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Genome-wide identification and expression analysis of the *GhIQD* gene family in upland cotton (*Gossypium hirsutum* L.)

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Abstract

Background: Calmodulin (CaM) is one of the most important Ca^{2+} signaling receptors because it regulates diverse physiological and biochemical reactions in plants. CaM functions by interacting with CaM-binding proteins (CaMBPs) to modulate Ca^{2+} signaling. IQ domain (IQD) proteins are plant-specific CaMBPs that bind to CaM by their specific CaM binding sites.

Results: In this study, we identified 102 *GhIQD* genes in the *Gossypium hirsutum* L. genome. The *GhIQD* gene family was classified into four clusters (I, II, III, and IV), and we then mapped the *GhIQD* genes to the *G. hirsutum* L. chromosomes. Moreover, we found that 100 of the 102 *GhIQD* genes resulted from segmental duplication events, indicating that segmental duplication is the main force driving *GhIQD* gene expansion. Gene expression pattern analysis showed that a total of 89 *GhIQD* genes expressed in the elongation stage and second cell wall biosynthesis stage of the fiber cells, suggesting that *GhIQD* genes may contribute to fiber cell development in cotton. In addition, we found that 20 selected *GhIQD* genes were highly expressed in various tissues. Exogenous application of MeJA significantly enhanced the expression levels of *GhIQD* genes.

Conclusions: Our study shows that *GhIQD* genes are involved in fiber cell development in cotton and are also widely induced by MeJA. Thw results provide bases to systematically characterize the evolution and biological functions of *GhIQD* genes, as well as clues to breed better cotton varieties in the future.

Keywords: *Gossypium hirsutum* L., *GhIQD* genes, Segmental duplication, Expression analysis

Background

Calcium signaling is one of the most important cytosolic second messages that mediates various developmental processes and the responses to biotic and abiotic stresses (Reddy et al. 2011). Cytoplasmic Ca^{2+} signals exert their functions through changes in the Ca^{2+} concentration with spatiotemporal specificity (Liu et al. 2019) and can be induced by extracellular stimuli such as drought, salt-alkali stress, and light, or intracellular stimuli such as plant hormones and pathogenic factors (Hernandez et al. 2004; Yuan et al. 2017; Salveson et al. 2019). Most Ca^{2+} receptor proteins contain helix-loop-helix fold (EF-hand) motifs that act as Ca^{2+}-binding domains. In higher plants, the calcium receptor proteins can be divided into four categories (Wei et al. 2019): Calmodulin (CaM), CaM-like proteins (CML), calcineurin B-like proteins (CBL), and calcium-dependent protein kinases (CDPK) (Ma et al. 2014), all of which contain EF-hand motifs.

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CaM is one of the most important Ca^{2+} signaling receptors (Zhou et al. 2018), and it regulates diverse physiological and biochemical reactions. However, CaM has no enzymatic activity and it transmits signals by interacting with CaM-binding proteins to modulate cellular physiology (DeFalco et al. 2009). CaM-binding proteins (CaMBPs) play an important role between Ca^{2+} and CaM and are the target proteins of the direct action of CaM. CaMBPs can be divided into two classes that are Ca^{2+}-dependent or Ca^{2+}-independent (Reddy et al. 2011). The IQ motif was the first identified Ca^{2+}-independent CaM-binding motif. In plants, the proteins containing IQ motifs include the myosin protein family, the calmodulin-binding transcription activator (CAMTA) protein family, the cyclic nucleotide-gated channel (CNGC) protein family, the IQ-motif (IQM) containing protein family, and the IQ67-domain (IQD) containing protein family (Steffen et al. 2013). *IQD* gene family members, which encoding plant-specific CaM/CML-binding proteins (CaMBPs), were firstly reported in *Arabidopsis* and rice (Abel et al. 2005). They are characterized by domains consisting of 67 amino acid residues (aa), such as the IQ67 domain, that are defined by a unique repetitive arrangement of the IQ motif; the Ca^{2+}-dependent CaM recruitment motifs exhibit 1–5-10 and 1–8-14 arrangements (Burstenbinder et al. 2013).

Plant-specific *IQD* gene families have been analyzed in *Populus trichocarpa* (Ma et al. 2014), *Arabidopsis thaliana*, *Oryza sativa* (Abel et al. 2005), *Phyllostachys edulis* (Wu et al. 2016), *Glycine max* (Feng et al. 2014), and *Solanum lycopersicum* (Huang et al. 2013), and the functions of a few *IQD* genes have been reported. In tomato, the *SUN* genes and other members of the *IQD* gene family exert their effects on organ shape by interacting with microtubules (Steffen et al. 2013). In *A. thaliana*, *AtIQD5* regulates pavement cell morphogenesis via Ca^{2+} signals (Liang et al. 2018). The tomato *IQD* gene *SUN24* regulates seed germination through the abscisic acid (ABA) signaling pathway (Bi et al. 2018). The AtIQD1 protein localizes to microtubules and interacts with kinesin in light chain-related protein 1 (KLCR1) to facilitate the cellular transport of specific cargo (Burstenbinder et al. 2013). *PtIQD* genes showed tissue-specific expression patterns and could also be regulated by drought and methyl jasmonate (MeJA) stresses (Ma et al. 2014). Additionally, some of the *GmIQD* genes expressed specifically and could be regulated by MeJA stress (Feng et al. 2014).

