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# The HD-Zip IV gene *GaHOX1* from cotton is a functional homologue of the *Arabidopsis GLABRA2*

Xue-Ying Guan, Qian-Jin Li, Chun-Min Shan, Shui Wang, Ying-Bo Mao, Ling-Jian Wang and Xiao-Ya Chen*

National Key Laboratory of Plant Molecular Genetics, Institute of Plant Physiology and Ecology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200032, China

**Correspondence**
*Corresponding author,
e-mail: xychen@sibs.ac.cn

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Most of the plant homeodomain-containing proteins play important roles in organ patterning and development, and *Arabidopsis* GLABRA2 (GL2), a member of the class IV homeodomain-leucine zipper (HD-ZIP) proteins, is a trichome and non-root hair cell regulator. Here we report the analysis of two cotton homeodomain-containing proteins, GaHOX1 and GaHOX2, isolated from the diploid cotton *Gossypium arboreum*. Both GaHOX1 and GaHOX2 belong to the class IV HD-ZIP family. When expressed under the control of the *GL2* promoter, *GaHOX1* rescued trichome development of an *Arabidopsis* glabrous mutant of *gl2-2* (SALK_130213), whereas *GaHOX2* did not. On the other hand, expression of *GaHOX1* with a Cauliflower mosaic virus (CaMV) 35S promoter in the wild-type *Arabidopsis* plants suppressed the trichome development just as the *GL2* ectopic expression. Expression analysis by Northern, RT-PCR and in situ hybridization indicated that *GaHOX1* is predominantly expressed in cotton fiber cells at early developmental stages, consistent with its putative role in regulating cotton fiber development, while *GaHOX2* is expressed in both fiber and other ovular tissues, including outer and inner integuments. Our results suggest that GaHOX1 is a functional homolog of GL2 in plant trichome development.

## Introduction

Trichomes, or epidermal hairs, are frequently found on the above-ground organs of terraneous plants. A number of angiosperm plant species, such as cotton (*Gossypium* spp.), willow (*Salix* spp.) and *Asclepias curassavica*, produce seed trichomes. Cotton seed hair is known as cotton fiber; both cotton fibers and *Arabidopsis* epidermal trichomes are of single cells, and cotton fibers are distinct in that they are highly elongated, rarely branched, and composed of nearly pure cellulose when mature. Investigations of molecular mechanisms that control cotton fiber development not only explore important genes for improving the cotton fiber quality and production, but also provide valuable data for understanding plant cell differentiation.

The molecular mechanism underlying *Arabidopsis* trichome development has been characterized in depth. A series of transcription factors involved in regulating trichome development have been isolated. Among the positive regulators characterized, there are two MYB factors, GLABRA1 (GL1) (Larkin et al. 1994) and AtMYB23 (Kirik et al. 2001), a WD40 protein, transparent testa GLABRA1 (TTG1) (Walker et al. 1999), two basic helix–loop–helix proteins, GLABRA3 (GL3) (Esch et al.

*Abbreviations* – CPC, caprice; EGL3, enhancer of GL3; ETC1, enhancer of TRY and CPC1; HD-ZIP protein, homeodomain-leucine zipper protein; GL2, GLABRA2; DPA, day postathesis; *fl*, fuzzless–lintless; START, steroidogenic acute regulatory protein-related lipid transfer; TCL1, trichomeless1; TRY, triptychon; TTG1, transparent testa GLABRA1.

2003; Payne et al. 2000) and enhancer of GL3 (EGL3) (Bernhardt et al. 2005) and a homeodomain protein, GLABRA2 (GL2) (Szymanski et al. 1998). All the loss-of-function mutants of these genes either are glabrous or have a decreased number of trichomes on epidermis. Three MYB proteins that lack a transactivation domain, triptychon (TRY) (Hülskamp et al. 1994, Schellmann et al. 2002), caprice (CPC) (Schellmann et al. 2002, Wada et al. 1997) and enhancer of TRY and CPC1 (ETC1) (Kirik et al. 2004a, Kirik et al. 2004b), are negative factors of trichome cell initiation. Recently a new single-repeat MYB-type transcription factor, trichomeless1 (TCL1), was also found to negatively regulate trichome formation in the inflorescence epidermis (Wang et al. 2007). Based on the interaction and genetic dissection of these transcription factors, a trichome promoting model has been proposed (Guan et al. 2007, Schiefelbein 2003), in which GL1, GL3, EGL3 and TTG1 form a regulatory core complex for trichome initiation. Once trichomes are initiated, this complex promotes the expression of downstream genes, such as *TRY* and *GL2*.

