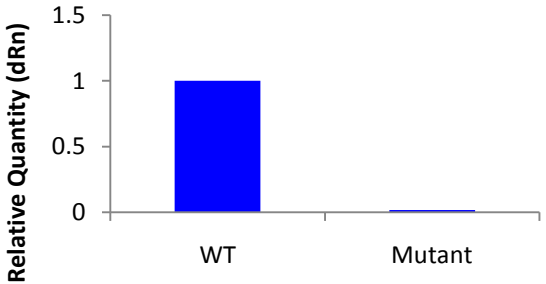


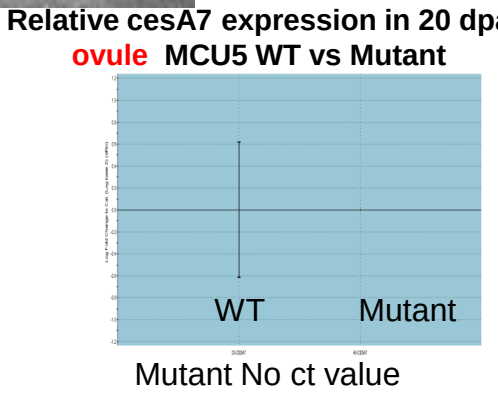
RT-PCR and qPCR analysis GhcesA7 in mutant and wild type MCU-5 genotype





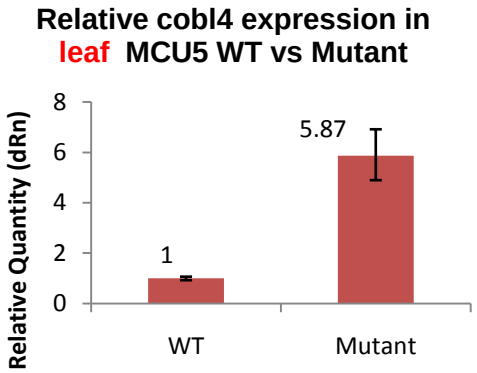
Actin

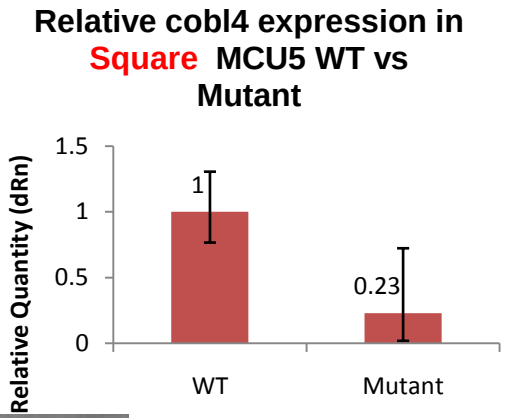


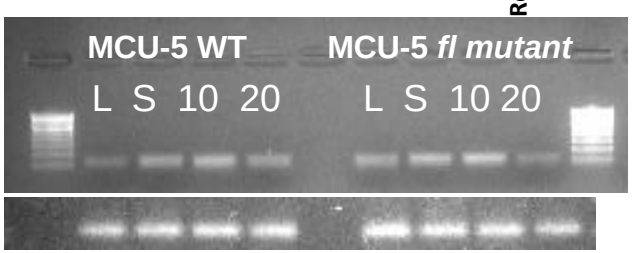


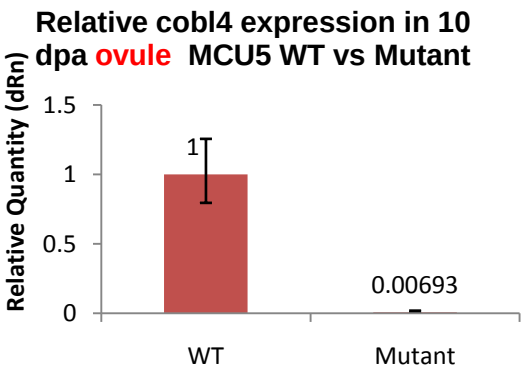
Mutant No ct value

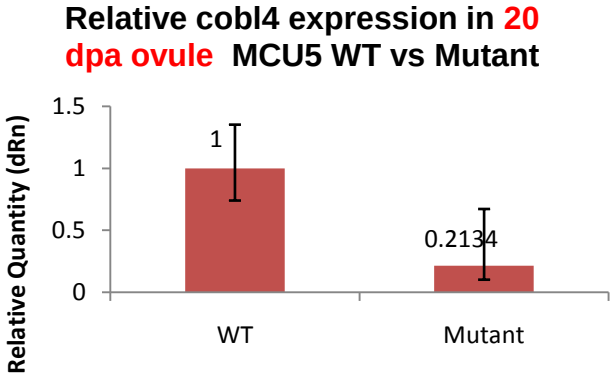
RT-PCR and qPCR analysis GhCOBL4 in mutant and wild type MCU-5 genotype