Gossypium hirsutum L. (AADD, 2n = 4x = 52) is one of the most important economic crops worldwide because it provides major raw materials for the textile industry as well as edible oil. The fiber length is determined during its elongation stage (0 to 26 days post-anthesis (DPA) to reach its final length (Zhao et al. 2019). The cellulose of the fiber cell wall is mainly synthesized from 15 to 40 DPA, which determines the fiber strength (Song et al. 2019). Therefore, *G. hirsutum* L. fiber production and quality are very important. However, the production is greatly affected by abiotic and biotic stresses. For example, in China, aphid infestation has been found to reduce *G. hirsutum* L. production by 30% (Li et al. 2018), and salt stress has been shown to decrease cotton fiber production by 20% (Shaban et al. 2018). IQD proteins are CaMBPs that play an important role in plant stress signal transduction. However, these proteins have not been reported in *G. hirsutum* L. In this study, we identified 102 *GhIQD* genes and determined their chromosomal locations, predicted protein physicochemical properties, duplications, phylogenetic relationships, and expression patterns during fiber development at 5, 10, 15, and 25 DPA. Twenty selected *GhIQD* genes were used for the analysis of tissue-specific expression patterns and their response to MeJA stress. These preliminary results for the *GhIQD* genes provide the foundation for further researches on the physiological and biochemical functions of IQD proteins in *G. hirsutum* L.

Materials and methods

Identification of *GhIQD* gene family members in *G. hirsutum* L.

To identify *GhIQD* gene family members in *G. hirsutum* L., the *G. hirsutum* L. genome (Hu et al. 2019) sequences were downloaded from the Cotton Functional Genomics Database (CottonFGD, https://cottonfgd.org/about/download.html). The AtIQD protein sequences from *A. thaliana* were downloaded from The *Arabidopsis* Information Resource (TAIR, https://www.arabidopsis.org/index.jsp), and the IQD protein sequences from *Glycine max* and *Solanum lycopersicum* were downloaded from Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html). The *G. hirsutum* L. genome sequences were searched using a Blastp search against reported IQD proteins with e-value less than 1e-5 (Chen et al. 2017; Sun et al. 2017), including 34 SlSUN, 27 OsIQD, 66 GmIQD, and 33 AtIQD proteins. We further used a Hidden Markov Model (HMM) with the default parameters to search the *G. hirsutum* L. genome for IQD proteins (PF00612) in the Pfam database (http://pfam.xfam.org), and the SMART databases (http://smart.embl-heidelberg.de) were used to confirm that all the candidate sequences were members of the IQD family (Lin et al. 2014). The identified *GhIQD* genes were named according to their physical locations on the chromosomes from *G. hirsutum* and visualized by MapChart 2.2 software (Voorrips 2002).

The isoelectric point (pI) and molecular weight (MW) were predicted for each protein with the online software

ExPASy (https://www.expasy.org/tools/). The subcellular localization of GhIQD proteins was predicted using WoLF PSORT (https://wolfpsort.hgc.jp).

GhIQD protein sequence alignment and phylogenetic analysis

Multiple alignments of all the predicted IQD protein sequences from maize, soybean, bamboo, *Arabidopsis*, tomato, and *G. hirsutum* L. was performed with MEGA version 7 (Kumar et al. 2016; Qin et al. 2018).

Gene duplication and synteny analysis of *GhIQD* genes

The duplication pattern of each *GhIQD* gene was analyzed using MCScanX software according to the manual (Wang et al. 2012). The whole-genome BLASTP analysis of *G. hirsutum* L. was performed using local Blast software considering e-values less than 1e-5, and output was produced (Knip 2012). The Blast search outputs and the positions of all protein-coding genes were imported into MCScanX software (http://chibba.pgml.uga.edu/mcscan2/), and the genes were classified into the various types of duplications, including segmental, tandem, proximal, and dispersed duplications (Jia et al. 2019), using the default parameters (You et al. 2015). Synteny relationships were visualized with CIRCOS software (Krzywinski et al. 2009). Nonsynonymous (*Ka*) and synonymous (*Ks*) substitution rates and the *Ka*/*Ks* ratio were estimated using DnaSP v5 software (Librado and Rozas 2009).

Expression analysis of the *GhIQD* genes

In the present study, RNA-seq data were downloaded from the public Cotton Functional Genomics Database (CottonFGD, https://cottonfgd.org/about/download.html), and the data were then used to survey the expression of the *GhIQD* genes (Zhu et al. 2017). The accession numbers of the RNA-Seq data for 5, 10, 20, and 25 DPA are SRR1695191, SRR1695192, SRR1695193, and SRR1695194, respectively, and all the expression values were standardized to fragments per kilobase per million (FPKM) values (Dong et al. 2018). The heatmap was performed to visualize gene expression patterns using OmicShare tools (https://www.omicshare.com/tools/Home/Soft/heatmap). R software was used to visualize the gene expression profiles, and the TCseq package (Feng et al. 2019) was used to cluster the *GhIQD* gene expression patterns.

