

1 **Title: ROS accumulation in cotton ovule epidermal cells is necessary for fiber initiation**

2 **Authors:** Mingxiong Pang^{1*}, Nicholas Sanford¹, Thea A Wilkins¹

3 **Affiliations:**

4 ¹Department of Plant & Soil Science, Texas Tech University, Lubbock, TX 79409 USA.

5

6 *Correspondences to: Mingxiong Pang. mingxiong.pang@ttu.edu.

7

8 **Abstract:** Cotton (*Gossypium hirsutum*) fiber, an extremely elongated and thickened single cell
9 of the seed epidermis, is the world's most important natural and economical textile fiber. Unlike
10 *Arabidopsis* leaf trichomes, fiber initials are randomly developed and frequently form in adjacent
11 seed epidermal cells and follow no apparent pattern. Numerous publications suggested cotton
12 fiber development shares a similar mechanism with *Arabidopsis* leaf trichome development.
13 Here we show that H₂O₂ accumulation in cotton ovule epidermal cells by NBT staining ovules
14 at different development stages between TM1 and N1n2, a lintless-fuzzless doubled mutant
15 originated from TM1. In contrast, *Arabidopsis* and cotton leaf trichomes do not show H₂O₂
16 content. By adding DPI (H₂O₂ inhibitor) and SHAM (H₂O₂ activator) in vitro ovule cultures,
17 we show fiber initiation directly involves with H₂O₂ accumulation. We propose that the
18 directional accumulation of H₂O₂ in cotton ovule epidermal cell is the drive for fiber initiation,
19 elongation.

20

21

1 **Main Text:**

2 Seed dispersal is sometimes split into autochory (when dispersal is attained using the
3 plant's own means) and allochory (when obtained through external means). Wind dispersal
4 (anemochory) is one of the autochories and more primitive means of dispersal and plant seeds
5 have a feathery tissue attached to their seeds and can be dispersed long distances. Primitively,
6 plants distributed the seeds majorly through autochory; as animals emerge and human activity,
7 some plants lost their ability to produce seed secondary tissues helpful for dispersal and some
8 plants gained the production for specific seeds tissues such as cotton seed trichomes as human
9 activity. But we do not understand how these tissues develop. Cotton (*Gossypium spp.*) fibers are
10 unicellular and single-celled seed trichomes of economic importance (1) and provide a special
11 single-cell functional genomics research platform (2, 3) for fundamental biological processes
12 such as organogenesis and cell wall development in plants (4, 5). Fiber development initiates on
13 the day of anthesis (0 dpa) and 30-40% of primordial cells (6) on the ovule epidermis form
14 cuboidal outgrowths called fiber “initials” (7). Unlike *Arabidopsis* leaf trichomes, fiber initials is
15 randomly distributed and follow no apparent pattern as fiber initials frequently form in adjacent
16 epidermal cells (8,9). Fiber initials rapidly elongate to produce highly tapered cells within 2-3
17 days of anthesis through a biased diffuse growth mechanism (10).

18 The initiation of single celled cotton cuboidal fiber cells is a biologically important and
19 complex process, but how the fiber initiate and how the genes involved are regulated remain
20 elusive. Zhang et al., (11) demonstrated by adding 30% H₂O₂ to in vitro cultured ovules of
21 XinFLM, a linted-fuzzless mutant, that the fiber initiation is partially rescued, but no evident
22 effect on N1, the dominant naked seed mutant from TM-1 genetic background. This implies the
23 H₂O₂ biosynthesis pathway may be defect and the signal transduction is still functional in

1 XinFLM. Like cuboidal outgrowth fiber initials, Andriunas et al., (12) shows a flavin-containing
2 enzyme (NAPDH oxidase) and superoxide dismutase cooperatively generate a regulatory H₂O₂
3 to govern ingrowth wall formation, a transfer cell (13), using *Visia Faba* cotyledon culture.
4 Forman et al., (14) verified that ROS accumulates in growing wild type root hairs but
5 dramatically decreases in *rhd2* short hair mutants and the generation of ROS is inhibited and
6 mimics *rhd2* mutant and further showed (15) a RhoGTPase GDP dissociation inhibitor controls
7 spatial ROS production during root hair development.

8 To determine whether H₂O₂ is necessary for cotton fiber initiation, we firstly used SEM
9 to observe fiber initiation and development dynamics starting ovules at 2 days of pre-anthesis to
10 2 days of post-anthesis. In the dominant N1 mutant (Fig. 1), no fiber initials are present on ovule
11 at anthesis and very rare fiber initials occur on the ovules at 2 days of post anthesis. The
12 recessive mutant n2 does not show significant change for fiber initials. The double mutants N1n2
13 generated by crossing N1 and n2 and selecting till 7 generation does not show any fiber initiation.
14 XZ142-fl, a lintless and fuzzless mutant originated from its wild type XZ142, also does not
15 produce any fibers.

16 In higher plants ROS can probably be produced by several alternative pathways (16)
17 including a cell-wall-localized peroxidase, amine oxidases and non-flavin NADPH oxidases, or
18 NADPH oxidases that resemble the flavin-containing type that is activated by Rac in leukocytes
19 (17). Simultaneously, a vast network of antioxidants is acting like ROS scavengers of H₂O₂ and
20 maintains ROS homeostasis (18). This scavenging system consists of catalase (CAT), ascorbate
21 (APX) and secretory peroxidases (POX), glutathione reductases (GR) and peroxiredoxines
22 (PRX), and non-enzymatic compounds like tocopherols, ascorbic acid and flavonoids. (19). Li et
23 al., (20) identified a cotton cytosolic APX1 (*GhAPX1*) to be highly accumulated during cotton