In cotton genes encoding several types of transcription factors are found expressed in developing fiber cells and some of them show high sequence similarities to *Arabidopsis* trichome regulators (Guan et al. 2007, Serna and Martin 2006). Three cotton *MYB* genes that encode R2R3 MYB proteins, *GhMYB109* (Suo et al. 2003), *GhMYB2* (Cedroni et al. 2003, Loguerico et al. 1999), *GaMYB2* (Wang et al. 2004) and *GhMYB25* (Wu et al. 2006), are all expressed in the initial and elongating cotton fibers. We previously reported that *GaMYB2*, isolated from the A-genome diploid cotton *Gossypium arboreum*, encodes an *Arabidopsis* GL1 homolog and is predominantly expressed in developing cotton fibers at initiation and early elongation stages. After transferring into the *Arabidopsis gl1* mutant, GaMYB2 rescued trichome formation (Wang et al. 2004). Four *TTG1*-like genes were isolated from the ancestral D diploid genome of the tetraploid upland cotton *Gossypium hirsutum*. Two of them, *GhTTG1* and *GhTTG3*, were able to restore trichome formation in *Arabidopsis ttg1* mutant plants (Humphries et al. 2005). These data suggest that cotton fiber and *Arabidopsis* trichome share a similar molecular process that requires orchestrated functions of transcriptional factors (Guan et al. 2007, Serna and Martin 2006). However, the molecular events during cotton fiber cell initiation are still poorly understood.

In *Arabidopsis* GL2 is a positive regulator downstream of the GL1/GL3/EGL3/TTG1 core complex in trichome development pathway, and it negatively regulates root hair development in a similar manner (Di Cristina et al. 1996, Ohashi et al. 2003, Schiefelbein 2003, Szymanski et al. 1998). *GL2* encodes a homeodomain protein of the class IV HD-ZIP (homeodomain-leucine zipper) family. Homeodomain proteins constitute a large transcription factor family which plays fundamental roles in a diverse set of biological processes, including body plan specification, pattern formation and cell fate determination during organism development (Gehring et al. 1994). Plant homeodomain proteins can be classified into five families (or subfamilies), KNOX, WOX, BLH, Zn-HD and HD-ZIP. The HD-ZIP family is further divided into four classes (Sessa et al. 1998), with HD-ZIP III and IV proteins sharing two conserved domains, a DNA-binding HD and a START (steroidogenic acute regulatory protein-related lipid transfer) domain that has a putative steroid-binding activity (Nakamura et al. 2006). Such structure is unique to plant kingdom (Gehring et al. 1994, Ruberti et al. 1991). Genetic and phenotypic analysis indicated that GL2 is the only homeodomain protein taking charge in the *Arabidopsis* trichome development, with no homologs that share its function. Yet the functional homologs of GL2 from other plant species have not been reported.

We have isolated two HD-ZIP IV genes, *GaHOX1* and *GaHOX2*, from the A-genome diploid cotton, *G. arboreum*. *GaHOX1* is highly similar to *Arabidopsis GL2* and expressed predominantly in cotton fiber cells. After introducing into *Arabidopsis* plants GaHOX1 could functionally substitute GL2. We suggest that GaHOX1 participates in regulating cotton fiber development.

## Materials and methods

### Plant material and growth conditions

Cotton plants of *G. arboreum* cv. Qingyangxiaozi, *G. hirsutum* cv. Xuzhou-142 and a fuzzless–lintless (*fl*) mutant identified from cv. Xuzhou-142 (Du et al. 2001) were grown in greenhouse. Ovules were harvested at various developmental stages, and fibers were collected by scraping the ovule in liquid nitrogen. Plants of *Arabidopsis thaliana* (Columbia-0) were grown in greenhouse at 22°C under continuous illumination. The T-DNA insertion mutant of *gl2* (SALK_130213) was obtained from ABRC (Ohio State University, Columbus, OH), and named *gl2-2*. *Arabidopsis* tissues above the hypocotyl of the 2-week-old seedlings (four pairs of leaves) were collected for analysis, unless otherwise indicated.

