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# The *PIN* gene family in cotton (*Gossypium hirsutum*): genome-wide identification and gene expression analyses during root development and abiotic stress responses

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## Abstract

**Background:** Cell elongation and expansion are significant contributors to plant growth and morphogenesis, and are often regulated by environmental cues and endogenous hormones. Auxin is one of the most important phytohormones involved in the regulation of plant growth and development and plays key roles in plant cell expansion and elongation. Cotton fiber cells are a model system for studying cell elongation due to their large size. Cotton is also the world's most utilized crop for the production of natural fibers for textile and garment industries, and targeted expression of the IAA biosynthetic gene *iaaM* increased cotton fiber initiation. Polar auxin transport, mediated by PIN and AUX/LAX proteins, plays a central role in the control of auxin distribution. However, very limited information about PIN-FORMED (PIN) efflux carriers in cotton is known.

**Results:** In this study, 17 PIN-FORMED (PIN) efflux carrier family members were identified in the *Gossypium hirsutum* (*G. hirsutum*) genome. We found that *PIN1–3* and *PIN2* genes originated from the At subgenome were highly expressed in roots. Additionally, evaluation of gene expression patterns indicated that *PIN* genes are differentially induced by various abiotic stresses. Furthermore, we found that the majority of cotton *PIN* genes contained auxin (AuxREs) and salicylic acid (SA) responsive elements in their promoter regions were significantly up-regulated by exogenous hormone treatment.

**Conclusions:** Our results provide a comprehensive analysis of the *PIN* gene family in *G. hirsutum*, including phylogenetic relationships, chromosomal locations, and gene expression and gene duplication analyses. This study sheds light on the precise roles of *PIN* genes in cotton root development and in adaption to stress responses.

**Keywords:** Auxin, PIN-formed, Cotton, Root development, Expression patterns, Abiotic stress

## Background

The plant phytohormone auxin (indole-3-acetic acid, IAA) plays an essential role in plant morphogenesis, organogenesis, apical dominance, embryo formation, vascular differentiation, and light and gravity perception

[1, 2]. Two critical pathways, including auxin transport and auxin signaling, are vital for plant development, playing a major role in both phototropism and gravitropism. At the cellular level, auxin negatively regulates the transcription factor AUXIN RESPONSE FACTOR (ARF), which mediates the expression of auxin-responsive genes [3, 4]. At the tissue level, auxin is synthesized and then transported from the site of biosynthesis to the sites of auxin action [5, 6]. Auxin efflux has been observed in specific tissues at different developmental stages, and plays a role in lateral organ initiation, root gravitropism, and root hair formation [7–9]. The cell to cell polar transport

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of auxin is mediated by specific influx and efflux carriers, resulting in asymmetric distribution of auxin [10, 11]. The auxin influx and efflux proteins in *Arabidopsis* can be grouped into 3 gene families: P-glycoprotein (MDR/PGP/ABCB) efflux/conditional transporters (PGP), auxin resistant 1/like aux1 (AUX1/LAX) influx carriers, and plant specific PIN-FORMED (PIN) efflux carriers [4, 12, 13]. The polar subcellular localization of PIN efflux proteins is responsible for directional auxin flow, and more and more evidence indicates that plasma membrane-localized PIN proteins are the rate-limiting step in auxin polar transport [14, 15]. PIN polarization and auxin polar transport play an important role in both plant phototropism and gravitropism [16, 17].

In *Arabidopsis*, the *PIN* gene family is comprised of 8 members, which have been reported to be involved in various developmental processes [18, 19]. PIN1, PIN2, PIN3, PIN14 and PIN7, located in the plasma membrane, are involved in the tropic response and root growth. In contrast, the endoplasmic reticulum (ER) localized PINs, including PIN5, PIN6 and PIN8, are mainly involved in intracellular auxin homeostasis [20, 21]. Among all PIN family members, loss-of-function *pin1* and *pin2* mutants show severe phenotypes, making them well suited for investigating auxin-dependent developmental processes [22]. PIN1 is mainly involved in the maintenance of embryonic auxin gradients, and organ initiation is severely affected in *pin1* mutants, which results in the formation of naked inflorescence stems [23]. Constitutive triple response 1 (CTR1), a protein that acts downstream of the ethylene receptors, is a negative regulator of ethylene signaling. *PIN2* functions downstream of CTR1 and primarily regulates root gravitropism [24]. The roots of *pin2* mutants are insensitive to ethylene and grow agravitropically [25, 26]. Recently, the *PIN* genes have been reported to play a role in the integration of hormone signaling and abiotic stress responses. In soybean, *PIN* genes are differentially regulated by both abiotic stresses and phytohormones [19]. In rice, *PIN* genes show tissue-specific expression and have been found to also be regulated by hormones [27]. In maize, the expression levels of most *ZmPIN* genes were induced in shoots and reduced in roots by various abiotic stress treatments, including drought, salt, dehydration and cold [15].

Cotton is an important source of both natural fibers in the textile industry and cotton seed oil used in the production of food and biodiesel fuel. Understanding the factors that regulate fiber initiation will drive the development of technologies to improve yield potentials. Prior work suggests that auxin plays an essential role in fiber cell initiation. Exogenous application of IAA to cotton ovules promoted cotton fiber initials during fiber cell initiation [28]. Furthermore, in vitro application of N-1-naphthylphthalamic acid (NPA), an inhibitor of auxin polar transport, reduces IAA

accumulation and inhibits fiber cell initiation [29]. Further, targeted expression of *iaaM*, an IAA biosynthetic gene, was showed to increase the number of lint fibers produced [29]. In addition, auxin regulates cotton fiber initiation via GhPIN-mediated auxin transport [30, 31]. Auxin is not synthesized in fiber cells, and is mainly transported from the outside of ovules to fiber cells via polar auxin transport.