1 fiber elongation by proteomic analysis and showed (21) ethylene is induced in in vitro cultured
2 ovules by endogenous H₂O₂ and found a feedback regulatory system between H₂O₂ and
3 ethylene in fiber development. Hovav et al., (22) found three genes modulating hydrogen
4 peroxide levels were consistently expressed in domesticated and wild cotton species with long
5 fibers, but not detected by q-PCR in wild species with short fibers. Potika et al., (23) Potikha et
6 al., 1999) speculated H₂O₂ may function as a developmental signal in the differentiation of
7 secondary walls in cotton fibers with support of coincidence of H₂O₂ generation with the onset
8 of secondary wall deposition and prevention the wall differentiation because of inhibition of
9 H₂O₂ production or removing the available H₂O₂. H₂O₂ modulates downstream signaling
10 events including calcium mobilization protein phosphorylation and gene expression (24) which
11 may lead to cotton epidermal cell fate determination. To determine whether H₂O₂ involves fiber
12 initiation, we used 0.1mg/mL NBT (Nitro Blue Tetrazolium), a chemical specifically staining
13 H₂O₂ in living cells (Fig.2). Fig.2A timely profiled a normal cotton fiber development starting 5
14 days of pre-anthesis to fiber maturation at 50 days of post-anthesis. Fig.2B demonstrated that
15 TM1 ovules stained darkly at 2 days of pre-anthesis to 2 days of post-anthesis has already
16 accumulated H₂O₂ in comparison to these from N1n2. Significantly, in contrast to TM1 ovules,
17 no any staining was detected on *Arabidopsis* and Cotton leaf trichomes (Fig.2C). We also used
18 cut cotton leaf edge as positive, dark staining was found at the cut leaf edge and spots with high
19 concentration of polyphenols (Fig.2C). To rule out the possibility of staining accessibility, we
20 conducted H₂O₂ production measurement using an Amplex red hydrogen peroxide/peroxidase
21 assay kit (Molecular Probes, <http://probes.invitrogen.com/>). We found significant decrease of
22 H₂O₂ in ovules of different stages of N1n2 comparing with those from TM, especially at the 2
23 DPA (Fig.2D).

1 To verify whether H₂O₂ directly influence epidermal cell differentiation and initiation,
2 we conducted in vitro ovule cultures by adding DPI (H₂O₂ inhibitor) and SHAM (H₂O₂
3 activator. The result (Fig.3A) shows the fiber initiation normally developed in the control (with
4 0.1% DMSO) in vitro ovule culture and NTB staining shows H₂O₂ accumulation in the fiber
5 initiation and epidermal cells. Fig.3B shows the fiber initiation was almost totally retarded in the
6 in vitro ovule culture with addition of 0.3 μM DPI and the NTB staining shows barely H₂O₂
7 accumulation. Interestingly, Fig.3C shows very similar results with Fig.3A. We cannot conclude
8 SHAM enhances fiber initiation as a more controlled experiment is needed for this result. Based
9 on this data, we can efficiently conclude that H₂O₂ directly involves cotton fiber initiation and
10 development.

11 To molecularly characterize the mechanism of fiber initiation, many researchers (25, 26)
12 proposed the mechanism similarity of cotton fiber development to *Arabidopsis* trichome and
13 study cotton fiber initiation by cloning cotton orthologs for *Arabidopsis* trichome development.
14 Three criteria were used to characterize whether the genes of interest relate to cotton fiber
15 initiation including (i) orthologs to *Arabidopsis* trichome related genes; (ii) highly expressed in
16 cotton ovules and fiber initials and (iii) specific expression in *Arabidopsis* transgenic Col-0
17 trichome by cotton orthologous promoter-driven GUS and complementation of trichome
18 development for *Arabidopsis* trichomeless mutants by overexpression of cotton ortholog and the
19 varied influences to cotton fiber development in loss-of-function and gain-of function transgenic
20 cotton (27). Not like *Arabidopsis* trichome, cotton fibers are initiated from seed epidermal cells,
21 their initiation and development highly synchronize and unbranch with extreme elongation (28).
22 To verify the reasonability of the speculation, we roughly grouped the documented cotton fiber

1 development related genes into three groups (S-table 1) including ROS-related, fiber initiation-
2 related and fiber elongation-related.

3 Transcript profiling for ROS-related genes including *Ghmir398*, with its two targets,
4 *GhCSD1* and *GhCSD2*, *GhAPX*, *GhRDL1* and *GhRDL2*, only *GhRDL1* and *GhRDL2* show
5 consistently lowest expression in the two lintless fuzzless mutants but significantly increase
6 expression starting 2 days of pre-anthesis and post-anthesis (fig.1S). The expression of these
7 genes helps maintain homeostasis of H₂O₂, an important signal for cotton fiber initiation and
8 development. We grouped cotton orthologs based on homology to Arabidopsis leaf trichome
9 development into cotton fiber-initiation-related genes. We do not find specific correlation of
10 these group genes to the two lintless fuzzless mutants. Interestingly, all of these genes express in
11 the cotton expanded leaves which produce trichomes (fig.2S). This result suggests these genes
12 may be cotton leaf trichome orthologs but not cotton seed fiber genes.

13 Interestingly, Walford et al., report (29) RNAi cotton transgenic plants for *GhMYB25-like*
14 resulted in fibreless seeds but developed with normal trichomes elsewhere, mimics the Xu142 fl
15 mutant. Furthermore, these plants had decreased expression of the fibers related MYBs including
16 *GhMYB25* and *GhMYB109*, suggesting these *MYBs* are downstream of *GhMYB25-like*.
17 *GhMYB25-like*, not an ortholog to Arabidopsis trichome genes, is identified from its reduced
18 expression in a fibreless mutant of cotton (Xu142 fl) (30) Du et al., 2001). These data implicate
19 cotton fiber initiation and development may share similar regulatory process with *Arabidopsis*
20 trichome but the involved genes are different. It is important to note that Arabidopsis seeds do
21 not have seed trichomes like cotton and thus cannot be considered as an ideal surrogate model to
22 evaluate the specificity of expression of cotton fiber genes. The search for cotton fiber initiation
23 and development genes is still elusive. We also detect the expression of the third group of

1 documented fiber related gene, fiber elongation genes, lowest to undetectable expression of
2 *GhMYB25* and *GhEXPI* (fig.3S) relate to the two lintless fuzzless mutants. This implies these
3 two genes are acting downstream and necessary for fiber elongation.