### DNA and RNA analysis

Genomic DNA and total RNA were extracted as described (Wang et al. 2004). For Northern blot analysis, a total of 20 μg of RNA was loaded per lane, and an ethidium bromide-stained gel was used to monitor the amount of RNA loaded. Blotting probes were generated

using the Prime-a-Gene Labeling System (Promega, Madison, WI), and the PCR primers used were GaHOX1-F (ATATTACCTTCAGGCTTCTC) and GaHOX1-R (TCACCCATCTTCACATTGCA), GaHOX2-F (GAAGCAAATGGGCATAATAT) and GaHOX2-R (CTATTTTTCTTTACCATTGT), respectively.

For RT-PCR analysis, total RNAs of 1 μg were used for reverse transcription in a 20-μl reaction system with the RNA PCR (AMV) kit (TaKaRa, Dalian, China). Cotton *Histone3* (*AF024716*) and *Arabidopsis Tubulin* (*At5g62690*) were used as internal controls, and the primers used were HIS3-F (GGCATACCTTGTGGGTCTTTTTGA), HIS3-R (CTACCACTACCATCATGGC), TUB2-F (TTTAACAGAAGACTTGGAGCAA) and TUB2-R (CGTGACAACCGAGCAGAA). *TRY* (*At5g53200*), *GL1* (*At3g27920*), *GL2* (*At1g79840*) and *GaHOX1* expression were analyzed by RT-PCR with the following primers, GL2-F (ATATGGGTGCTGCAAGACAG), GL2-R (GGAGGATTTGGATGTTGCTT), GL1-F (CTCCACCGTCATTGTTCATC), GL1-R (ATACGACGCCGTTAAAGCTC), TRY-F (GAGAAACAGTGAAGGCTTTGC), TRY-R (ACGGTGAGGCTTGGTATGTT), GaHOX1-F and GaHOX1-R.

cDNAs of *GaHOX1* and *GaHOX2* were isolated from *G. arboreum* based on the EST data, and the accession numbers are EU328266 and EU330401, respectively. The genes or gene fragments were amplified by PCR from the reverse transcription product using Pyrobest DNA polymerase (TaKaRa), with the primers of GaHOX1-cDNA-F (ATGACTAACCCACCCACCAAA), GaHOX1-cDNA-R (CCCATCTTCACATTGCAAGCT), GaHOX2-cDNA-F (ATGACTGTGAGAGCAACAAAA) and GaHOX2-cDNA-R (TTTTTCTTTACCATTGTCA).

### Plant transformation

The cDNAs of *GaHOX1* and *GaHOX2* were cloned into pCAMBIA1301 vectors, respectively, after digestion with *Bam*HI and *Sma*I. Promoter of *GL2* (2.1 kb upstream of ATG) was amplified from *Arabidopsis* genomic DNA with the primers of GL2-PROM-F (TTGAATTGTAGATAAATC) and GL2-PROM-R (TGACATACAAATCCTGTCCCTAGCTA). The *GL2* or CaMV 35S promoter was inserted into pCAMBIA1301, respectively, after digestion with *Bam*HI and *Hin*dIII. *NOS* terminator was isolated from pBI121 by PCR with the primers of NOS-F (ACCGAGCTCGAATTTCCCCGAT) and NOS-R (AGTGAATTCCCGATCTAGTAACATA), then inserted into the *Eco*RI–*Sac*I site. Binary constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 and subsequently transferred into *Arabidopsis* using a floral dip method (Clough and Bent 1998). Transgenic plants were selected on media containing 50 mg $l^{-1}$ of hygromycin.

### In situ hybridization and scanning electron microscopy (SEM)

*G. arboreum* ovules were collected at 0 DPA (day postanthesis) and embedded in paraffin. *GaHOX1* and *GaHOX2* mRNAs were detected with a digoxigenin-labeled riboprobe using the method as described (Long and Barton 2000; http://www.wisc.edu/genetics/CATG/barton/protocols.html), and the probe templates used were the same as those for Northern analyses.

The cotton ovule surface was examined under a JSM-6360LV scanning electron microscope (JEOL, Tokyo, Japan).