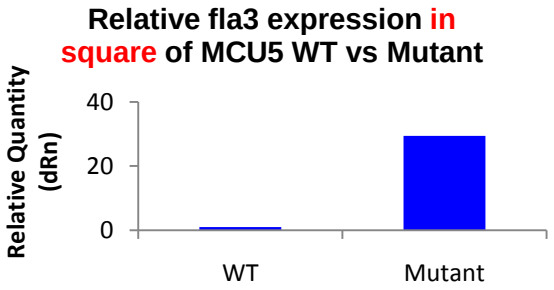


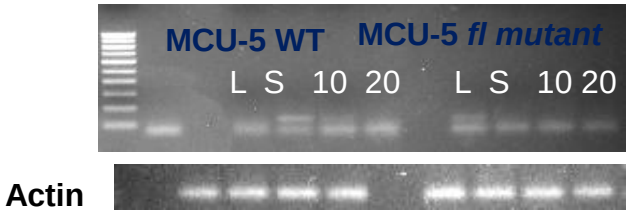


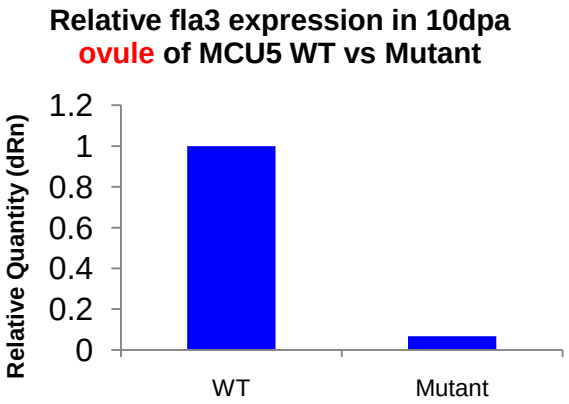


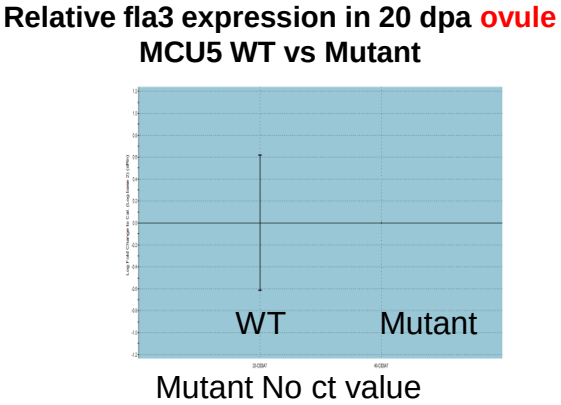


RT-PCR and qPCR analysis Ghfla3 in mutant and wild type MCU-5 genotype

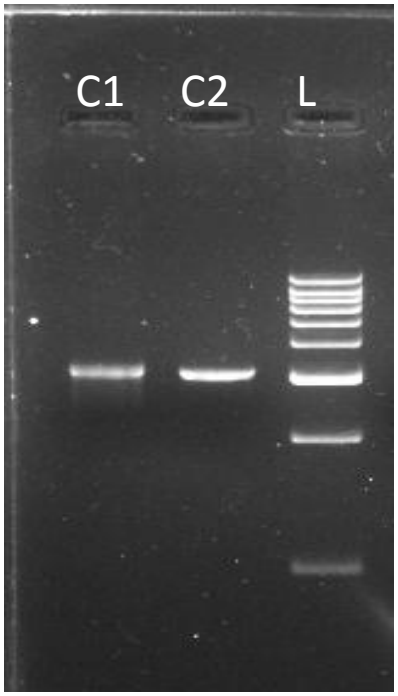




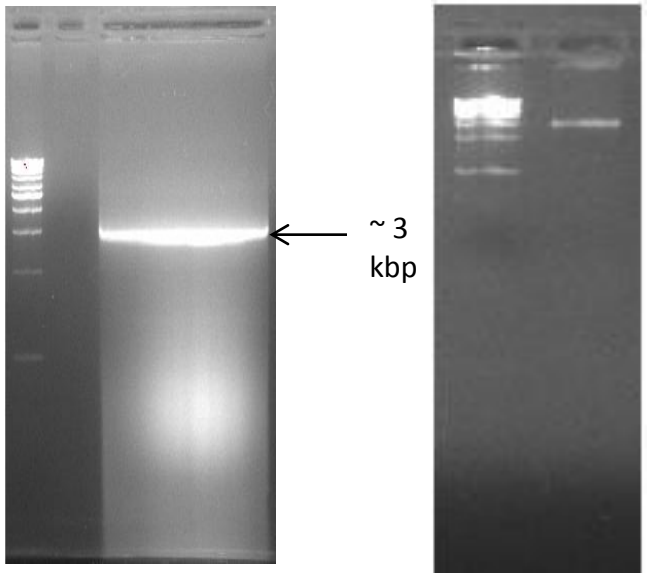