Plant materials and treatments

The *G. hirsutum* L. cultivar TM-1 was grown in the field in Anyang, Henan province, China. Leaves, stems, roots, and hypocotyl tissues were collected at the seedling stage. Stigma, petal, pollen, and calyx samples were collected at the flowering stage. The *G. hirsutum* L. cultivar TM-1 was also grown in a greenhouse under a 14 h light / 10 h dark photoperiod at 30 °C (day) and 28 °C (night) (Wang et al. 2018). Seedlings at the five-leaf stage were sprayed with 0.5 mM MeJA and the fifth leaf was sampled at seven time points (0 h, 3 h, 6 h, 12 h, 24 h, 48 h, and 72 h) after treatments (Yang et al. 2017).

The cotton cultivars TM-1 was obtained from the State Key Laboratory of Cotton Biology, Institute of Cotton Research of Chinese Academy of Agricultural Sciences. All tissue samples were immediately frozen in liquid nitrogen and stored at − 80 °C, and three biological replicates were conducted for each sample.

RNA extraction and quantitative real-time PCR (qRT-PCR) analysis

Total RNA from the collected samples was extracted using the Tiangen RNAprep Pure Plant Plus Kit (Tiangen, China) as directed by the manufacturer. First-strand cDNA was synthesized via reverse transcription of 2 μg of total RNA using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Oligo 7 software was used to design gene-specific primers for qRT-PCR (Additional file 1: Table S1). The *GhHis3* gene (AF024716) was used as an internal reference control for gene expression (Wang et al. 2013). The qRT-PCR experiments were performed with the TB Green™ Premix Ex Taq™ II RNaseH Plus kit (TaKaRa, Japan) on an ABI7500 real-time PCR system (Applied Biosystems, USA) with three replicates per sample. qRT-PCR assays were performed in a volume of 20 μL, which contained 2 μL of each primer, 1 μL of cDNA and 7 μL of ddH_2O. The amplification conditions were as follows: initial denaturation at 95 °C for 2 min (Step 1), followed by 40 cycles of 10 s at 95 °C, 15 s at 58 °C, and 15 s at 72 °C (Step 2). The relative expression levels of the *GhIQD* genes were calculated using the $2^{-\Delta\Delta C}{}_T$ method (Livak and Schmittgen 2001). Statistical analyses were conducted using the one-way analysis of variance as implemented in SPSS software (Sun et al. 2018).

Results

Identification of *GhIQD* gene family members in *G. hirsutum* L.

In order to identify IQD gene family members in *G. hirsutum* L., 33 *Arabidopsis* IQD protein sequences were used as queries to search in the upland cotton genome database. After the further selection of conserved domains, a total of 102 *IQD* genes from the *G. hirsutum* L. genome were identified as members of the *GhIQD* gene family. The chromosomal locations of the *GhIQD* genes were then determined using the upland cotton genome information (Hu et al. 2019). As a result, all of the *GhIQD* genes were mapped to the *G. hirsutum* L. chromosomes and named GhIQDA01.1 to GhIQDD13.12 based on their relative positions on the chromosomes (Table 1, Fig. 1).

Table 1 Information of *GhIQD* gene family members in *G.hirsutum* L., their sequence characteristics and subcellular location

Gene ID	Gene name	length /aa	ORF length /bp	Mass /Da	pI	Predicted location	Exon number
GH_A01G1101	GhIQDA01.2	838	2 517	92 762.8	6.45	nucl	6
GH_A01G2332	GhIQDA01.3	466	1 401	51 680.07	10.04	nucl	5
GH_A02G0100	GhIQDA02.1	540	1 623	60 671.48	11.13	nucl	5
GH_A02G1393	GhIQDA02.2	385	1 158	43 210.99	10.19	nucl	3
GH_A04G0950	GhIQDA04.1	467	1 404	51 973.41	10.46	nucl	5
GH_A05G0263	GhIQDA05.1	411	1 236	45 861.25	10.34	nucl	3
GH_A05G0790	GhIQDA05.2	434	1 305	47 787.02	9.98	nucl	6
GH_A05G1339	GhIQDA05.3	403	1 212	44 740.05	10.14	nucl	3
GH_A05G1378	GhIQDA05.4	447	1 344	50 742.09	10.1	nucl	4
GH_A05G1577	GhIQDA05.5	306	921	34 962.1	10.42	nucl	5
GH_A05G1877	GhIQDA05.6	900	2 703	99 312.5	5.27	nucl	6
GH_A05G3022	GhIQDA05.7	339	1 020	37 749.85	10.8	nucl	4
GH_A05G3607	GhIQDA05.8	574	1725	52 858.66	10.51	nucl	5
GH_A05G4277	GhIQDA05.9	464	1 395	52 514.35	10.14	nucl	5
GH_A06G0050	GhIQDA06.1	659	1980	72 053.71	6.26	nucl	5
GH_A06G1837	GhIQDA06.2	442	1 329	50 167.53	9.86	nucl	5
GH_A06G1940	GhIQDA06.3	382	1 149	43 725.98	10.81	nucl	5
GH_A07G0094	GhIQDA07.1	414	1 245	45 320.56	10.21	nucl	3
GH_A07G0227	GhIQDA07.2	437	1 314	49 258.14	10.51	nucl	5
GH_A07G0720	GhIQDA07.3	651	1956	72 594.59	9.72	nucl	6
GH_A08G1336	GhIQDA08.2	539	1 620	60 952.52	9.78	nucl	4
GH_A08G2765	GhIQDA08.4	360	1 083	39 811.23	10.4	nucl	3
GH_A08G2816	GhIQDA08.5	418	1 257	47 014.72	10.52	nucl	5
GH_A09G1366	GhIQDA09.2	409	1 230	46 558.51	10.2	nucl	5
GH_A09G1685	GhIQDA09.3	452	1 359	50 179.15	10.12	nucl	5
GH_A09G2303	GhIQDA09.5	511	1 536	56 997.69	11.39	nucl	5
GH_A09G2394	GhIQDA09.6	404	1 215	45 045.51	9.94	nucl	3
GH_A10G0319	GhIQDA10.1	311	936	35 184.76	10.9	nucl	5
GH_A10G1560	GhIQDA10.2	523	1 572	58 681.88	10.66	nucl	5
GH_A11G0174	GhIQDA11.1	293	882	33 829.1	11.24	nucl	3
GH_A11G0321	GhIQDA11.2	767	2 304	84 830.18	9.77	nucl	6
GH_A11G1049	GhIQDA11.3	531	1 596	59 599.5	10.51	nucl	5
GH_A11G1587	GhIQDA11.4	381	1 146	43 192.29	9.28	nucl	6
GH_A12G1325	GhIQDA12.1	500	1 503	55 878.89	10.73	nucl	5
GH_A12G2604	GhIQDA12.2	519	1 560	59 126.62	9.88	nucl	5
GH_A12G2690	GhIQDA12.3	451	1 356	49 874.94	9.74	nucl	4
GH_A13G0768	GhIQDA13.2	549	1 650	60 060.1	9.99	nucl	6
GH_A13G0771	GhIQDA13.3	550	1 653	60 669.01	10	nucl	6
GH_A13G1240	GhIQDA13.5	306	921	34 548.98	10.4	nucl	3
GH_A13G1987	GhIQDA13.6	362	1 089	41 511.44	10.45	nucl	3
GH_A13G2167	GhIQDA13.7	326	981	38 128.29	10.38	nucl	5
GH_D01G1150	GhIQDD01.2	838	2 517	92 819.9	6.13	nucl	6
GH_D01G2410	GhIQDD01.3	466	1 401	51 732.91	10.07	nucl	5
GH_D02G0104	GhIQDD02.1	543	1 632	61 081.99	11.09	nucl	5