## Results

### Sequence analysis of *GaHOX1* and *GaHOX2* from cotton

GhHOX1 (AAM97321), a homoedomain-containing protein of *G. hirsutum* L., shows a high identity of protein sequences to GL2. We then isolated the cDNA from the diploid A-genome cotton, *G. arboreum*, and named it *GaHOX1* (Wang et al. 2004). *GaHOX1* and *GhHOX1* are 99% identical at the nucleotide and 98% at the deduced amino acid sequence level. Searching of the database revealed that GaHOX1 and GhHOX1 are most similar to *Arabidopsis* GL2, with 66% amino acid sequence identities.

GhHOX2 is another homeodomain-containing protein, sharing 47 and 45% identities of amino acid sequences with *Arabidopsis* PDF2 and ATML1, respectively. We also isolated the cDNA of this gene from *G. arboreum*, and named it *GaHOX2* (Wang et al. 2004). The sequence identity between *GaHOX1* and *GaHOX2* is 50% at the nucleotide and 35% at the deduced amino acid sequence level. GaHOX2 and GhHOX2 are distantly related to GL2, with an amino acid sequence identity of 36%. We then used *GaHOX1* and *GaHOX2* throughout this investigation, as both were isolated from *G. arboreum*, which was used here for expression analysis.

In a recent analysis, GhHOX1 and GhHOX2 were placed in the HD-ZIP IV group (Nakamura et al. 2006). The *Arabidopsis* genome contains 16 genes encoding the class IV HD-ZIP proteins, including *GL2*, *PDF2* and *ATML1* (Nakamura et al., 2006). All the previously identified HD-ZIP IV proteins contain two domains: a HD and a START domain, so do the two cotton HOX proteins. The highest sequence identity is found in the HD, which is 94.2% between GaHOX1 and GL2 and 83.1% between GaHOX2 and PDF2. GaHOX2 and GL2 share a 64.4% sequence identity in the HD, and their overall identity is 36%. While GL2 plays a key role in trichome

development, PDF2 and ATML1 function in the shoot epidermal cell differentiation (Abe et al. 2003).

### Expression patterns of the *GaHOX1* and *GaHOX2*

To gain insights into the functions of *GaHOX1* and *GaHOX2*, we first analyzed their expression patterns. Examination of different tissues of *G. arboreum* plants by RT-PCR showed that *GaHOX1* was expressed in the 0 DPA ovule which contained the fiber initials. The transcripts were also present in other organs examined, although at a lower level, and the expression was particularly weak in the hypocotyl (Fig. 1A). In the ovule, *GaHOX1* expression was promoted as early as before anthesis and the transcript level increased along with cotton fiber development in the early stage (Fig. 1B). To further examine the spatial pattern of expression, we separated the fiber from the ovule by liquid nitrogen treatment. Northern blot showed that *GaHOX1* transcripts were enriched in fiber cells, and in the ovules from which the fibers were stripped off the transcripts were barely detectable (Fig. 1C). The fiber-preferential distribution of *GaHOX1* transcripts was also supported by analyzing the ovule of a fiberless-seed mutant, *fl* of *G. hirsutum*, in which the *HOX1* gene expression was extremely weak (Fig. 1C). In addition, from 3 to 12 DPA the transcript abundance of *GaHOX1* was gradually decreasing (Fig. 1C). These results indicate that *GaHOX1* is highly expressed in cotton fiber cells during fiber initiation and early elongation stages.