4 Cell morphogenesis is the process by which cells acquire shape during development and
5 is central to organogenesis in multicellular organisms. Based on the currently available results,
6 Qin et.al. (37) speculated that fiber cells may elongate via a combination of both tip-growth and
7 diffuse-growth modes and named linear cell-growth mode. Using NBT staining ovules at
8 different development stages between TM1 and N1n2, a lintless-fuzzless doubled mutant
9 originated from TM, we show that H₂O₂ accumulation in cotton ovule epidermal cells but no
10 H₂O₂ staining in *Arabidopsis* and cotton leaf trichomes. We propose that the directional
11 accumulation of H₂O₂ in cotton ovule epidermal cell is the drive for cotton fiber initiation and
12 elongation.

13

14 **References and Notes**

- 15 1. L. M. A. Dirk, T. A. Wilkins, J. M. Stewart, *et al.*, Eds. *Physiology of Cotton*, (2010).
- 16 2. D. W. Galbraith, *Comp. Funct. Genom.* **4**, 208–15 (2003).
- 17 3. B. M. Lange, *Curr. Opin. Plant Biol.* **8**, 236–41 (2005).
- 18 4. T. A. Wilkins, J. A. Jernstedt, in *Cotton Fibers*, A. S. Basra, Ed. (1999), vol. 269, pp. 1–34.
- 19 5. A. B. Arpat *et al.*, *Plant Mol. Boil.* **54**, 911–29 (2004).
- 20 6. Y. L. Ruan, D. J. Llewellyn, R. T. Furbank, *Plant Cell* **13**, 47–60 (2001).
- 21 7. J. M. Stewart, *Amer. J. Bot.* **62**, 723–730 (1975).

- 1 8. N. Yu *et al.*, *Plant Cell* **22**, 2322–35 (2010).
- 2 9. J. Holt, J. M. Stewart, *Proc. Beltwide cotton conf.* 1320–1324 (1994).
- 3 10. S. C. Tiwari, T. A. Wilkins, *Canad. J. Bot.* **73**, 746–757 (1995).
- 4 11. D. Zhang, T. Zhang, W. Guo, *J. Plant Physiol.* **167**, 393–9 (2010).
- 5 12. F. A. Andriunas *et al.*, *J. Exp. Bot.* **63**, 3617–29 (2012).
- 6 13. C. E. Offler *et al.*, *Annu. Rev. Plant Biol.* **54**, 431–54 (2003).
- 7 14. J. Foreman *et al.*, *Nature* **422**, 442–6 (2003).
- 8 15. R. J. Carol *et al.*, *Nature* **438**, 1013–6 (2005).
- 9 16. A. C. Allan, R. Fluhr, *Plant Cell* **9**, 1559–1572 (1997).
- 10 17. T. Xing, V. J. Higgins, E. Blumwald, *Plant Cell* **9**, 249–59 (1997).
- 11 18. V. D. Petrov, F. Van Breusegem, *AoB Plants* **2012**, 1–13 (2012).
- 12 19. G. Miller *et al.*, *Plant, Cell & Environ.* **33**, 453–67 (2010).
- 13 20. H. B. Li *et al.*, *New Phytol.* **175**, 462–71 (2007).
- 14 21. Y.M. Qin *et al.*, *Plant Signal. & Behav.* **3**, 194–196 (2008).
- 15 22. R. Hovav *et al.*, *PLoS Genet.* **4**, e25 (2008).
- 16 23. T. S. Potikha *et al.*, *Plant Physiol.* **119**, 849–58 (1999).
- 17 24. L. Xiong *et al.*, *Plant Cell* **14**, S165–S183 (2002).
- 18 25. L. Serna, C. Martin, *Trends Plant Sci.* **11**, 274–80 (2006).
- 19 26. S. Wang *et al.*, *Plant Cell* **16**, 2323–2334 (2004).

- 1 27. X.Y. Guan *et al.*, *Physiol. Plantar.* **134**, 174–82 (2008).
- 2 28. H. J. Kim, B. A. Triplett, *Plant Physiol.* **127**, 1361–1366 (2001).
- 3 29. S. A. Walford, *et al.*, *Plant J.* **65**, 785–97 (2011).
- 4 30. X. Du *et al.*, *Plant Breed.* **120**, 519–522 (2001).
- 5 31. Y.-M. Qin, Y.-X. Zhu, *Curr. Opin. Plant Biol.* **14**, 106–11 (2011).

6

7 **Acknowledgments:** We thank Dr. Nouredine Abidi for help with Scanning Electron
8 Microscopy (SEM) at Fiber and Biopolymer Research Institute (FBRI) at Texas Tech University
9 and funding by the Texas Governor's Emerging Technology Superior Research Award granted to
10 T.A.W.

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

1 Fig. 1. Altered fiber initiation and development in two pair of naked seed mutants with their wild
2 types. (A) Almost all fiber initials occur at 0 DPA in wild type TM1. (B) Few fiber initials occur
3 at 0 and 2 DPA in dominant mutant N1. (C) Many fiber initials occur at 0 and 2 DPA in receive
4 mutant *n2*. (D) No fiber initials developed on *N1n2* double mutant. (E) Almost all fiber initials
5 occur at 0 DPA in wild type XZ142. (F) No fiber initials produced on ovules of XZ142-fl. Black
6 Bar = 200um.