In this work, we performed a comprehensive analysis of the *PIN* gene family based on data gathered from recent whole genome sequencing results [32–36]. Comparative analysis of allotetraploid cotton (*G. hirsutum*) with its diploid ancestor (*G. arboreum*) indicated that *PIN1–3* and *PIN2* may play an important role in root development. In addition, we carried out expression profiling of cotton *PIN* genes in response to different hormonal treatments and abiotic stresses. The results showed that the majority of cotton *PIN* genes contained auxin response elements (AuxREs) and salicylic acid (SA) responsive elements in their promoter regions, which were significantly up-regulated by exogenous hormone treatment.

## Methods

### Plant materials and growth conditions

*Gossypium hirsutum* (Xuzhou 142) and *Gossypium arboreum* (Shixiya 1) seeds were acquired from the Institute of Cotton Research of the Chinese Academy of Agricultural Sciences (Anyang, China). The seeds were planted into sand containers (one seedling per container) and grown in a climate-controlled greenhouse (16-h light and 8-h dark cycle at 30 °C) located at Shaanxi Normal University. For each assay, samples were collected from the equivalent growth stages of *G. hirsutum* and *G. arboreum*. A total of 9 seedlings of each species were used for each treatment and three biological triplicates were performed per assay.

### Identification and phylogenetic analysis of cotton *PIN* genes

Genome sequences of *G. hirsutum*, *G. arboreum* and *G. barbadense* have recently become available [32–38]. A genome browser for the cotton genome, termed Cottongen, is accessible online (<https://www.cottongen.org>). The *A. thaliana* genome sequence was acquired from TAIR 10 (<http://www.arabidopsis.org>). Putative cotton PINs were identified by blast searches against the three reference genomes using *A. thaliana* PIN protein sequences as queries. Next, candidate PINs were further filtered based on their conserved domains using SMART (<http://smart.embl-heidelberg.de>) and Pfam (<http://pfam.xfam.org/search#tab-view=tab0>) database analyses [39, 40]. The phylogenetic relationships between putative cotton PINs and *A. thaliana* PIN proteins were determined using the neighbor-joining algorithm using default parameters with 1000 bootstrap analyses by MEGA 5.0 (<https://www.megasoftware.net>). Based on the phylogenetic analysis, the putative cotton PINs were named

according to their respective clades. Multiple sequence alignments of all newly identified PINs in this study were performed using ClustalX with default parameters [41].

### Chromosomal location and gene duplication analysis

Chromosome position information of the putative PINs in *G. hirsutum* was obtained from gene annotation files downloaded from the CottonGen website (<https://www.cottongen.org>). The map of *PIN* gene distribution along the chromosomes is shown from top to bottom [42]. Duplication analysis of *G. hirsutum* *PIN* genes and genomic synteny was identified and displayed with the Synteny Mapping and Analysis Program (SyMAP) v3.4 [43].

### Stress and hormonal treatments

Plants used for stress and hormone treatments were grown under the same greenhouse conditions as reported previously [44]. The same protocols were used for drought, dehydration and salt (250 mM) abiotic stress treatments [44]. For the plant hormone treatments, the methods were modified from the methods utilized by Chai et al. [45]. Briefly, two-week old seedlings were irrigated and sprayed with 10  $\mu$ M NAA and 0.5 mM salicylic acid, respectively. After treatment, root and shoot tissues were harvested at indicated times (0.5, 1, 3 and 5 h). The samples were frozen in liquid nitrogen immediately after collection and kept at  $-80^{\circ}\text{C}$ . For each treatment, three individual samples were collected and analysis was performed on biological triplicates.