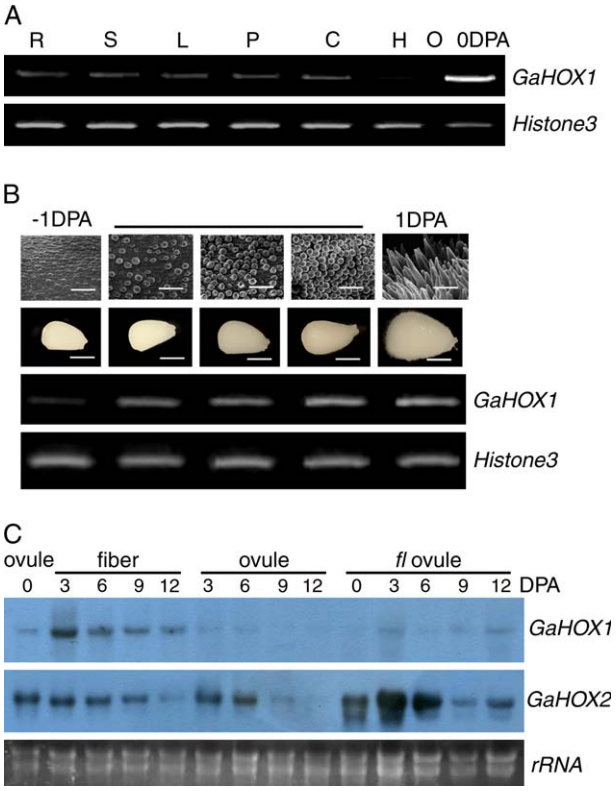


**Fig. 1.** *GaHOX1* expression pattern. (A) RT-PCR analysis of *GaHOX1* transcripts in *G. arboreum* roots (R), stems (S), leaves (L), petals (P), cotyledons (C), hypocotyls (H) and ovules (O, 0 DPA); PCR was performed in 32 cycles for *GaHOX1* and 25 cycles for *Histone3*. The roots, cotyledons and hypocotyls were collected at 10 days after germination, leaves and stems were collected at 15 days after germination. (B) Scanning electron micrographs showing the cotton fiber cells at an 8-h interval from 23:00 h of −1 DPA to 07:00 h of 1 DPA, and RT-PCR showing *GaHOX1* expression levels during this stage; (C) Northern blot of *GaHOX1* and *GaHOX2* transcripts in the cotton fiber and ovule during development, fiber and ovule were separated by stripping off the fiber in liquid nitrogen; the *fl* mutant ovule was also analyzed.

To further localize the *GaHOX1* transcripts, we performed in situ RNA hybridization. A high level of *GaHOX1* transcripts was observed in the *G. arboreum* ovule epidermal cells at 0 DPA and in fiber cells at 1 DPA (Fig. 2A). No obvious signal of *GaHOX1* transcripts was detected in the ovule of *fl* mutants from 0 to 1 DPA on which no fiber cells developed (Fig. 2B).

Expression of *GaHOX2* was also investigated. *GaHOX2* transcript level was high in both fiber and other ovular cells at early developmental stages. In the *fl* ovule, the expression level of *GaHOX2* was even higher (Fig. 1C). In the RNA in situ hybridization experiment, the *GaHOX2* signal was clearly detected in the inner and outer integument cells of the *G. arboreum* ovule at 0 DPA (Fig. 2C). A similar expression pattern was also detected in the *fl* mutant ovule (Fig. 2C).

### Complementation of *Arabidopsis gl2* mutant phenotypes by *GaHOX1*

To answer whether the sequence and structural similarities between *GaHOX1* and *GL2* reflect functional similarity, the ability of the cotton *GaHOX1* gene to complement *gl2* mutant phenotypes was tested. A T-DNA insertion mutant, *gl2-2* (SALK_130213), which harbors a T-DNA in the second intron of *GL2* gene, was used for complementation. The *gl2-2* plants exhibited a glabrous phenotype (Fig. 3B).

Two constructs, *35S::GaHOX1* and *PGL2::GaHOX1*, in which *GaHOX1* was driven by the CaMV 35S and *GL2* promoter, respectively, were introduced into the *gl2-2* plants. The transgenic plants harboring *35S::GaHOX1* remained glabrous as the *gl2-2* (Fig. 3D). This observation was similar to an earlier report that the GL2 coding region placed behind the CaMV 35S promoter failed to complement defects in the *gl2-1* mutant (Ohashi et al. 2002). On the contrary, in over 90% of the plants transformed with *PGL2::GaHOX1* the

