Characterization of *PROFILIN* genes from allotetraploid (*Gossypium hirsutum*) cotton and its diploid progenitors and expression analysis in cotton genotypes differing in fiber characteristics

Apostolos Kalivas[1], Anagnostis Argiriou[2], Georgios Michailidis[3] and Athanasios Tsaftaris[2]

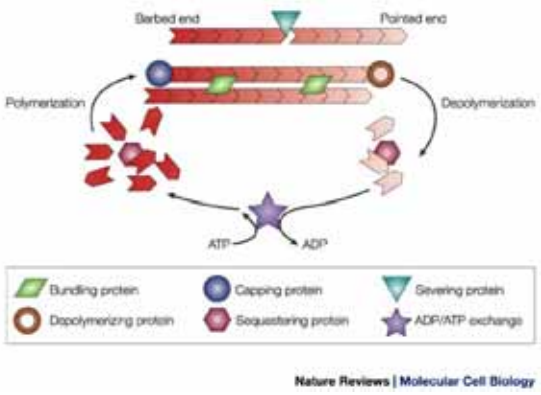
[1] Hellenic Agricultural Organisation – DEMETER, Cotton and Industrial Plants Institute, PO Box 60406, Post code 57001 Thermi Thessaloniki Greece, Tel: 0030-2310-471544, e-mail: dir.cotton@nagref.gr

[2] Institute of Applied Biosciences, Center for Research and Technology Hellas, 6th Km Charilaou Thermi Road, Thermi GR-570 01, Greece. tsaft@certh.gr

[3] Laboratory of Physiology of Reproduction of Farm Animals, Department of Animal Production, School of Agriculture, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece. michageo@agro.auth.gr

Profilin

- **Low molecular weight actin bindind protein (12-15KD) .**
- **Profilin facilitates actin polymerization by binding monomeric G-actin and promoting assembly by catalysing ADP-to-ATP exchange on actin monomers.**



Profilin

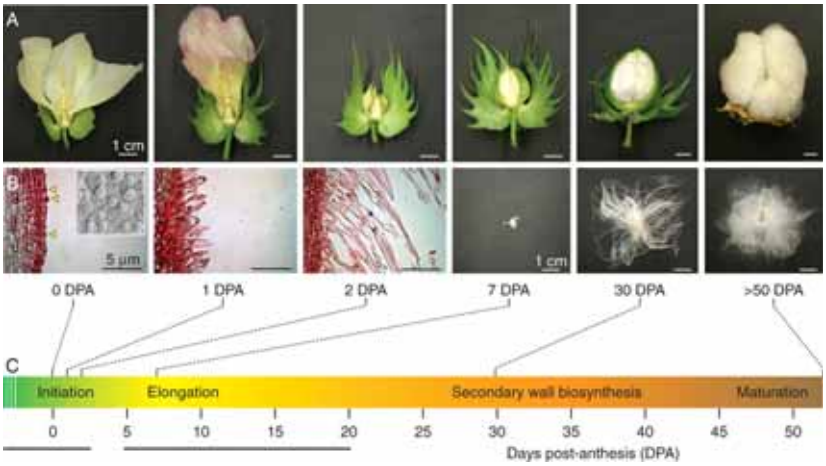
Analysis of arabidopsis transgenic plants overexpressing sense and antisense PRF indicated that PRFs play a role in flowering time and polarized growth of root cell elongation, cell shape maintenance, determination of hair and trichomes

Cotton fibers are single, elongated epidermal cells of the outer integument of the ovule. The development of cotton fibers is a complex process that can be divided into four overlapping stages: initiation, elongation, secondary wall synthesis and maturation and involves numerous genes functioning in concert in various biochemical pathways in the cell.

Recent studies have identified a relevant number of cotton fiber ESTs found to be preferentially expressed cotton fibers and some of these sequences encode components related directly cell growth. Among them a sequence corresponding to a PRF gene was identified (GhPFN1) encoding an isoform of cotton PRF, and suggested a possible role of PRF in cotton fiber cell elongation.

Overexpression of this GhPFN1 gene in transgenic tobacco cells resulted in the formation of elongated cells that contained thicker and longer microfilament cables.

Fibre initiation and elongation stages



Lee J J et al. Ann Bot 2007;100:1391-1401

Material and Methods

Profilin

Plant material: 79/BH47 (*G. arboreum*), Ulbrich (*G. raimondii*), Acsj2 (38 % fiber percentage), 138F (60 % fiber percentage), Giza 7 (*G. barbadense*)

Isolation, cloning and sequencing: PCR, PGEM T easy vector, ABI3730

Protein sequence alignment - Clustal W

Phylogenetic analysis - MEGA 4 (Neighbor-Joining)