Table 1 Information of *GhlQD* gene family members in *G.hirsutum* L., their sequence characteristics and subcellular location *(Continued)*

Gene ID	Gene name	length /aa	ORF length /bp	Mass /Da	pI	Predicted location	Exon number
GH_D03G0607	GhlQDD03.2	385	1 158	43 307.07	10.25	nucl	3
GH_D04G0099	GhlQDD04.1	464	1 395	52 501.4	10.16	nucl	5
GH_D04G0861	GhlQDD04.2	474	1 425	52 953.74	10.51	nucl	5
GH_D04G1282	GhlQDD04.3	470	1 413	52 267.63	10.25	nucl	5
GH_D05G0268	GhlQDD05.1	411	1 236	45 920.33	10.28	nucl	3
GH_D05G0787	GhlQDD05.2	435	1 308	48 163.42	10.09	nucl	6
GH_D05G1342	GhlQDD05.3	403	1 212	44 653.93	10.36	nucl	3
GH_D05G1385	GhlQDD05.4	447	1 344	50 640.2	10.09	nucl	4
GH_D05G1607	GhlQDD05.5	306	921	34 947.05	10.39	nucl	5
GH_D05G1915	GhlQDD05.6	900	2 703	99 360.68	5.3	nucl	6
GH_D05G3028	GhlQDD05.7	427	1 284	47 736.21	10.29	nucl	5
GH_D06G0037	GhlQDD06.1	664	1995	72 524.53	6.89	nucl	5
GH_D06G1866	GhlQDD06.2	442	1 329	50 104.22	9.81	nucl	5
GH_D06G1971	GhlQDD06.3	386	1 161	44 138.29	10.65	nucl	5
GH_D07G0100	GhlQDD07.1	415	1 248	45 440.73	10.2	nucl	3
GH_D07G0234	GhlQDD07.2	437	1 314	49 274.22	10.48	nucl	5
GH_D07G0707	GhlQDD07.3	650	1953	72 097.97	9.75	nucl	6
GH_D08G1206	GhlQDD08.3	540	1 623	60 919.52	9.78	nucl	4
GH_D08G2761	GhlQDD08.5	359	1 080	39 873.37	10.43	nucl	3
GH_D08G2809	GhlQDD08.6	395	1 188	44 794.15	10.56	nucl	5
GH_D09G1141	GhlQDD09.1	436	1 311	47 553.9	10.08	nucl	6
GH_D09G1315	GhlQDD09.2	414	1 245	47 099.05	10.12	nucl	5
GH_D09G1630	GhlQDD09.3	446	1 341	49 661.4	10.1	nucl	5
GH_D09G2241	GhlQDD09.4	511	1 536	57 044.68	11.27	nucl	5
GH_D09G2334	GhlQDD09.5	406	1 221	45 218.73	9.98	nucl	3
GH_D10G0332	GhlQDD10.1	311	936	35 020.59	10.74	nucl	5
GH_D10G1333	GhlQDD10.2	523	1 572	58 604.9	10.63	nucl	5
GH_D11G0175	GhlQDD11.1	293	882	33 561.81	11.06	nucl	3
GH_D11G0334	GhlQDD11.2	767	2 304	84 784.04	9.8	nucl	6
GH_D11G1078	GhlQDD11.3	531	1 596	59 590.38	10.55	nucl	5
GH_D11G1617	GhlQDD11.4	411	1 236	47 034.9	9.46	nucl	7
GH_D12G1345	GhlQDD12.1	501	1 506	56 136.15	10.76	nucl	5
GH_D12G2626	GhlQDD12.3	518	1 557	58 961.41	9.89	nucl	5
GH_D12G2716	GhlQDD12.4	451	1 356	49 960.99	9.73	nucl	4
GH_D13G0741	GhlQDD13.2	522	1 569	60 358.36	9.98	nucl	6
GH_D13G0744	GhlQDD13.3	552	1 659	60 897.05	9.98	nucl	6
GH_D13G1947	GhlQDD13.5	376	1 131	43 085.2	10.43	nucl	3
GH_D13G2149	GhlQDD13.6	377	1 134	42 426.34	10.51	nucl	5
GH_A01G0547	GhlQDA01.1	285	858	32 302.82	10.15	mito	5
GH_A02G2016	GhlQDA02.3	120	363	13 689.84	11.79	mito	3
GH_A03G0236	GhlQDA03.1	488	1 467	53 976.96	9.96	mito	4
GH_A13G0956	GhlQDA13.4	480	1 443	53 068.34	9.98	mito	5
GH_D01G0541	GhlQDD01.1	285	858	32 287.85	10.11	mito	5