### RNA extraction and quantitative RT-PCR (qRT-PCR) analysis

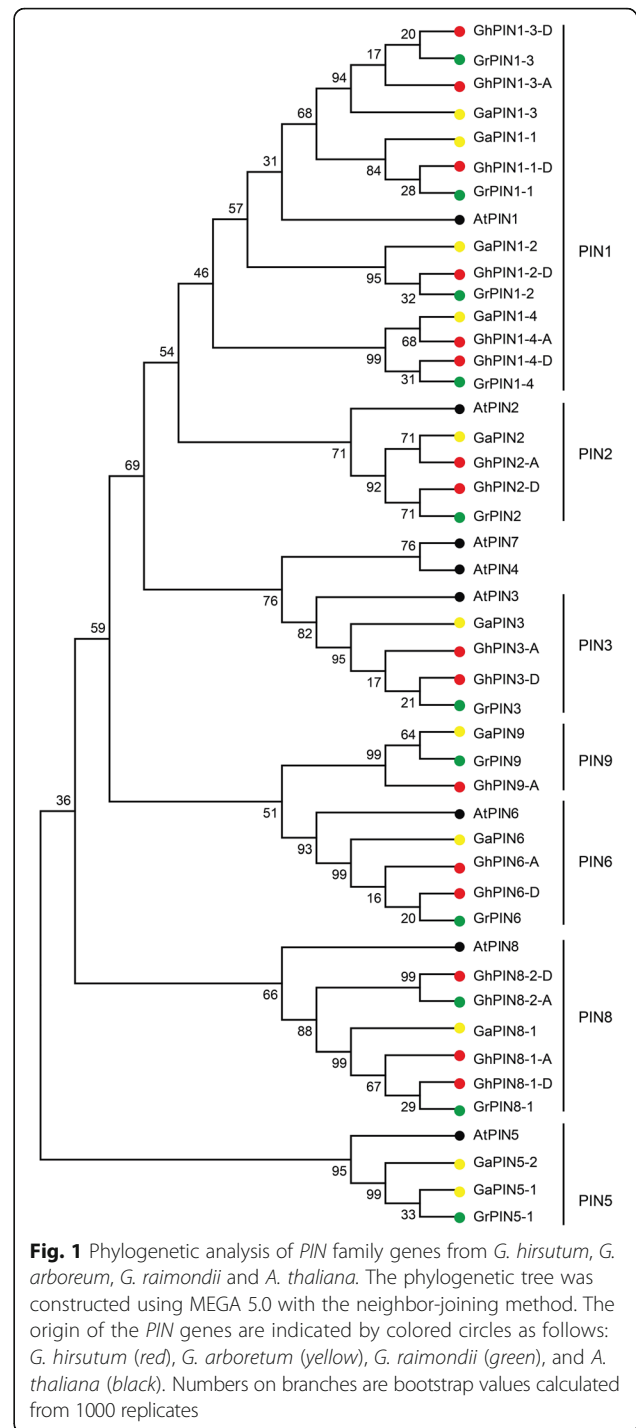
Cotton samples were ground to fine powder with a mortar and pestle in liquid nitrogen. Total RNA was isolated using a modified CTAB method as described [46], and 5  $\mu$ g of total RNA was converted into cDNA using the cDNA Synthesis SuperMix (Transgen, China) according to the manufacture instructions. For qRT-PCR experiments, cotton *UBQ7* (GenBank No. AY189972) was used as an internal control. Primers for qRT-PCR analysis are listed in Additional files 1: Table S4.

### Analysis of regulatory elements in the promoter region

Identified *GhPIN* genes including their predicted promoter sequences were downloaded from the CottonGen website (<https://www.cottongen.org>). The regulatory elements in the promoter regions were predicted using PLACE and PlantCARE software as previously [47, 48].

### Accession numbers

The TAIR accession numbers for the *Arabidopsis* PIN sequences used in this study are as follows: At1g73590 (*AtPIN1*), At5g57090 (*AtPIN2*), At1g70940 (*AtPIN3*),



At2g01420 (*AtPIN4*), At5g16530 (*AtPIN5*), At1g77110 (*AtPIN6*), At1g23080 (*AtPIN7*), At5g15100 (*AtPIN8*).

## Results

### Genome-wide identification of PIN proteins in cotton

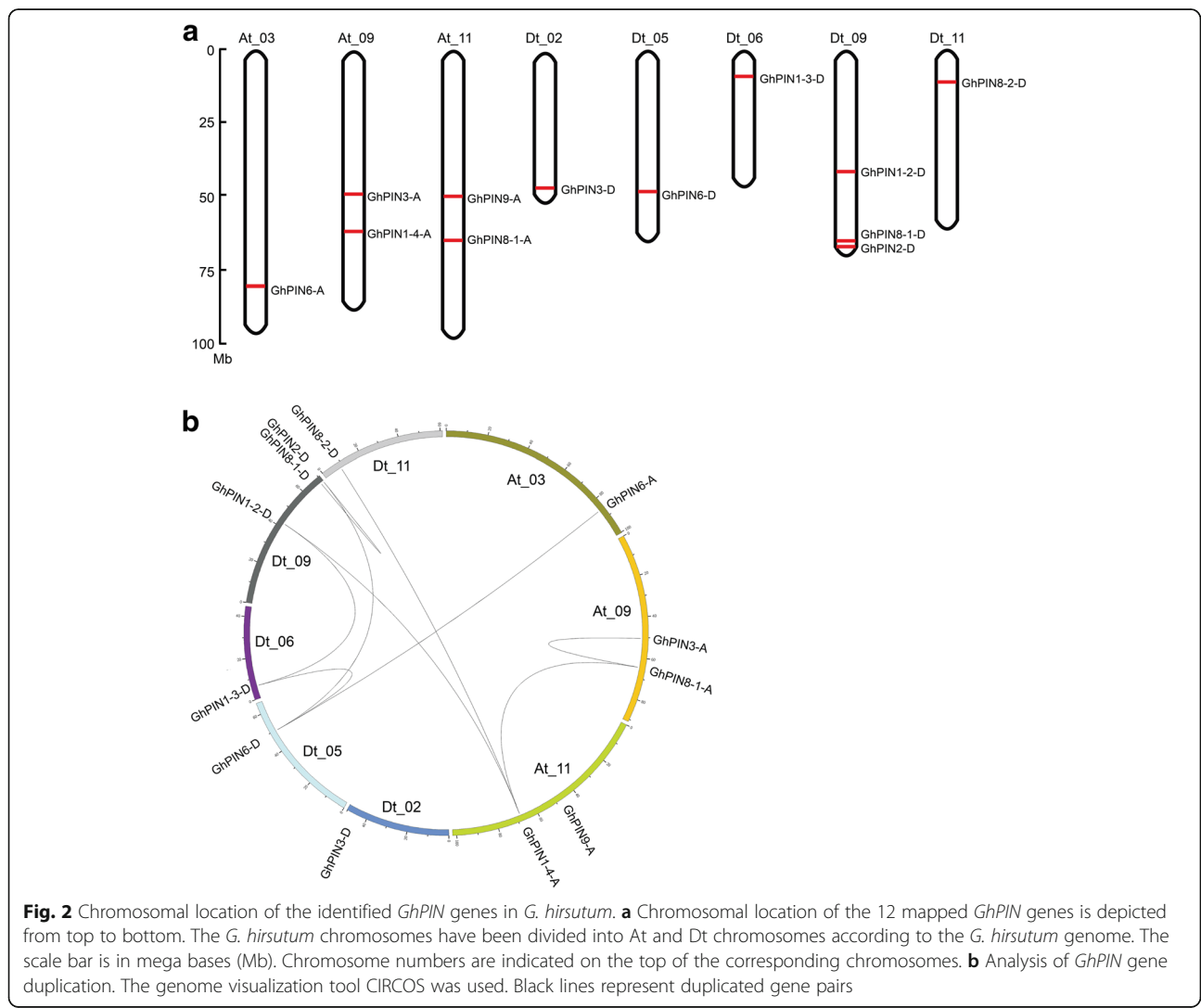
Three cotton genomes, including two diploid (*G. arboreum* and *G. raimondii*) and one allotetraploid (*G. hirsutum*), have recently been completed and published [33–36]. A genome