Cloning of CesA1 and A2 gene from *Gossypium hirsutum*

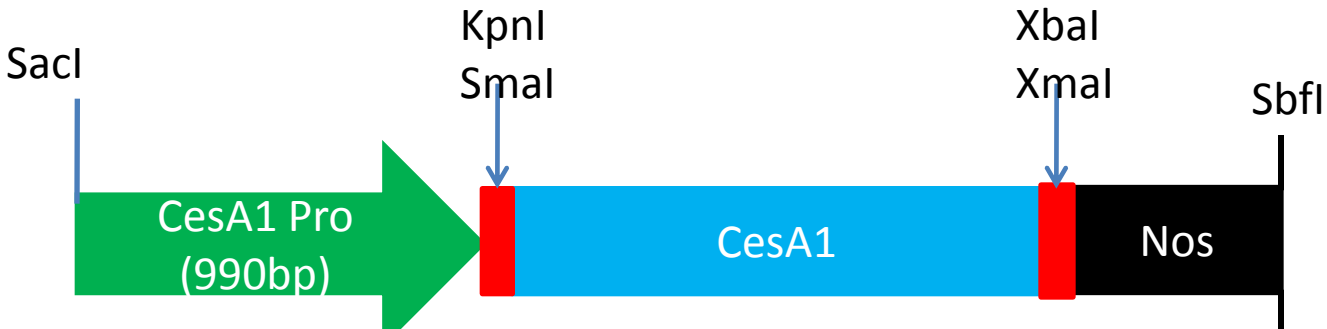


Agarose gel electrophoresis for CESA 2 gene product for gel extraction

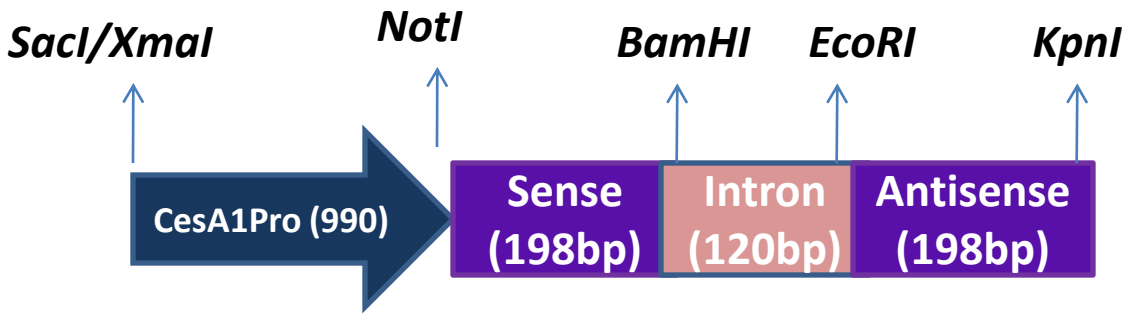


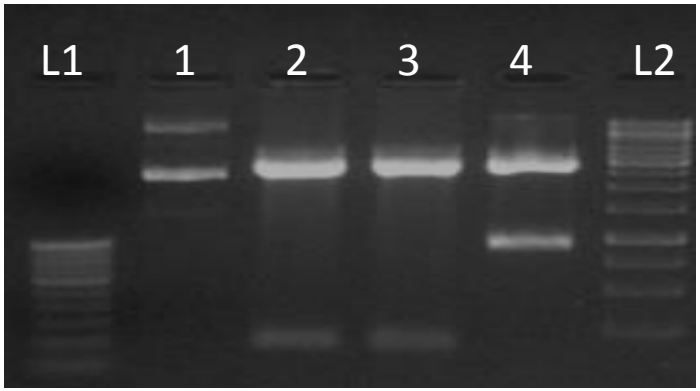
After gel extraction and purification *CesA2* gene product