Table 1 Information of *GhIQD* gene family members in *G.hirsutum* L., their sequence characteristics and subcellular location *(Continued)*

Gene ID	Gene name	length /aa	ORF length /bp	Mass /Da	pI	Predicted location	Exon number
GH_D03G0046	GhIQDD03.1	133	402	15 440.04	11.65	mito	2
GH_D03G1733	GhIQDD03.3	489	1 470	54 209.22	10.02	mito	4
GH_D12G2109	GhIQDD12.2	315	948	36 162.76	10.74	mito	4
GH_D13G1022	GhIQDD13.4	480	1 443	52 890.99	9.94	mito	5
GH_A08G1119	GhIQDA08.1	445	1 338	49 676.63	9.38	E.R.	4
GH_D08G1102	GhIQDD08.1	445	1 338	49 457.39	9.38	E.R.	4
GH_A09G1181	GhIQDA09.1	436	1 311	47 752.06	10	chlo	6
GH_A08G2051	GhIQDA08.3	317	954	35 369.75	10.06	chlo	4
GH_A09G1949	GhIQDA09.4	461	1 386	51 179.31	10.33	chlo	4
GH_A10G2676	GhIQDA10.3	384	1 155	43 186.39	9.93	chlo	4
GH_A13G0121	GhIQDA13.1	385	1 158	44 219.64	10.37	chlo	5
GH_D08G1176	GhIQDD08.2	456	1 371	50 866.22	10.21	chlo	4
GH_D08G2064	GhIQDD08.4	508	1 527	56 482.43	9.83	chlo	6
GH_D10G2782	GhIQDD10.3	398	1 197	45 120.65	9.88	chlo	4
GH_D13G0120	GhIQDD13.1	385	1 158	44 188.66	10.27	chlo	5

nucl indicates nucleus, *mito* indicates mitochondria, *chlo* indicates chloroplast, *E.R.* indicates endoplasmic reticulum

The number of amino acids (aa) in the predicted GhIQD protein sequences ranged from 120 (GhIQDA02.3) to 900 aa (GhIQDA05.6) with an average length of 458 aa, and the open reading frames (ORFs) ranged from 363 base pairs (bp) to 2 703 bp with an average length of 1 377 bp. The molecular weights (MWs) of the proteins encoded by these proteins varied from 13 689.84 Da (Daltons) (GhIQDA02.3) to 99 360.68 Da (GhIQDD05.6), with an average MW of 51 151.02 Da. Based on isoelectric point (pI) analysis, the calculated pIs of the 96 *GhIQD* genes were > 7.0 (with an average of 10.27), whereas six *GhIQD* genes were predicted to encode proteins with pIs < 7.0 (average of 6.05), including *GhIQDA01.2*, *GhIQDA05.6*, *GhIQDA06.1*, *GhIQDD01.2*, *GhIQDD05.6*, and *GhIQDD06.1*. The predicted subcellular localizations showed that 82 GhIQD proteins localized to the nucleus,