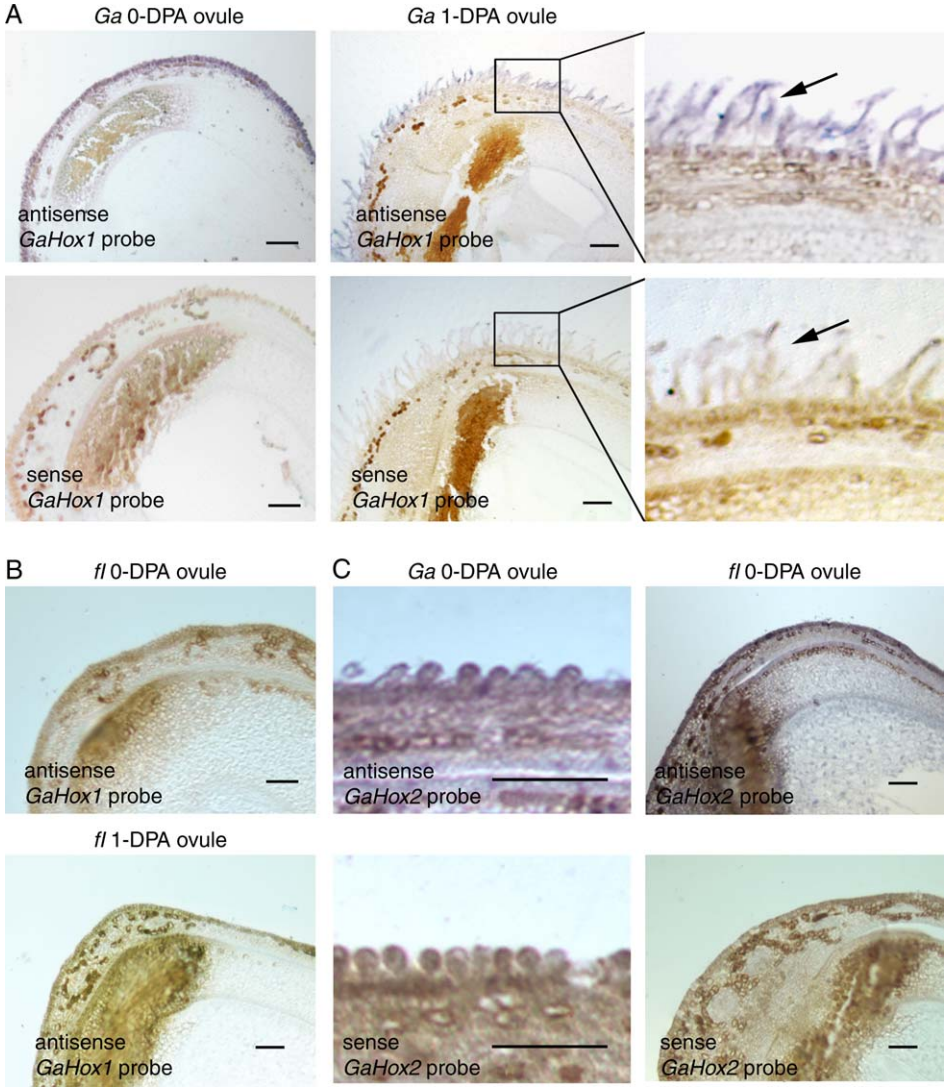


**Fig. 2.** RNA in situ hybridization showing the distribution of *GaHOX1* and *GaHOX2* transcripts in the cotton ovule and fiber. (A) In *G. arboreum GaHOX1* signal was detected in epidermal cells of the 0 DPA ovule and in fiber cells at 1 DPA; (B) *GaHOX1* signal was not detected in the 0 and 1 DPA ovule of the *fl* mutant; (C) *GaHOX2* transcripts were detected in the integument and fiber cells of the 0 DPA ovule of *G. arboreum*, and in the integument of the *fl* ovule at 0 DPA. *Ga*: *G. arboreum*; *fl*: the *fl* mutant. Bars in (A, B) = 100 μm.

trichome development was restored to the wild-type plant level (Fig. 3E). Our result that *GaHOX1* rescues the *gl2* function in the same way as *GL2* suggests that GaHOX1 is functionally equivalent to GL2 in *Arabidopsis* trichome initiation and development, and a proper spatial expression of the HD-ZIP IV gene is necessary for trichome initiation.

Constructs of *35S::GaHOX2* and *PGL2::GaHOX2* were also introduced into the *gl2-2* mutant plants. Although both transgenes were expressed to a detectable level (Fig. 3H), all the transgenic plants remained glabrous (Fig. 3F, G), suggesting that GaHOX2 is functionally different from GL2 and GaHOX1.

It was reported that in the wild-type genetic background, *35S::GL2* caused a *gl2*-mutant-like phenotype with reduced viability (Ohashi et al. 2002). To examine whether the GaHOX1 leads to similar consequences, we expressed *35S::GaHOX1* in the wild-type *Arabidopsis* plants. Among the 37 independent transgenic lines, 26 were indistinguishable from the wild-type plants, and the remaining 11 exhibited suppressed trichome formation in stems and cauline leaves (Fig. 4A). The trichome densities on rosette leaves were also reduced, though to a less degree (Supplementary material Fig. S1B).