Southern blot

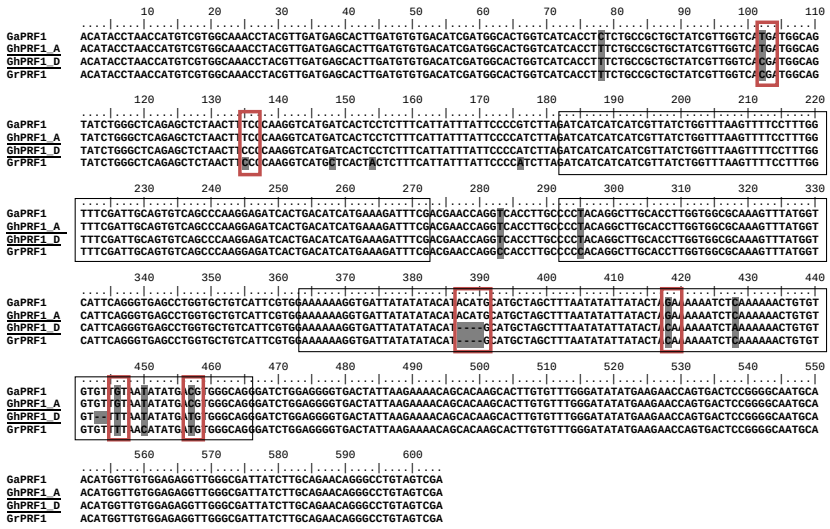
RNA isolation – cDNA synthesis – Profilin's gene Expression analysis

Real-time Quantitative PCR analysis

Construction of Genome Walker DNA libraries

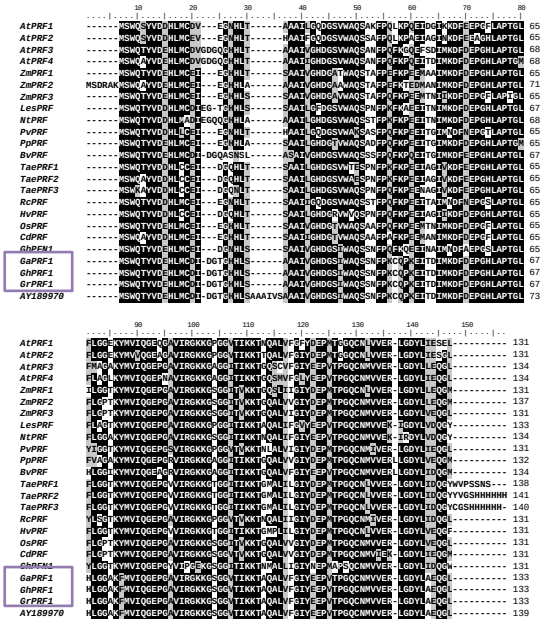
Results

Amino Acid Sequences Alignment

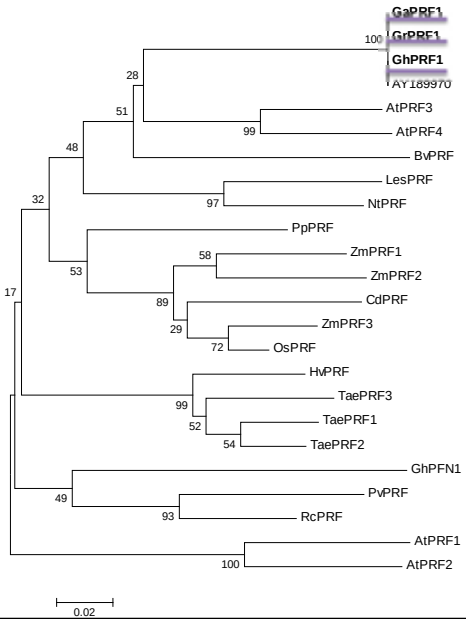


Alignment of cotton PRF profilin genomic sequences. Nucleotide sequence alignment was performed using the Clustal method. Boxed areas represent introns, boxed grey and light grey areas show the nucleotide differences between the four sequences. Start and stop codons, are in bold.

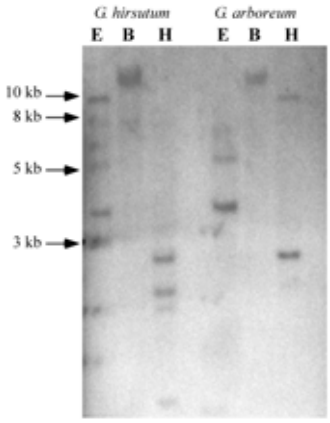
Amino Acid Sequence Alignment



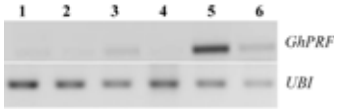
Phylogenetic analysis



Southern blot- Expression analysis



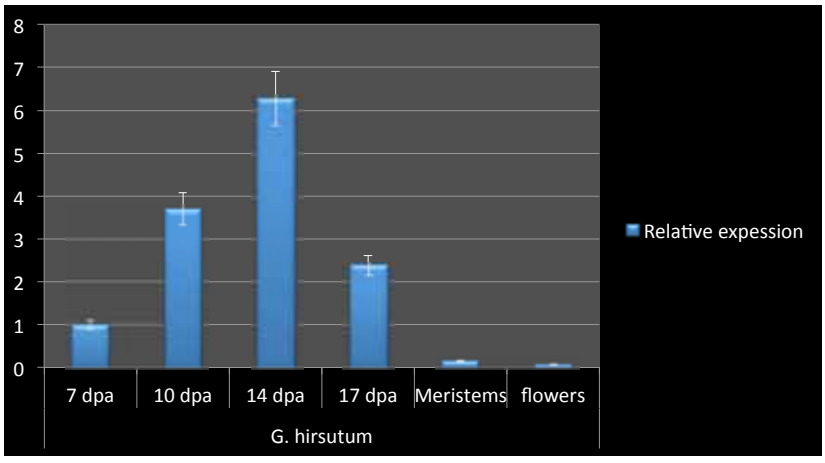
Southern Blot for *profilin* gene in *G. hirsutum* and *G. arboreum.*
E: *Eco*RI
B: *Bam*HI
H: *Hind*III.