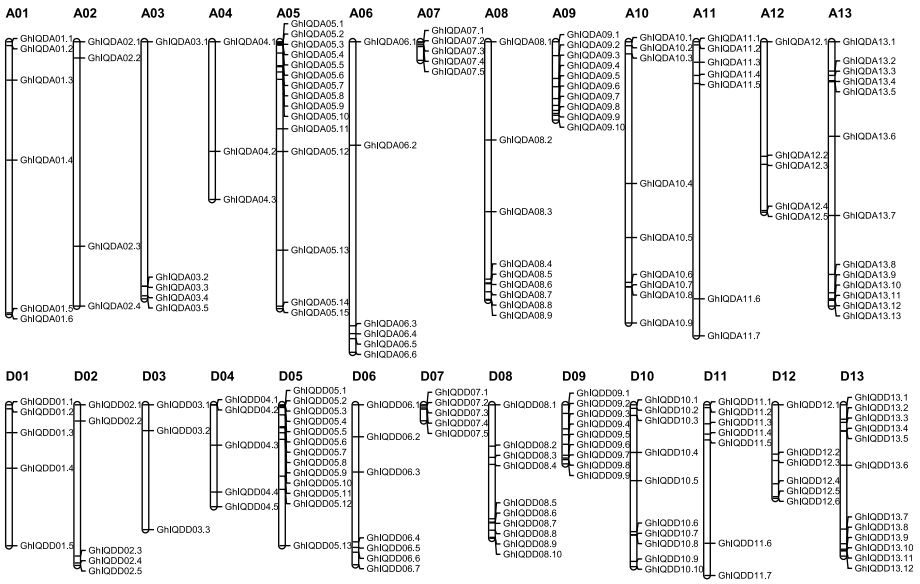

Fig. 1 Chromosomal locations of cotton GhIQD genes on the *G. hirsutum* L. chromosomes. A01-A13 and D01-D13 indicate chromosomes from the A-subgenome and D-subgenome, respectively. The chromosome number is shown above each chromosome, and the relative locations of the *GhIQD* genes are indicated on the chromosomes

nine GhIQD proteins localized to the mitochondria, nine GhIQD proteins localized to the chloroplasts, and two GhIQD proteins were found localized to the endoplasmic reticulum (ER) (Table 1).

Phylogenetic analysis of GhIQD proteins

To examine the molecular evolutionary relationships among plant IQD proteins, the amino acid sequences of the IQD proteins from *Arabidopsis*, tomato, soybean, and *G. hirsutum* L. were used in phylogenetic analysis. As shown in Fig. 2, a phylogenetic tree was constructed with the Neighbor-joining (NJ) method from an alignment of all complete IQD protein sequences. The NJ tree showed that the IQD proteins group could be divided into four clusters (I, II, III, and IV). Cluster III is the largest one with 18 GhIQDs and cluster Ib is the smallest subcluster with 2 GhIQDs.

IQD proteins are reported to specifically bind to calcium via CaM-binding sites (Cunwu et al. 2018). To better explore the biological functions of GhIQD proteins, the CaM-binding sites of the GhIQD proteins were predicted using the online Calmodulin Target Database software. As a result, GhIQD proteins are predicted to contain CaM-binding sites. Multiple consecutive strings of amino acid residues with scores > 7 are given in Additional file 1: Table S2. This result suggests that all GhIQD proteins contain CaM-binding sites with 1–3 strings of high-scoring amino acid residues.

Evolutionary analysis of *GhIQD* genes

To analyze the evolution of IQD gene family in *G. hirsutum* L., gene duplication events were analyzed. Based on the whole-genome duplication analysis in *G. hirsutum* L., 5 926 (8.14%) and 55 707 (76.56%) genes originated from tandem and segmental duplication, respectively. Therefore, we investigated the role of duplication events in the evolution of *GhIQD* genes. As shown in Fig. 3, among the 102 *GhIQD* genes identified in *G. hirsutum*