browser is available at the Cottongen website (<https://www.cottongen.org>). Putative cotton PIN proteins were initially identified by Blastp searches against the three reference genomes using *A. thaliana* PIN protein sequences as queries. After protein conserved domain and gene structure selection, a total of 17, 12, and 10 predicted protein coding sequences (CDSs) were identified in *G. hirsutum*, *G. arboreum* and *G. raimondii*, respectively (Additional file 2: Table S1). Most *G. hirsutum* PIN genes contained at least 3 introns in their open reading frames, except *PIN1-4-D* and *PIN8-2-A*, which have only one intron and two introns, respectively. The protein coding sequences of all *GhPINs* are listed in Additional file 3: Table S2. The deduced GhPIN proteins varied in length from 127 amino acids (*GhPIN8-2-A*) to 678 amino acids (*GhPIN2-D*). Conserved domain analysis showed they have an auxin efflux carrier (ACE) domain at the amino-terminal region and a membrane transport (MT) domain at the carboxy-terminus (Additional file 4: Figure S1). Although the predicted sizes of the putative

*GhPIN* proteins varied markedly, these two domains were found to be consistently present in all analyzed sequences, indicating that these two domains are likely to be crucial for biochemical function.

**Phylogenetic analysis and chromosomal distribution of *GhPIN* genes**

In order to investigate the evolutionary relationships of the identified PIN proteins, an unrooted phylogenetic tree was generated using the predicted full length amino acid sequences from *G. arboreum*, *G. raimondii*, *G. hirsutum* and *A. thaliana*. As illustrated in the Neiboring-Joining phylogenetic tree (Fig. 1), a similar organization for the cotton and *A. thaliana* PIN proteins and some orthologous relationships between both species were identified. Based on this analysis, cotton PIN proteins were named based on their relationships to known *A. thaliana* PINs. Compared with *A. thaliana*, the *PIN1* subfamily was found to be extensively expanded in cotton, indicating they may play an