Expression analysis of *GhPRF1* in different cotton organs.

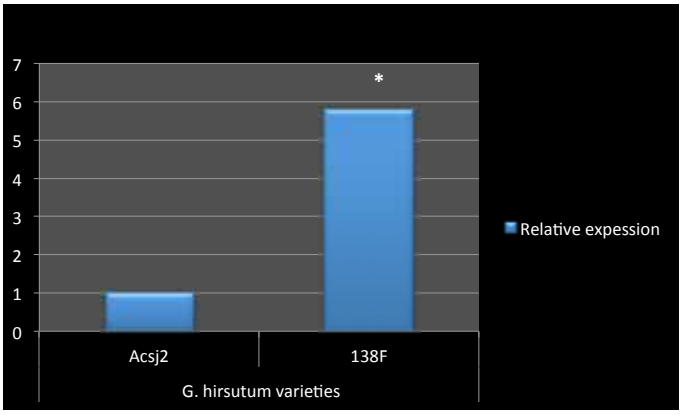
1: Leaves
2: Cotyledons
3: Flowers
4: Petals
5: Fibers 10 dpa
6: fibers 25 dpa

Expression analysis with Real-time PCR



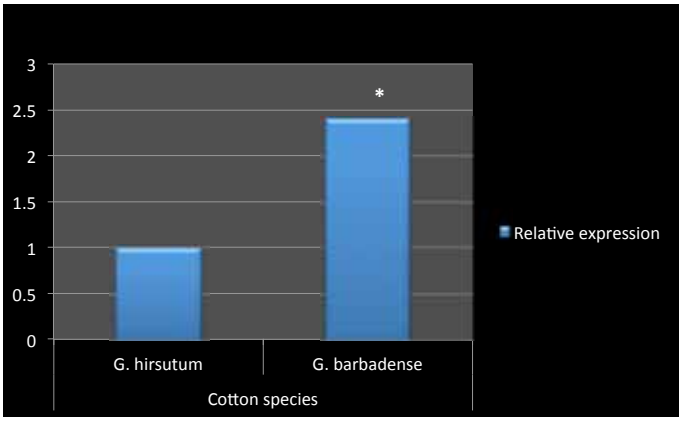
Quantitative real-time PCR expression analysis of GhPRF1 gene in different G. hirsutum cotton fiber dpa and cotton tissues and organs. Expression levels is expressed relatively to 7 dpa.

Expression analysis with Real-time PCR



Quantitative real-time PCR expression analysis of *GhPRF1 gene between the G. hirsutum varieties Acala and 138F.*

Expression analysis with Real-time PCR



Quantitative real-time PCR expression analysis of *GhPRF1 between the cotton species G. hirsutum (variety Acala) and G. barbadense (variety Giza7).* Asterisk indicates the statistically significance of values.

Profilin's promoter

```
-450 AAATTTAATATTCAAAATATAAATTTATTAATAATTTATCAACTTTGACCAAAATTTCCCTTA
-387 AGAAATGGTGCAAATGACGTAAACGTAAAAATAGAAGAAAAGAATGTGGGCAATAAGACACGT
-324 GAAGAGTGAGAGAGAAAAGGAAAAATAAAGGGAAAAAAAAGTGTGGGGAACTTGGGAAGGGTC
-261 TATGGGGGCTTATATGGGTTGCTAAAATAAAGGCGGACGTGACTGGTAGTAATGAAAATAAGC
-198 AAAATGGACGGTTGAGAAATCATCACATCAGCTTTTTCAGTGCCCTTCCCCCTACCTTCACTT
-135 CCTTATTTATCGTCTCCCTCCCCCCCCTTCCCTTCCCCATTCACCATTACCCTTCTCCCCCTT
                          CAAT                    Inr
 -72 CCTCCCATATTCCTCTTTCCCAATACCCTTTCACCCTTGCTAATAAAACCACCTTATTAACA
                +1
  -9 TACCTAACCATGTCG
```

Nucleotide sequence of the putative cotton PRF promoter region. Potential regulatory elements of the cotton PRF promoter are boxed. CAAT and Inr motifs are shown in boxes. Conserved motifs between the *G. hirsutum and A. thaliana's* PRF promoters are shown in bold boxes. Numbering is relative to the first base before the ATG codon.

Conclusions

PRF gene is probably a positive regulator of both cotton fiber elongation and density and suggest that over-expression of this gene in cotton species with low fiber yield would probably result in improvements of cotton fiber characteristics.