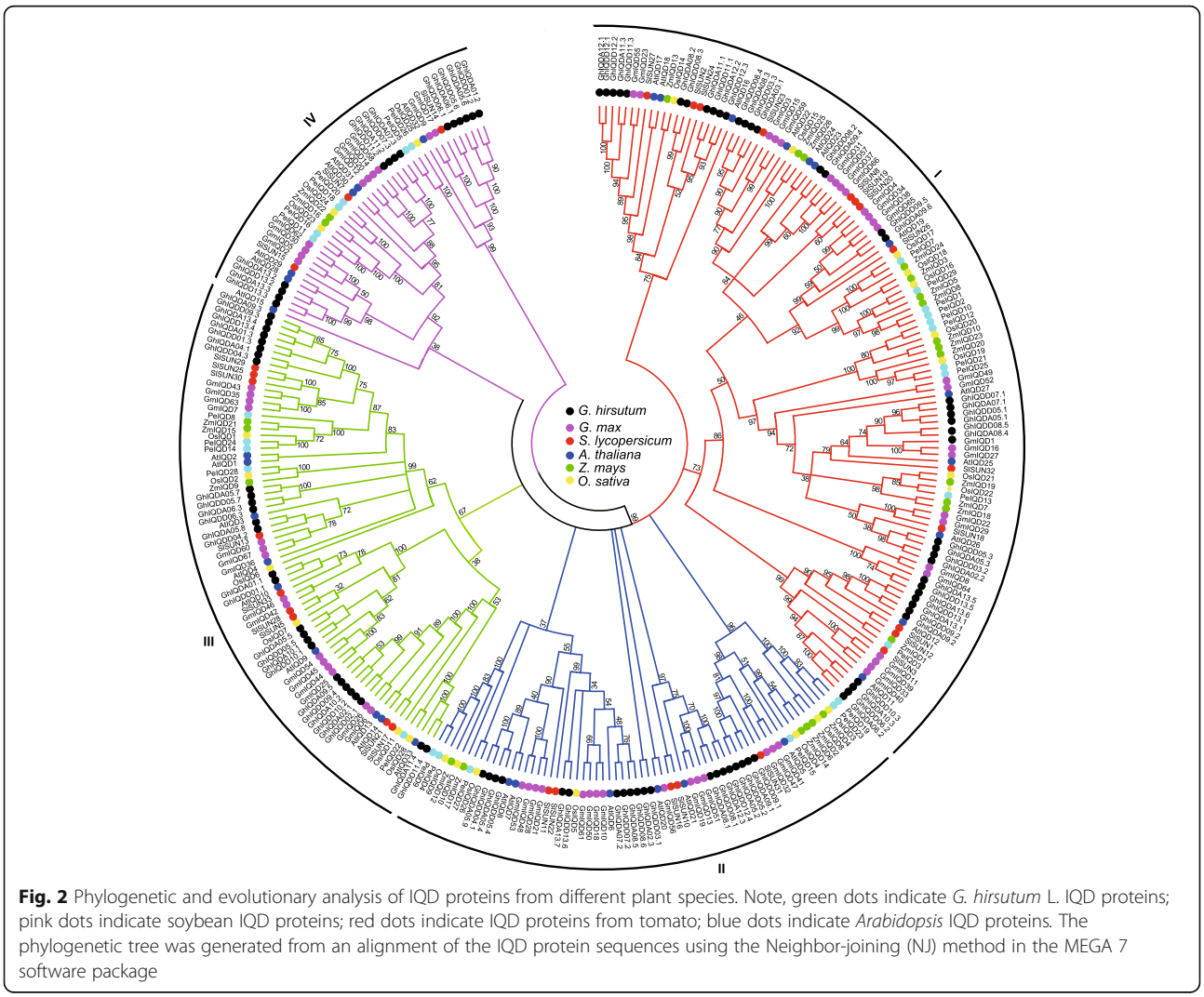

Fig. 2 Phylogenetic and evolutionary analysis of IQD proteins from different plant species. Note, green dots indicate *G. hirsutum* L. IQD proteins; pink dots indicate soybean IQD proteins; red dots indicate IQD proteins from tomato; blue dots indicate *Arabidopsis* IQD proteins. The phylogenetic tree was generated from an alignment of the IQD protein sequences using the Neighbor-joining (NJ) method in the MEGA 7 software package

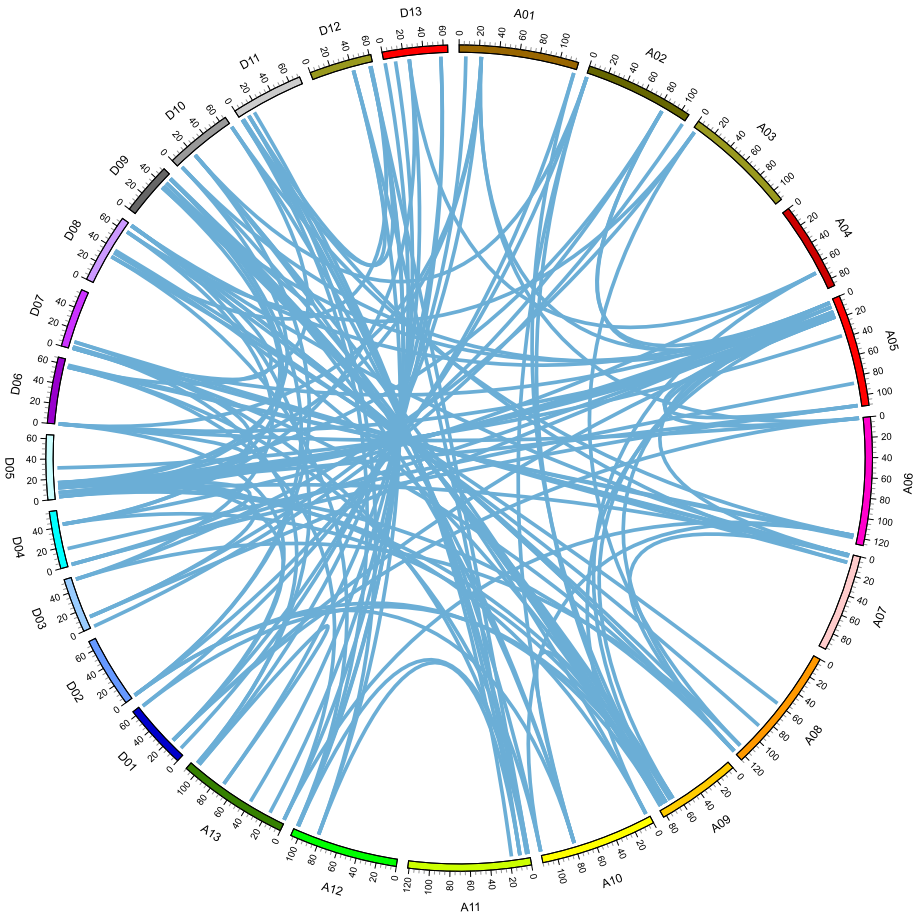

Fig. 3 Circos figure of *GhIQD* gene pairs that arose from segmental duplication. The *G. hirsutum* L. chromosomes from the A- and D-subgenomes are shown in different colors, and the gene pairs involved in segmental duplication are linked by blue lines. The CIRCOS genome visualization tool was used to construct this figure

L., 100 (98.04%) derived from segmental duplication events, and only two genes (*GhIQDA13.3* and *GhIQDD13.3*) derived from proximal duplication. In contrast, none of the *GhIQD* genes was found to have arisen from tandem duplication events (Additional file 1: Table S3 and 4). These results indicate that segmental duplication is the main driving force in the expansion of the *GhIQD* genes.