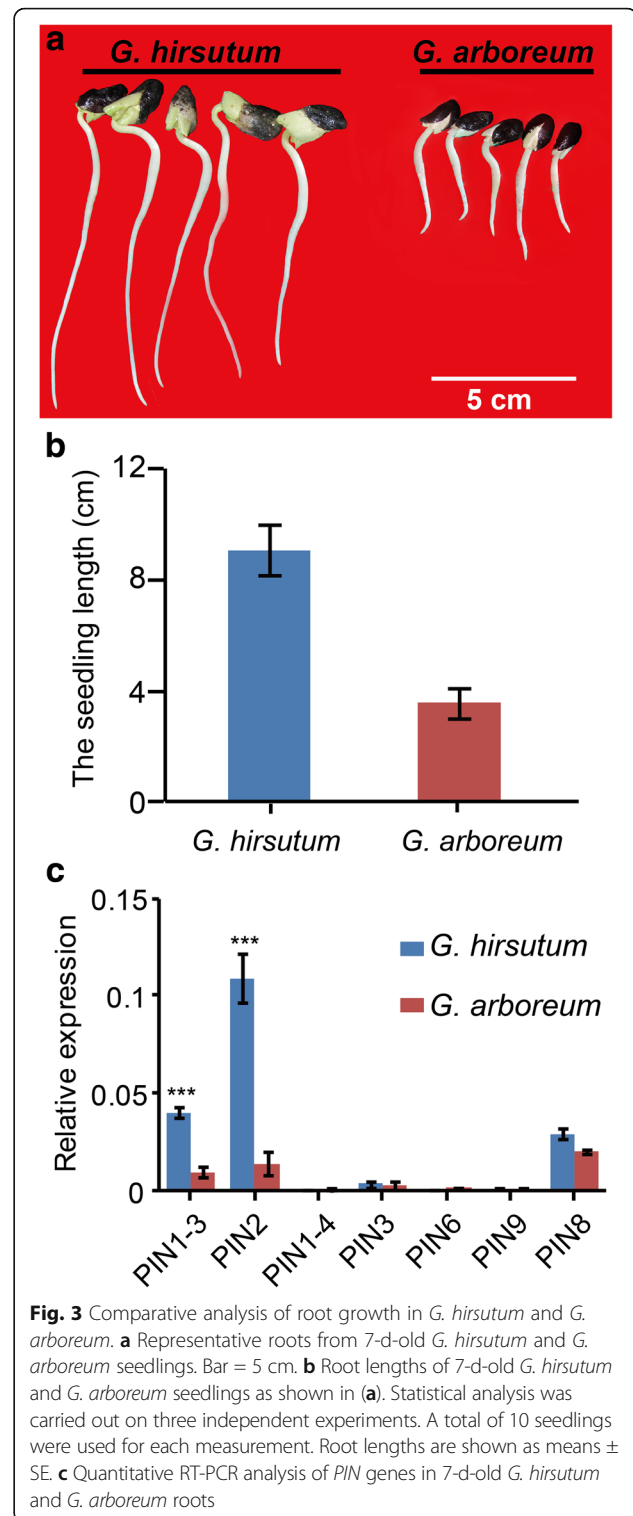


important role in cotton. Interestingly, *PIN5* subfamily genes were found in *G. arboreum*, *G. raimondii* and *A. thaliana*, but all of them were lost in *G. hirsutum* and *G. barbadense* (Additional file 5: Table S3), implying that a gene loss event occurred in the *PIN* family genes after polyploidization in the allotetraploid cotton.

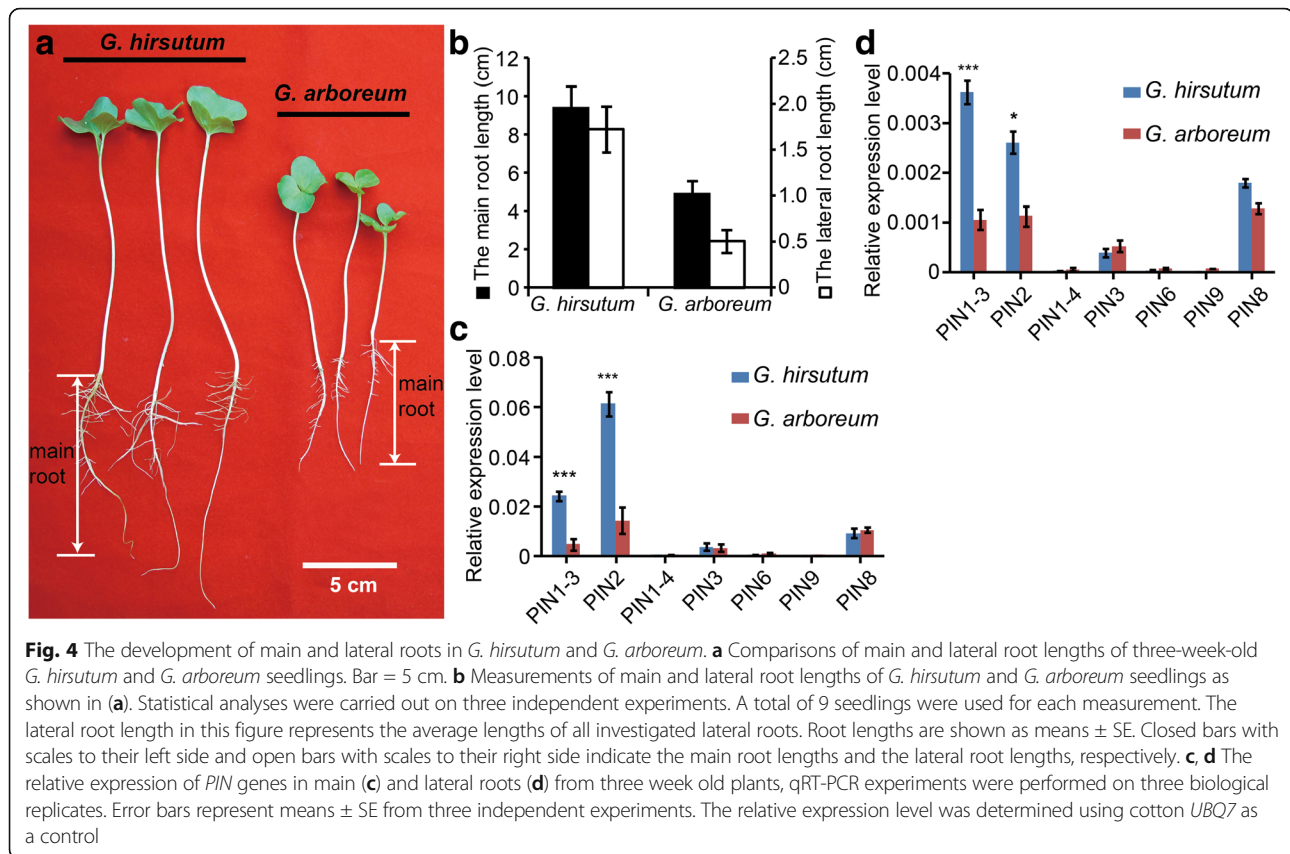
*GhPIN* genes were mapped onto chromosomes to provide insights into their organization in the cotton genome. This data indicated that 12 genes were distributed among 8 chromosomes, including 5 *GhPIN* genes on 3 chromosomes from the At-subgenome and 7 genes found on 5 chromosomes from the Dt-subgenome (Fig. 2a). Two whole-genome duplication (WGD) events have been observed in cotton [36], which resulted in the occurrence of gene duplication. The relationships and duplication events between the 12 *PIN* genes were investigated. These combined data suggested that segmental duplication and dispersed duplication might be the main types of *PIN* gene duplication in cotton (Fig. 2b).