7
8
9 Fig.2 Alteration of H₂O₂ accumulation by NBT staining and measurement. (A) The time scale
10 indicates the duration of epidermal cell initiation, fiber initial elongation, cellulose biosynthesis
11 and maturation. (B) Ovules from TM1 and N1n2, stained with NBT (C). Leaf trichomes from
12 Arabidopsis and TM1 and TM1 leaf cut edge, stained with NBT. (D). More H₂O₂ accumulation
13 in ovules from TM1 at different stages comparing to those from N1n2. Red Bar=1mm.

14
15 Fig.3. In Vitro Ovule Cultures Treated with DPI and SHAM and Stained with NBT.
16 Cotton ovules from TM1 plants were collected at 0 DPA, treated with DPI (inhibitor at 0.3μM)
17 or SHAM (activator at 1.5mM) dissolved in DMSO separately for 48 Hrs, and stained with NBT
18 for 2.5 Hrs. Red Bar=1mm.

19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

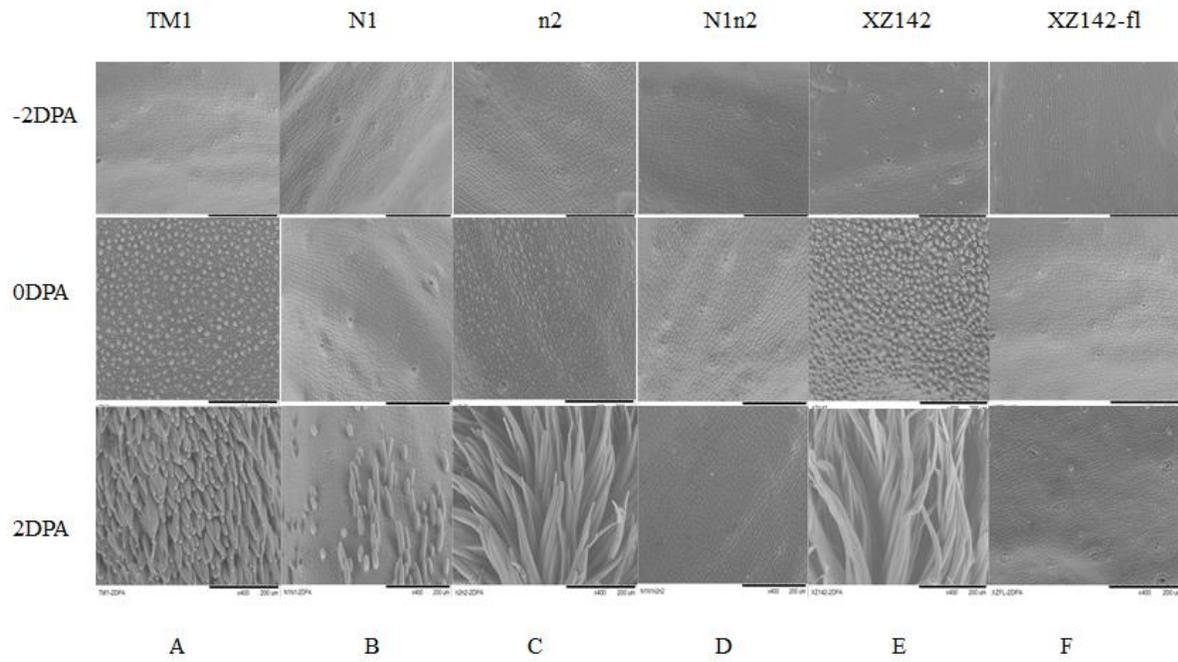


Fig.1.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26

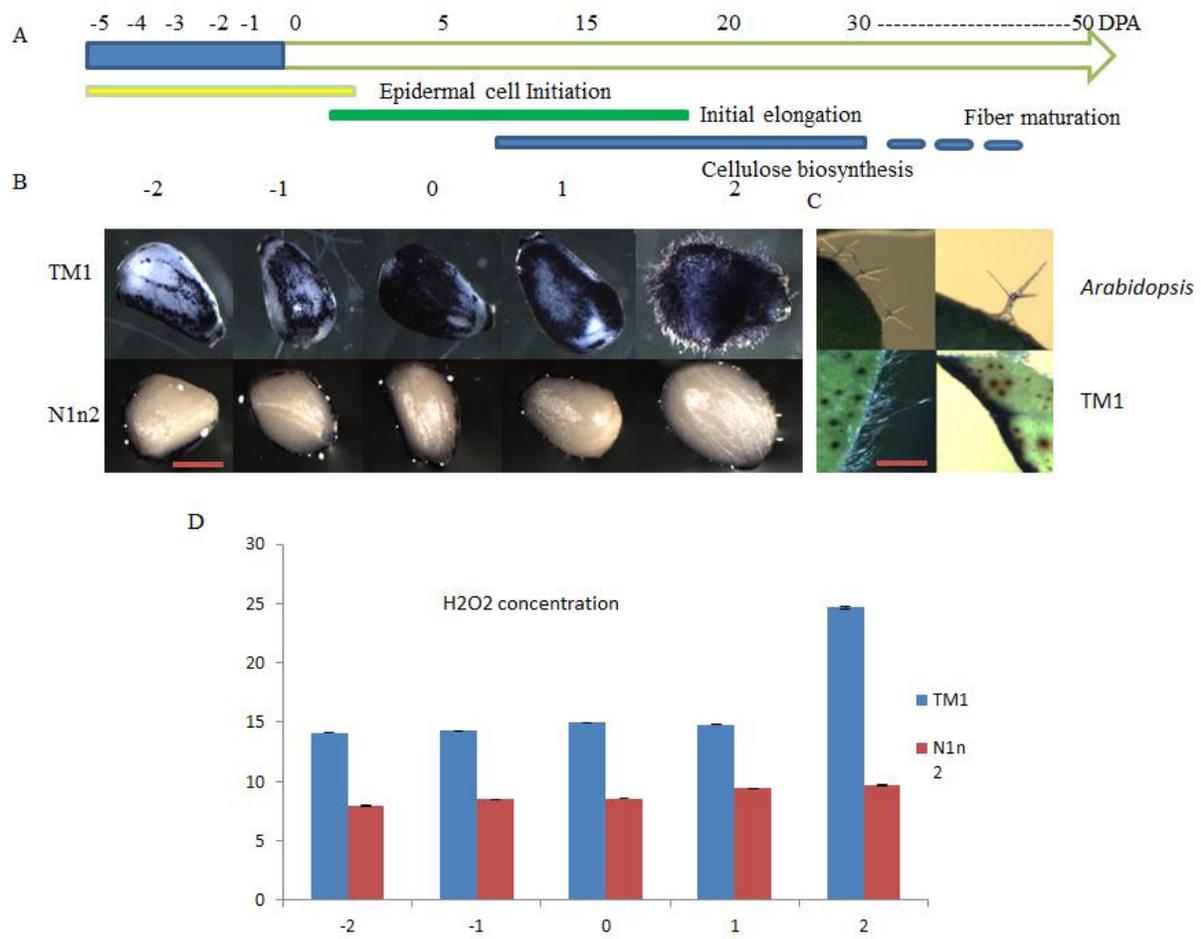


Fig. 2.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21

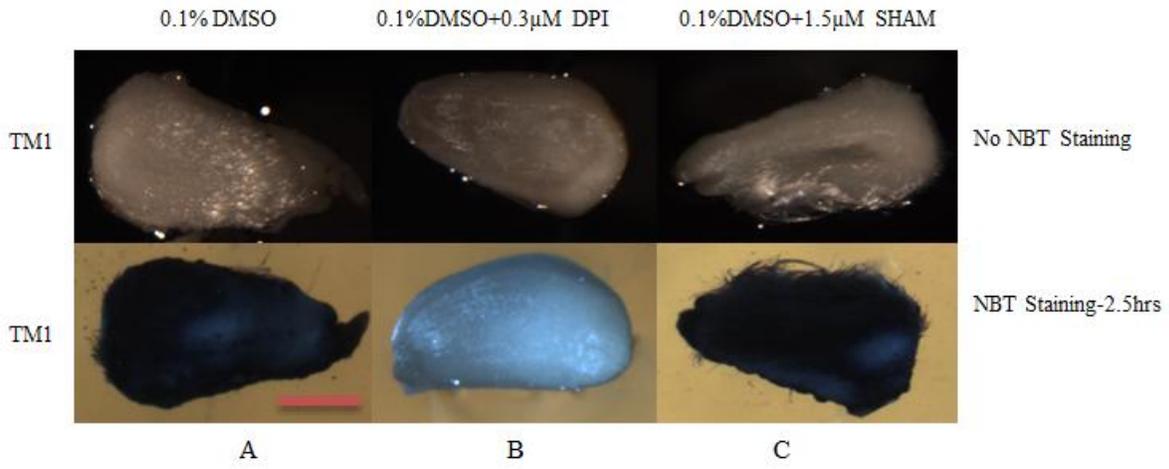


Fig.3.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32

1 Supplementary Materials

2

3

4 Materials and Methods

5

6 Figs. S1 to S3

7

8 Tables S1

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

1 **Materials and Methods**

2 **Plant Materials and Scanning Electron Microscopy**

3 Upland cotton (*Gossypium hirsutum* L. cv TM-1), its isogenic naked seed mutants (N1N1 and
4 n2n2), the double mutant N1n2 and XZ142 with its fiberless fuzzless mutant XZ142-fl were
5 grown in the greenhouse under 32/21°C (day/night) temperature regime. Ovules were collected
6 at -2, 0 and 2 dpa flowers at 9 and 10 A.M. Hitachi tabletop TM-1000 scanning electron