Amplification of GhcesA1



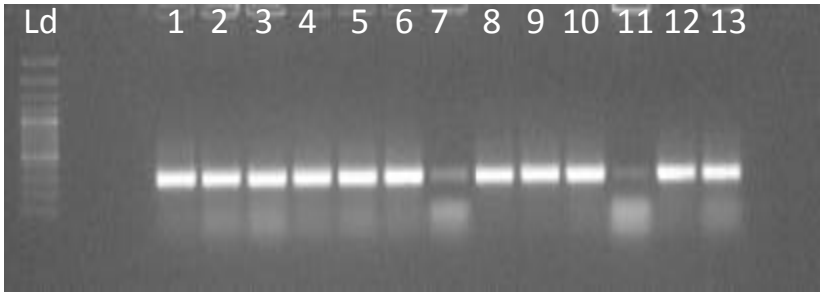
GhCesA1



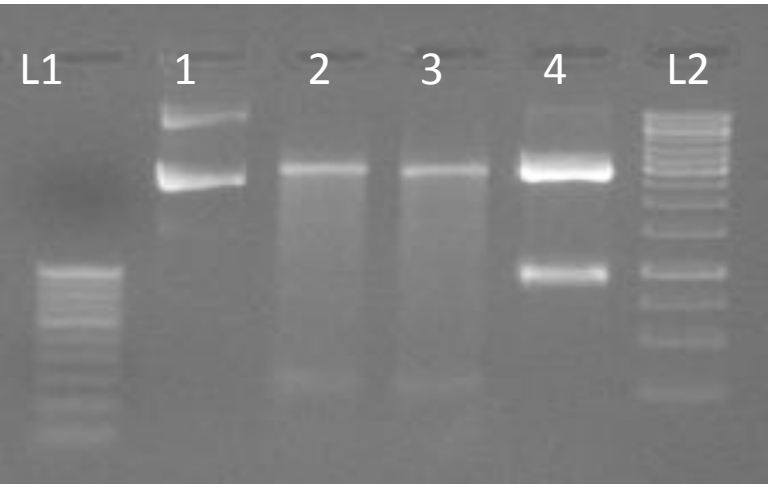


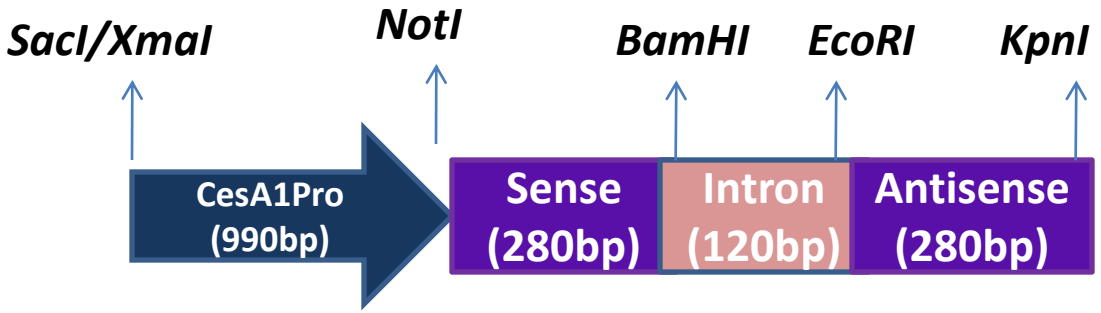
L1= (100bp)
1= uncut Pbsk, 2= Digested with SacI and BamHI, 3=Digested with EcoRI and KpnI
4=Digested with XmaI and NotI, L2= 1Kb Ladder

GhcesA2



Colony PCR
L=Ladder Colonies no.=1,2,3,4,5,6,7,8,9,10,11,12,13





L1= (100bp) 1= uncut pBSK 2= Digested with SacI and BamHI 3=Digested with EcoRI and KpnI 4=Digested with XmaI and NotI L2= 1Kb Ladder


Thank you