#### *GhPIN1-3* and *GhPIN2* are required for cotton root development

Roots play an essential role in plant growth by absorbing water and inorganic nutrients from the ground. Auxin, which is transported and regulated by auxin efflux transporters, has been reported as a positional cue for root cell type determination [49]. To evaluate the roles of *PIN* genes in cotton root development, a comparative analysis of root growth between *G. hirsutum* and *G. arboreum* was carried out. 7 days after germination, a significant difference in root length was observed between *G. hirsutum* and *G. arboreum* (Fig. 3a). The roots of *G. hirsutum* reached an average length of 8 cm, while roots of *G. arboreum* were less than 4 cm (Fig. 3b). Next, we tested the expression of *GhPIN* genes during root development. qRT-PCR results showed that the expression levels of *GhPIN1-3* and *GhPIN2* in *G. hirsutum* were much higher than their predicted orthologs in *G. arboreum* (Fig. 3c), indicating that *GhPIN1-3* and *GhPIN2* genes may be required for early root development. After 3 weeks, both the main and lateral roots of *G. hirsutum* were significantly longer than those of *G. arboreum* (Fig. 4a and b). However, no difference in the number of lateral roots was observed between *G. hirsutum* and *G. arboreum* (Additional file 6: Figure S2). Among the 7 *PIN* genes analyzed, *GhPIN1-3* and *GhPIN2* were found to be higher expression in both main and lateral roots of *G. hirsutum* relative to *G. arboreum* (Fig. 4c and d), indicating that *GhPIN1-3* and *GhPIN2* may play key roles in regulating the late-stage development of cotton roots. Furthermore, the expression data of *GhPIN2* suggests that it may contribute more than *GhPIN1-3* in regulating cotton root development (Figs. 3c and 4c). However, *GhPIN1-3* was found to be more highly expressed in lateral roots, suggesting that



it plays more an important role in regulating lateral root growth (Fig. 4d). The observed differences in gene expression during root development indicate possible functional differentiation of *PIN* genes.



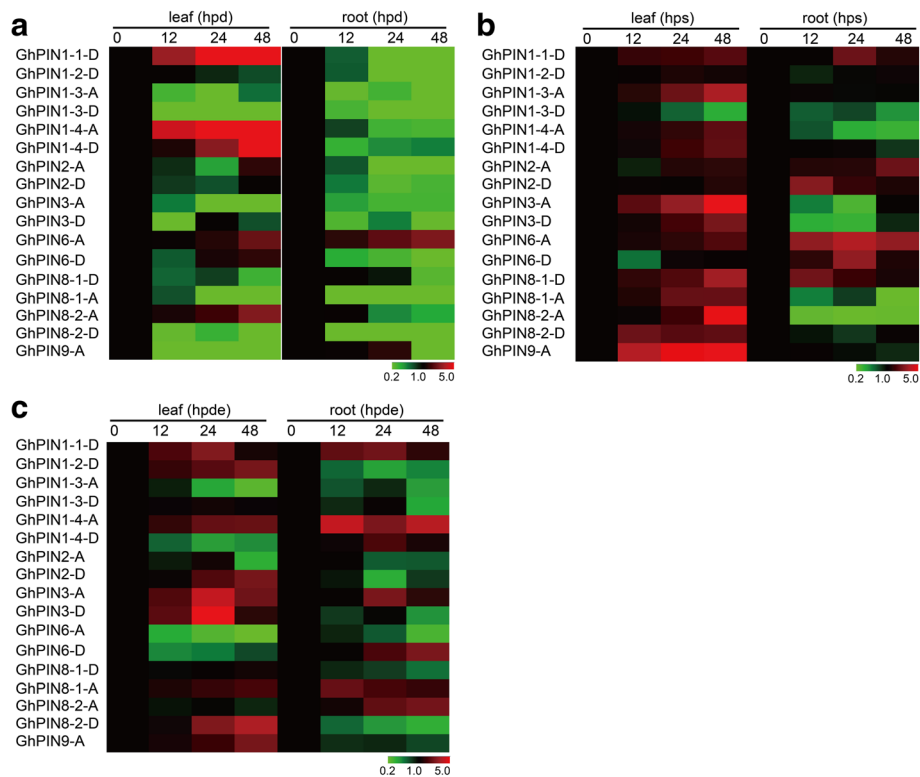
### Expression of *GhPINs* in response to drought, salt and dehydration

It has been reported that *PIN* genes are involved in plant response to diverse abiotic stresses, such as drought, salt and dehydration [50]. In order to identify potential roles of individual cotton *PIN* genes in different abiotic stress responses, quantitative real-time PCR (qRT-PCR) analysis was performed to identify changes in gene expression in leaves and roots of plants that underwent different stress treatments. *GhPIN1-1-D* and *GhPIN1-4-A* were found to be highly differentially expressed. More *GhPINs* were up-regulated in both leaves and roots that underwent salt and dehydration treatment, relative to plants subjected to drought stress. After cotton seedlings were grown in drought conditions for 48 h, 5 genes were induced in leaves (list genes) while only *GhPIN6-A* was up-regulated in roots (Fig. 5a). *GhPIN1-1-D*, *GhPIN1-4-A* and *GhPIN1-4-D* were highly upregulated in leaves under drought stress, but concomitantly down-regulated in roots. More genes showed significant changes in gene expression in leaves, when compared to roots, and almost all genes (13 out of 17) were highly expressed in leaves in plants grown in saline conditions (Fig. 5b). Interestingly, the number of *GhPIN* genes up-regulated in leaves and roots were nearly identical after drought stress (Fig. 5c). In general, *GhPIN* genes were widely induced by drought, salt and

dehydration stresses, suggesting that *GhPINs* play a role in abiotic stress responses in cotton.