7 microscope was used for imaging the fresh ovules.

8 **Grouping and QRT-PCR Primers of Potential Genes Related to Fiber Development**

9 We grouped the potential genes related to fiber development into three categories
10 including ROS-related, fiber initiation and elongation (Table-S1).

11 **Imaging and ROS Staining for TM1 and N1n2 Ovules at -2DPA to 2DPA**

12 Ovules from TM1 and N1n2 at -2, -1, 0, 1 and 2 DPA were covered with freshly made
13 NBT (nitroblue tetrazolium) solution (in 0.1M potassium phosphate at pH7). Plates were left for
14 2.5 hrs at room temperature for the color to develop. Ovules were imaged under bright-field
15 illumination using an Olympus SZH10 Zoom Stereo Microscope.

16 **H2O2 Measurement**

17 To measure H2O2 concentration, ovules at different stages were ground in liquid
18 nitrogen, and 200 mg of ground tissues from each sample was added to 200 µL of 20 mM
19 sodium phosphate buffer (pH 6.5) and mixed well and centrifuged at 9500g for 10 min. at 4°C.
20 The supernatants were used for assay. H2O2 concentration was measured using an Amplex red
21 hydrogen peroxide/peroxidase assay kit (Molecular Probes: <http://probes.invitrogen.com/>).

22

23

1 ***In Vitro Ovule Culture and Treatment with H₂O₂ Inhibitor and Activator***

2 Cotton ovules from TM1 plants were collected at 0 DPA, soaked in 70% ethanol for 1
3 min, rinsed in distilled and deionized water and placed in liquid media formulated by Beasley
4 and Ting (S8) in a 6-well plate under aseptic conditions. The ovules were cultured in vitro, as
5 described by Meinert and Delmer (S9). DPI (inhibitor at 0.3μM) or SHAM (activator at 1.5mM)
6 dissolved in DMSO was added to medium separately. The final concentration of DMSO was
7 0.1% in all of the samples.