To gain more data for trichome-suppression by *35S::GaHOX1*, we performed RT-PCR to analyze the

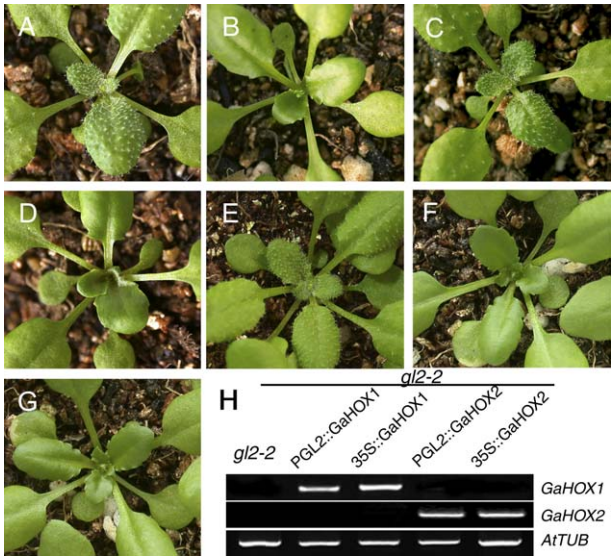


**Fig. 3.** Complementation of the *gl2-2* mutant by *GaHOX1*. (A) Wild-type plant; (B) *gl2-2* plant showing a glabrous phenotype; (C) the *gl2-2* mutant plant expressing *PGL2::GL2*, trichome development was restored; (D) the *gl2-2* mutant plant expressing *P35S::GaHOX1*, trichome development was not restored; (E) the *gl2-2* mutant plant expressing *PGL2::GaHOX1*, trichome development was restored; (F) the *gl2-2* mutant plant expressing *P35S::GaHOX2*, trichome development was not restored; (G) the *gl2-2* mutant plant expressing *PGL2::GaHOX2*, trichome development was not restored; (H) RT-PCR analysis of the expressions of the *GaHOX1* and *GaHOX2* in the *gl2-2* mutant and the transgenic plants. *AtTUB* (*At5g62690*) was used as an internal control.

expression levels of several transcription factor genes of the trichome development pathway. We found that, in the trichome-repressed lines, *GL2* expression level was decreased and the expression of *GL1* and *TRY* remained largely unchanged (Fig. 4B). These results suggest that misexpression of *GaHOX1* affected the endogenous *GL2* gene expression and function. It has been found that the ectopically expressed *GL2* can interrupt endogenous *GL2* function in trichome development and be toxic to plants (Ohashi et al. 2002). In our experiments, *GaHOX1* misexpression in the wild-type *Arabidopsis* also led to a *gl2*-mutant like phenotype. However, the viability of these transgenic plants harboring *35S::GaHOX1* was not affected in our greenhouse conditions. On the contrary, misexpression of *GaHOX2* in the wild-type *Arabidopsis* plants did not affect trichome development (Supplementary material Fig. S1D).

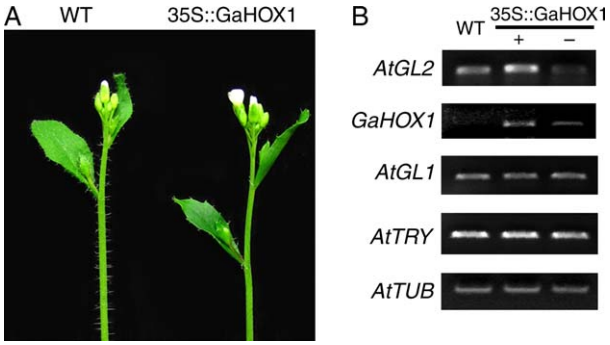


**Fig. 4.** Expression of *GaHOX1* with 35S promoter in wild-type *Arabidopsis*. (A) Trichome development on stem was suppressed in the *35S::GaHOX1* plants; (B) RT-PCR analysis of the expressions of the trichome development pathway genes in the wild-type and the transgenic plants (+, transgenic plants of normal trichome development; −, transgenic plants with suppressed trichome development). *AtTUB* (*At5g62690*) was used as an internal control.