### Gene expression analysis of *GhPINs* in response to auxin and salicylic acid treatment

The phytohormone auxin is known to play important roles in plant development [51]. Emerging evidence indicates that salicylic acid (SA), a pivotal signaling molecule involved in plant immune responses, is involved in both local and systemic disease resistance responses [52]. In order to explore the effects of auxin and SA on *GhPINs* expression, *GhPIN* gene expression analysis was carried out on shoots and roots after treatment with plant hormones were analyzed. *GhPINs* were differentially expressed upon treatment with SA and the auxin analogue NAA. The results showed that a total of 8 and 10 *GhPIN* genes were responsive to NAA treatment in shoots and roots, respectively (Fig. 6a). Further, we have also shown that 9 genes in shoots and 10 genes in roots were responsive to SA (Fig. 6b). Auxin responsive elements (AuxREs) and SA responsive elements (SAREs) have been shown to be involved in response to auxin and SA stimuli, respectively [53, 54]. In order to build our knowledge regarding how these genes are induced by NAA and SA, a comprehensive analysis of *GhPIN* promoters was performed. Our results showed that 6 out of 8 highly expressed genes possessed at



**Fig. 5** Gene expression analysis of *GhPINs* after abiotic stresses treatments. **a** Fold changes of *GhPIN* gene expression under drought treatment. **b** Fold changes of *GhPIN* gene expression under salt treatment. **c** Fold changes of *GhPIN* gene expression under dehydration treatment. All treatments were performed with three biological and three technical replicates. Hpd, hours post drought; hps, hours post salt; hpde, hours post dehydration. The relative gene expression levels were determined using cotton *UBQ7* as a control

least one AuxRE in their promoter regions (Fig. 6c). In addition, 4 genes, which were up-regulated in both shoots and roots under SA treatment, contained a predicted SARE in their promoter regions (Fig. 6d).

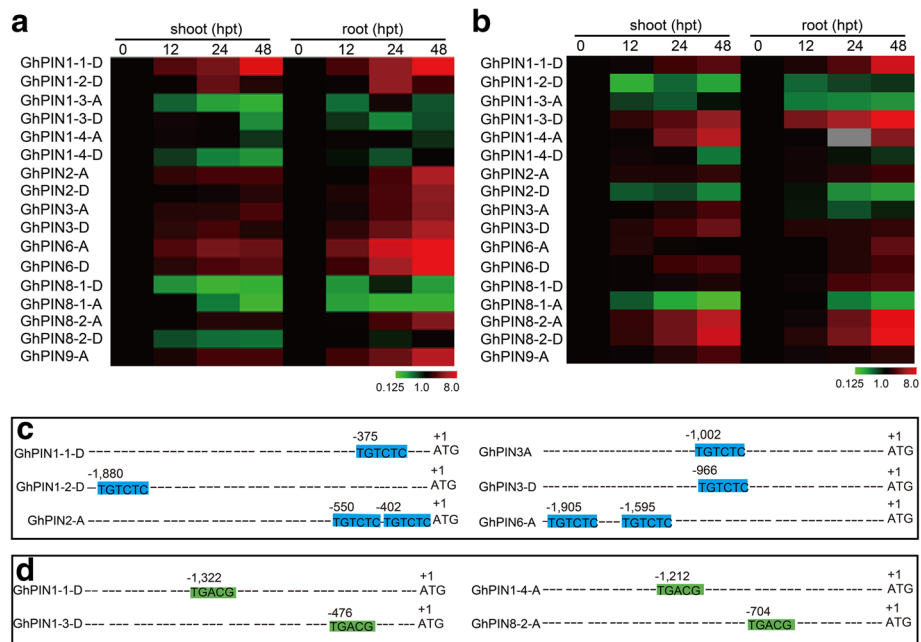
## Discussion

In this work, 17 *PIN* genes were identified in *G. hirsutum*, including 8 that originated from the At subgenome and 9 from the Dt subgenome (Additional file 2: Table S1). Among them, 5 genes from the A genome and 2 from the D genome were lost in the At and Dt subgenomes, which is consistent with previous results that the allotetraploid genome suffered a higher rate of gene loss relative to the diploid genomes, and that more genes were lost in the At subgenome during polyploidization [34, 35]. Genes in the *PIN5* group were observed in *G. arboreum* and *G. raimondii*, but not found in *G. hirsutum* (Fig. 1), suggesting that these genes evolved from a common ancestor, but were then lost in *G. hirsutum*. Chromosomal distribution of *GhPIN* genes showed that 12 out of the 17 genes are distributed across 8 chromosomes (Fig. 2a).