8 ***RNA Preparation and QRT-PCR***

9 Total RNA was extracted from ovules, young expanded leaves using the Spectrum™
10 Plant Total RNA Kit and on-column DNase I digestion, following the manufacturer's
11 recommendations (Sigma-Aldrich). RNA was quantified using nondrop-1000 and the integrity of
12 total RNA was confirmed by denaturing agarose gel electrophoresis. QRT-PCR amplification
13 reactions were performed using SYBR Green detection chemistry and run in triplicate on 96-
14 wells plates with the 7500 Real Time PCR system (Applied Biosystems). Reactions were
15 prepared in a total volume of 20 μL containing: 5 μL of template, 1 μL of each amplification
16 primer (10uM), 10 μL of 2× Power SYBR Green PCR Master Mix (Applied Biosystems) and 3
17 μL PCR-quality ddH₂O. Cotton Histone-3 (AF024716) was used to normalize the amount of
18 gene-specific RT-PCR products. All PCR reactions were conducted in three replicates and
19 monitored the absence of primer dimers using a dissociation analysis. The amplification data
20 were analyzed using ABI7500 SDS software (version 1.2.2), and the relative expression levels to
21 expression of Histone-3 (fold changes) were calculated. For detecting expression of
22 *GhmiRNA398*, protocol followed (S7) using QRT-PCR primer GTCGTATCCA
23 GTGCAGGGTCCGAGGTATTCGCACTGGATAACGACAAGGGG and forward primer

1 ACATTCAAGTGTGTTCTCAGGTCA and universal QRT-PCR miRNA reverse primer
2 GTGCAGGGTCCGAGGT.

3 **Fig.S1.** Transcripts profiling for ROS homeostasis-related genes. (A) Gene expression for
4 *Ghmir398a+b* in different varieties and development stages. (B) Gene expression for
5 *GhCSD1(S2)* in different varieties and development stages. (C) Gene expression for *GhCSD2(S2)*
6 in different varieties and development stages. (D) Gene expression for *GhAPX1(1δ)* in different
7 varieties and development stages. (E) Gene expression for *GhRDL1(2δ)* in different varieties
8 and development stages. (F) Gene expression for *GhRDL2(2δ)* in different varieties and
9 development stages.

10 **Fig.2S.** Transcripts profiling for potential fiber initiation related genes. (A) Gene expression for
11 *GhMYB2(2δ)* in different varieties and development stages. (B) Gene expression for *GhCPC(S3)*
12 in different varieties and development stages. (C) Gene expression for *GhHOX1(2δ)* in different
13 varieties and development stages. (D) Gene expression for *GhHOX2(2δ)* in different varieties
14 and development stages. (E) Gene expression for *GhHOX3(2δ)* in different varieties and
15 development stages. (F) Gene expression for *GhDEL65(S4)* in different varieties and
16 development stages.

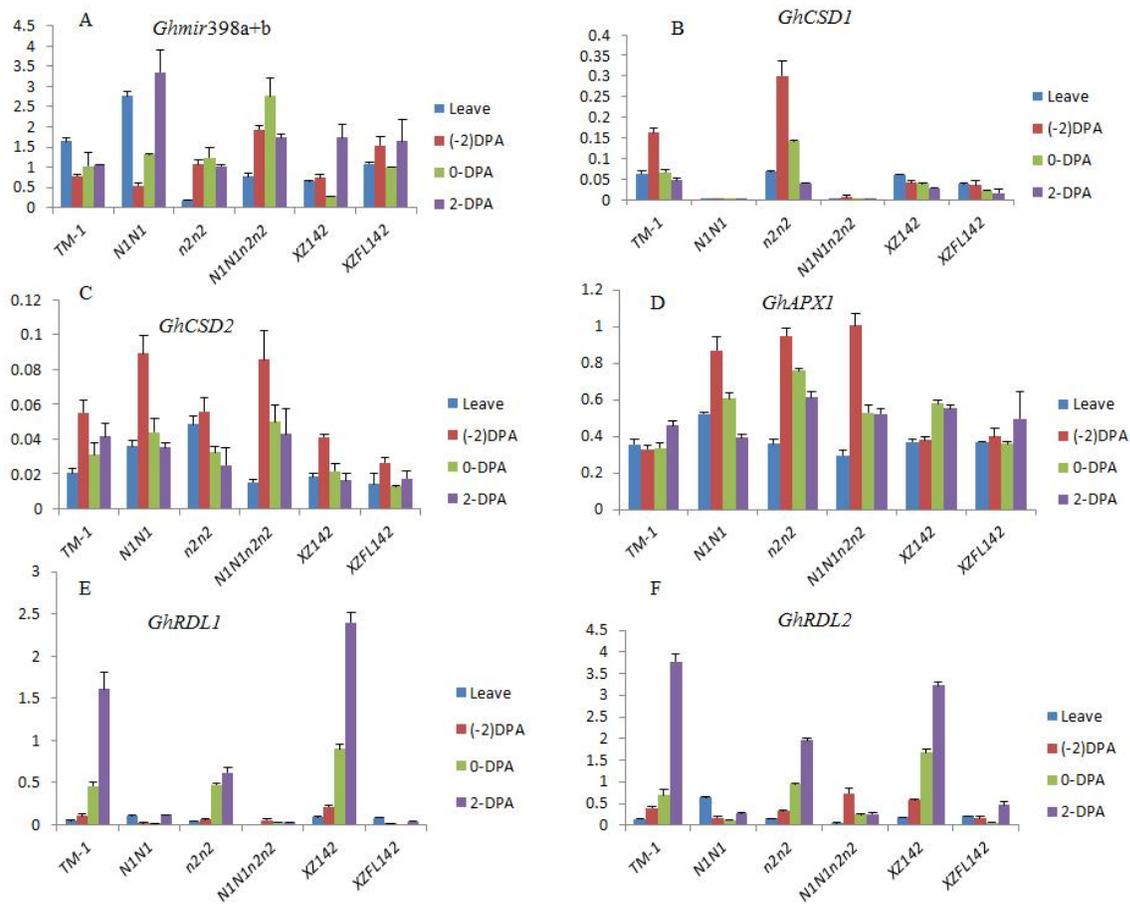
17 **Fig.3S.** Transcripts profiling for potential fiber elongation related genes. (A) Gene expression for
18 *GhMYB25(29)* in different varieties and development stages. (B) Gene expression for
19 *GhMYB25-L(29)* in different varieties and development stages. (C) Gene expression for
20 *GhPDF1(S5)* in different varieties and development stages. (D) Gene expression for *GhMYB109*
21 *(S6)* in different varieties and development stages. (E) Gene expression for *GhEXP1(S7)* in
22 different varieties and development stages.