## Discussion

In this report, we describe two cotton homeodomain-containing genes, *GaHOX1* and *GaHOX2*, which belong to the HD-ZIP IV family. Comparison with the *Arabidopsis* HD-ZIP IV proteins showed that GaHOX1 is the closest homolog of GL2, whereas GaHOX2 is more similar to ATML1 and PDF2 than to GL2. HD-ZIP IV genes have been isolated from several plant species, including *G. hirsutum* (Wang et al. 2004), *Picea abies* (Ingouff et al. 2003) and maize (Ingram et al. 2000). Among the *Arabidopsis* HD-ZIP IV proteins, GL2 is the only one that is required for trichome cell and non-root hair cell development. We show for the first time that a GL2 homolog from cotton could fully rescue the *gl2* glabrous phenotype. The functional complementation was observed only when *GaHOX1* was driven by the *GL2* promoter, whose activity is predominantly detected in trichome (Szymanski et al. 1998). Together, these results suggest that spatial location of the HD-ZIP IV protein is important for specifying epidermal trichome development, and that GaHOX1 protein could substitute GL2 in regulating trichome cell differentiation in *Arabidopsis*. Expression of *GaHOX1* with the 35S promoter led to repression of trichome development in wild-type *Arabidopsis*. It has been reported that the ectopically expressed GL2 also can repress the trichome development and be toxic to the plants. We suppose the misexpressed GaHOX1 interrupt the trichome formation in the similar pathway as the GL2 ectopic expression.

Both of *Arabidopsis* trichomes and cotton fibers are derived from single epidermal cells. Transcriptional profiling analysis in cotton fiber and ovule of *G. arboreum* and *G. hirsutum* showed that, during the cotton fiber initiation and fast-elongation stage, the genes related to cell wall structure and biogenesis, cytoskeleton and energy/carbohydrate metabolism are

upregulated (Arpat et al. 2004, Gou et al. 2007, Samuel Yang et al. 2006, Shi et al. 2006, Udall et al. 2006). Ethylene, brassinosteroid and auxin signaling and response genes are also highly active in fiber cells during this stage (Gou et al. 2007, Samuel Yang et al. 2006, Shi et al. 2006). And genes encoding several transcription factors, such as those of MYB, WRKY and HOX, show increased expression during the fast-elongation stage of cotton fiber development (Samuel Yang et al. 2006). *GaHOX1* is expressed predominantly in epidermal cells of cotton ovule at 0 DPA and in fiber cells at the early development stage. Only a very weak signal of *GaHOX1* expression could be detected in the ovule of the *fl* mutant. Together with the fact that GaHOX1 has the same function as GL2 when properly expressed in *Arabidopsis* plants, these data propose a role of GaHOX1 in cotton fiber development.

GaHOX2 shows high sequence identities to *Arabidopsis* PDF2 and ATML1. In *Arabidopsis* ATML1 and PDF2 are functionally overlapping in regulation of shoot epidermal cell differentiation, and *atml1 pdf2* double mutants failed to form the epidermis (Abe et al. 2003). *GaHOX2* did not exhibit the activity of promoting trichome development when expressed in *Arabidopsis*. In developing cotton ovules *GaHOX2* is expressed in integuments, and its expression level is even higher in the *fl* mutant ovule. The integumental expression, together with the sequence similarity with *Arabidopsis PDF2* and *ATML1* suggests that GaHOX2 may play a role in seed coat development. However, transcripts of several other *HOX* genes, including *GaHOX2* (Fig. 1C) and *GhHOX3* (Wang et al. 2004), were also present, although not enriched, in developing cotton fiber cells. GhHOX3 and GaMYB2 operating together can active the *RDL1-P3* promoter in *Arabidopsis* plants (Wang et al. 2004). Whether these HOX proteins jointly participate in regulating cotton fiber development is an interesting question for further investigation.

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## Supplementary material

The following supplementary material is available for this article:

**Fig. S1**. Trichome development was suppressed by *35S::GaHOX1* in the wild-type *Arabidopsis*. (A) wild-type plant; (B) approximately 30% of the plants transformed with *35S::GaHOX1* showed reduced trichome densities on the rosette leaves; (C) approximately 70% of the plants transformed with *35S::GaHOX1* showed a wild-type trichome production; (D) expression of *35S::GaHOX2* in wild-type plants did not change trichome formation.

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