Genetic analysis in *Arabidopsis* has shed light on the function of several *PIN* genes. Several members of the *PIN* gene family of auxin efflux carriers, such as *AtPIN1*,

*AtPIN2*, *AtPIN3*, *AtPIN4*, and *AtPIN7*, are well-known to be involved in cell-to-cell auxin transport in the root [55]. In particular, *AtPIN1* and *AtPIN3* were found to be the major auxin transport facilitators mediating polar auxin re-allocation from the shoot to the root tip [12, 55, 56]. The two *PIN* genes showed high expression in root, thereby determining meristem size and consequently growth rates of the primary root [56, 57]. In this study, we found that both main and lateral roots in *G. hirsutum* were longer than those from its diploid ancestor (Figs. 3a and 4a). qRT-PCR analysis revealed that *GhPIN1-3* and *GhPIN2* were strongly expressed in both main and lateral roots of *G. hirsutum* (Fig. 4c). The differential and high-level expression of these genes seems to contribute to increased root length in *G. hirsutum*. Lodging, referred to as the permanent displacement of aboveground parts, greatly depends on root length and strength [15]. Lodging is a common phenomenon that causes yield reductions and makes plants difficult to harvest. *GhPIN1-3* and *GhPIN2* are required for cotton root development, which can be further used in breeding programs to selecting genotypes that are lodging-resistance.

Previous results revealed that accumulation of the plant hormone indole 3-acetic acid (IAA) in the epidermis of



**Fig. 6** Quantitative RT-PCR analysis of *GhPINs* from roots and shoots of cotton plants treated with auxin or salicylic acid. **a** Expression of *GhPIN* genes after NAA treatment. **b** Transcript abundance of *GhPIN* genes under salicylic acid treatment. Hpt indicates hours post treatment in **(a)** and **(b)**. **c** Analysis of *GhPINs* with AuxRE elements present in their promoter regions. The element (TGCTCT) is the putative ARF binding site, which is one of the key auxin responsive elements (AuxREs). **d** Analysis of *GhPINs* with SA responsive elements present in their promoter regions. The element (TGACG) is the putative SA responsive element

cotton ovules significantly increased the number of lint fibers, an important component of fiber yield [29]. In *Arabidopsis*, mutation of genes involved in auxin polar transport facilitators resulted in defects in distal organization of roots [39]. Our results showed that *GhPIN1-3* and *GhPIN2* genes might play an important role in regulating both main and lateral root development (Figs. 3 and 4). Auxin may function in both the promotion of both fiber initiation and cotton root growth, implying that auxin may play a dual functional role in cotton development.

Soybean *PIN* genes were previously shown to be induced by various abiotic stresses and plant hormones [19]. *PIN* genes in *Sorghum bicolor* were also found to be differentially up-regulated after phytohormone treatment and under abiotic stress [58]. Additionally, auxin transporter gene families in maize were reported to be responsive to different abiotic stresses [15]. In the present study, several *PIN* genes were shown to be highly expressed in cotton plants grown under drought, salt and dehydration treatments (Fig. 5). Further gene-specific overexpression or analysis of *PIN* knockout plants may be helpful to unravel their functions. 10 out of 17 *PINs* expressed in cotton roots were induced by NAA and SA treatment (Fig. 6a and b). When analyzing the promoter regions of the 10 *PIN* genes upregulated by hormone treatment, 6 and 4 were found to contain AuxREs and SAREs in their promoter regions, respectively (Fig. 6c and d),

indicating these elements are very important for *PIN* genes to respond to NAA and SA stimuli. These findings provide clues towards the identification of more candidates with potential roles in phytohormone stimuli.

**Conclusions**

Our study provided a comprehensive analysis of the *PIN* gene family in *G. hirsutum*. We showed that *PIN1-3* and *PIN2* are involved in cotton root development. This study will help us to elucidate the precise role of *PIN* genes in cotton root development and in adaption to abiotic stress. Our findings will also further help breeding efforts to develop and select the lodging-resistant varieties in the future.

**Additional files**

- Additional file 1: Table S4.** Primers for qRT-PCR experiments. (PDF 52 kb)
- Additional file 2: Table S1.** Analysis of *G. hirsutum PIN* genes and their corresponding orthologues in the AA and DD genomes. (PDF 16 kb)
- Additional file 3: Table S2.** The coding sequences of 17 GhPIN genes identified in *G. hirsutum*. (PDF 31 kb)
- Additional file 4: Figure S1.** Multiple sequence alignment of the deduced amino acid sequences of predicted PINs from *G. hirsutum*. (PDF 2431 kb)
- Additional file 5: Table S3.** The number of *PIN* genes identified across 7 plant species. (PDF 89 kb)
- Additional file 6: Figure S2.** Measurements of lateral root number of three-week-old *G. hirsutum* and *G. arboreum* seedlings. (PDF 100 kb)



### Abbreviations

ARF: Auxin response factor; AuxRE: Auxin-responsive element; CTAB: Hexadecyl trimethyl ammonium Bromide; IAA: indole-3-acetic acid; NAA: 1-Naphthaleneacetic acid; SA: Salicylic acid; UBCQ: Ubiquinone; WGD: whole-genome duplication

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### Availability of data and materials

The cotton genome data analyzed during this article is available from CottonGen (<https://www.cottongen.org>) and the *A. thaliana* genome sequence is accessible at TAIR 10 (<http://www.arabidopsis.org>). The nucleotide sequences of all *PIN* genes in *G. hirsutum* can be found in the Additional file 3: Table S2.

### Authors' contributions

GHX and JNY designed the experiment; GHX and PH performed experiments and supervised data analysis; PH, PZ and LMW analyzed data, YZZ and XSW wrote the manuscript. JNY and HX revised the manuscript. All authors contributed to the research and read and approved the final manuscript.

### Ethics approval and consent to participate

All the cotton lines used and analyzed for this study were collected from the Institute of Cotton Research of the Chinese Academy of Agricultural Sciences, which were public and available for non-commercial purpose. This article did not contain any studies with human participants or animals performed by any of the authors.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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