23 **Table S1.** QRT-PCR primers for potential fiber initiation and development genes
24

1 **Supplementary References**

- 2 S1. E. Varkonyi-Gasic, *et al.*, *Plant meth.* **3**, 12 (2007).
- 3 S2. H. J. Kim, N. Kato, S. Kim, B. Triplett, *Planta* **228**, 281–92 (2008).
- 4 S3. E. W. Taliercio, D. Boykin, *BMC plant boil.* **7**, 22 (2007).
- 5 S4. X.-X. Shanguan, B. Xu, Z.-X. Yu, L.-J. Wang, X.-Y. Chen, *J.Exp. Bot.* **59**, 3533–42 (2008).
- 6 S5. F. Deng *et al.*, *Plant physiol.* **158**, 890–904 (2012).
- 7 S6. L. Pu, Q. Li, X. Fan, W. Yang, Y. Xue, *Genetics* **180**, 811–20 (2008).
- 8 S7. S. E. Harmer, S. J. Orford, J. N. Timmis, *Mol. Genet. Genom.* **268**, 1–9 (2002).
- 9 S8. C.A. Beasley, I.P. Ting, *Am. J. Bot.* **60**, 130–9 (1973).
- 10 S9. M. C. Meinert, D. P. Delmer, *Plant Physiol.* **59**, 1088–97 (1977).

11
12
13
14
15
16
17
18
19
20
21
22
23
24
25



1

2

3 Fig.S1.

4

5

6

7

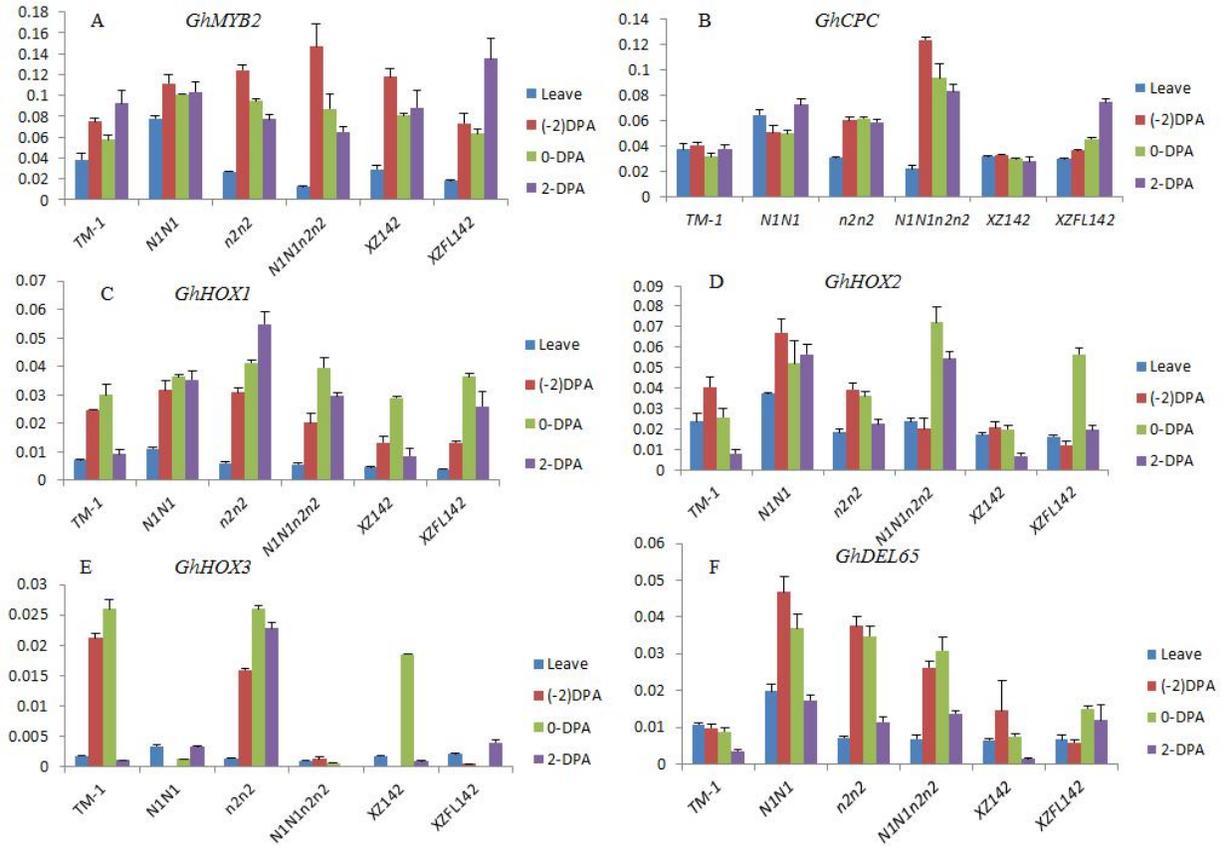
8

9

10

11

12



1

2 Fig.S2.

3

4

5

6

7

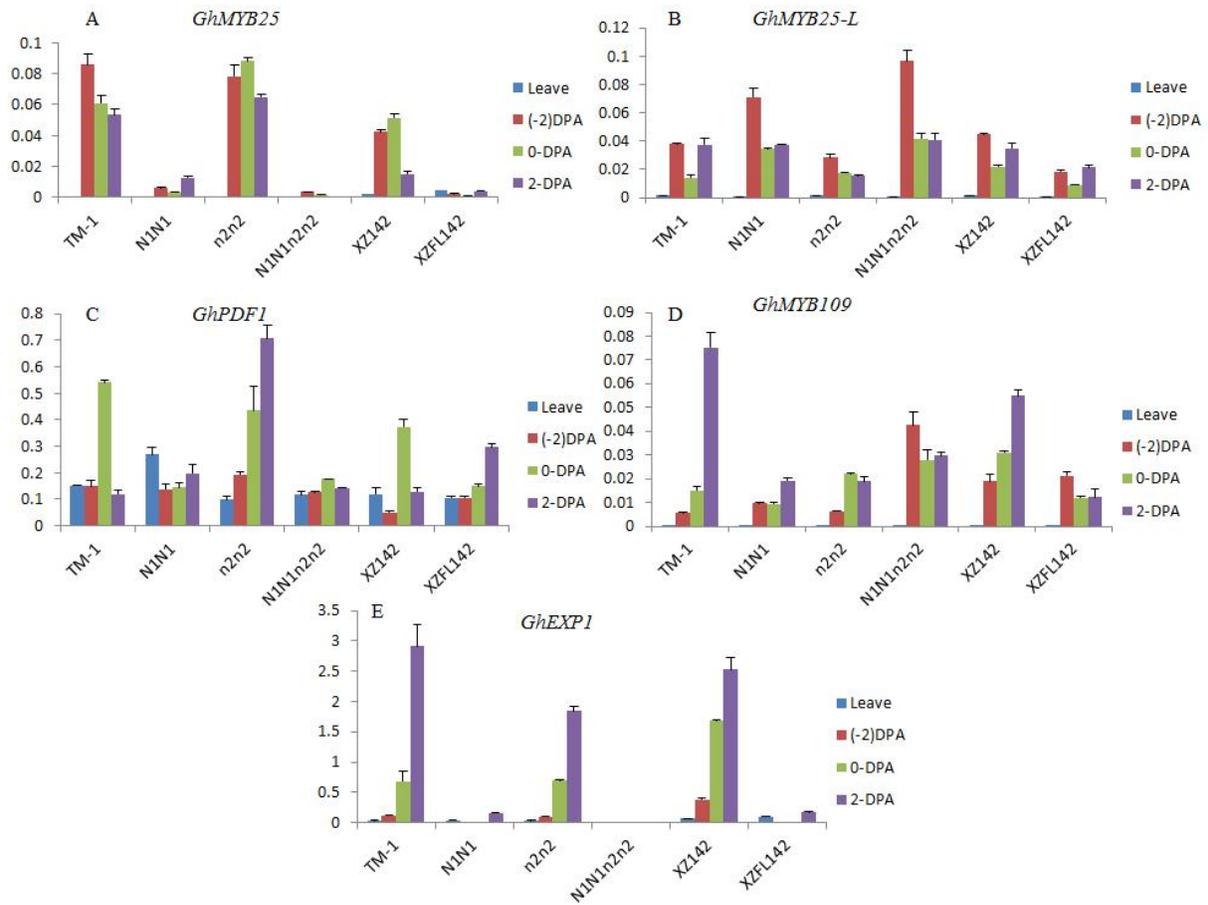
8

9

10

11

12



1

2

3

4 Fig.S3.

5

6

7

8

9

10

11

12

1

2 Table S1. QRT-PCR primers for potential fiber initiation and development genes

3

Categories	Genes	NCBI-ID	Forward primers	Reverse primers	
ROS-related	<i>GhCSD1</i>	F415203	TCTTCTCCCAAGAAGGAGATGG	TTTGTCGGTGATGGAGAAGCTA	
	<i>GhCSD2</i>	EU597268	TGCGGGTGACCTGGGAAACATAAT	ATGCTCTTCCAACAACCGCATTGG	
	<i>GhAPX1</i>	EF432582	TGATTGGTCGACTCAAAACG	GCCCCAATATTTCCCATCTT	
	<i>GhRD22-L1</i>	AY072821	AACAAACCCCAATGCAAAAAG	TTGGGTTCCATGCTGATGTA	
initiation	<i>GhRD22-L2</i>	AY641991	CAGCTGGAGTGCAGAAGATG	GTGTGACAGACTGCTACTGC	
	<i>GaMYB2</i>	AY626160	ATGGCTCCAAAGAAGGATGGAGT	GCCGCATCGATTCAAACCTGA	
	<i>GhCPC</i>	FJ402930	TGGCTGACTCTCAACATTCCT	GTCTACCGGGATTCTTCC	
	<i>GaHOX1</i>	AF530913	GAAATTGGCCAGAGAAATGA	GAGTCGGAGAGACAGGCAAC	
	<i>GaHOX2</i>	AF530914	TGCGAAAGGGTTGCTAGTCT	ATTGGGTTGACCTGCTTAC	
	<i>GaHOX3</i>	AY626159	CCTTCAAAGGATGTGCGAAT	ATTCGAACCGGAAAGTGTG	
	<i>GhDEL65</i>	AF336280	CGAAAGGTCGTTCTCTTTG	GCTTCAAGCCATTTTCTGC	
	<i>GhPDF1</i>	AF141375	GTCCCCCACTACTCCCATT	CAAGCTCATGCTTGTTCCAA	
	<i>GhMYB25</i>	AF336283	CAATGGGAGAGTGCTCGTTT	CAACGTTGACGTAGGGGACT	
	<i>GhMYB25-L</i>	HM134081	GAGAAATCGAGCCAAGTTGC	ATCCCCAGAATCACAAACCA	
	<i>GhMYB109</i>	AY072821	TGGAACAACCACTGAAACCA	TGGTCAATCATGGCAAGTGT	
	elongation	<i>GhEXP1</i>	AF043284	TCCAGAACAGGATGGCTACC	AGTTTGTCCGAATTGCCAAC

4

5

6

7

8