A *Ka*/*Ks* ratio > 1 indicates that paralogous gene pairs were produced by positive selection, a ratio < 1 indicates that paralogous gene pairs were under purifying selection and a ratio equal to 1 indicates that paralogous gene pairs were not subjected to selection pressure (Verma et al. 2017). To explore the type of selection pressure experienced by the duplicated *GhIQD* genes, paralogous *GhIQD* gene pairs were used to calculate synonymously (*Ks*) and non-synonymously (*Ka*) substitution rates to assess the ratio of non-synonymous to synonymous substitutions. As shown in Fig. 2, 50 paralogous gene pairs were identified. The *Ka*/*Ks* ratios of 48 members were < 1.0, and the *Ka*/*Ks* ratios for the remaining two paralogous gene pairs were > 1 (Additional file 1: Table S5), suggesting that the *GhIQD* paralogous gene pairs were mainly produced by purifying selection. Furthermore, the *Ks* ratio is stable and is usually used to estimate the evolution divergence time. The *Ks* ratios of 50 *GhIQD* gene pairs ranged from 0.009 40 to 0.379, and duplication events occurred from approximately 1.81 million years ago (MYA) to 72.87 MYA.

Transcriptome analysis of *GhIQD* genes during fiber development

Gene expression profiling can provide us with clues about the possible biological functions of genes. Therefore, we analyzed the gene expression profiles of *GhIQD* genes using the transcriptome data downloaded from the publicly available CottonFGD database. As shown in Fig. 4, a total of 20 *GhIQD* genes expressed during the developmental process in fiber cells. Cotton fiber development is divided into four overlapping stages: initiation,

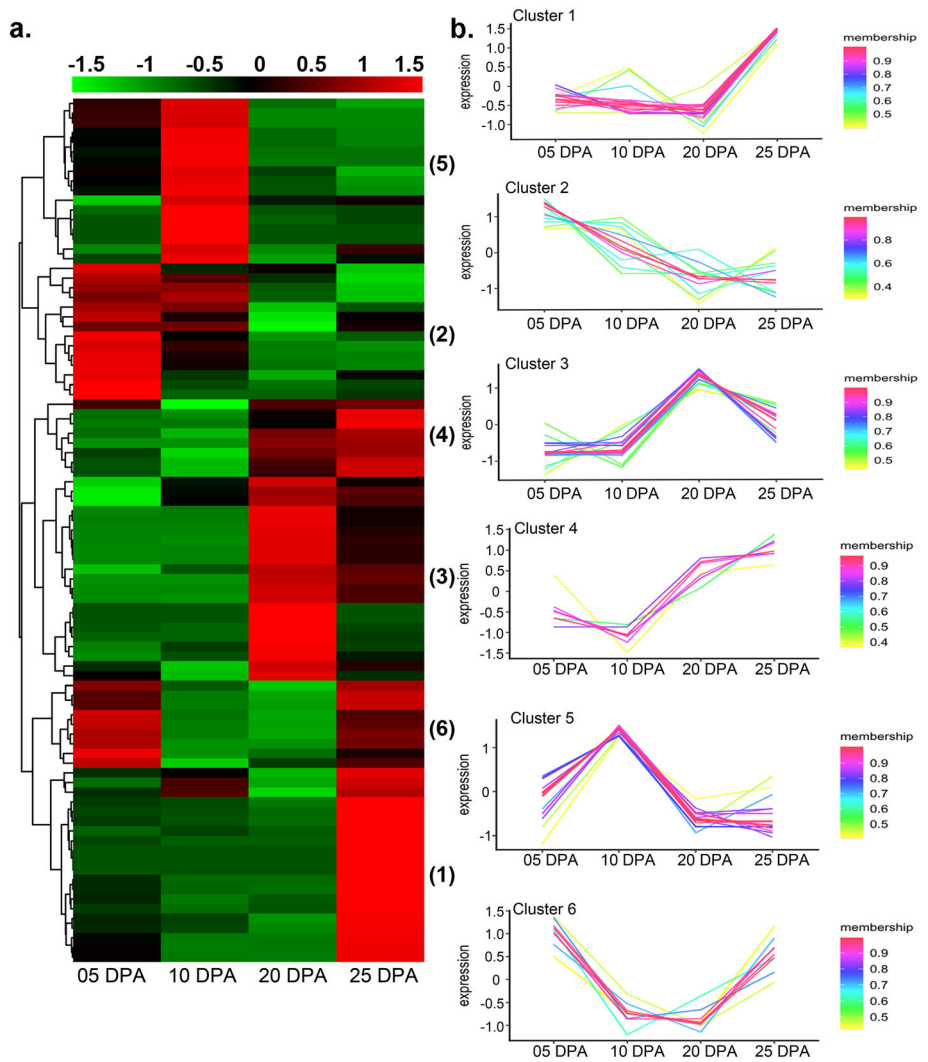

Fig. 4 Expression profiling of *GhIQD* genes during four developmental stages of the cotton fiber cell. **a** A hierarchical clustering heatmap of *GhIQD* gene expression in fiber cells at 5, 10, 20, and 25 DPA. These data were obtained from the transcriptome data available on the Cottongen website (https://www.cottongen.org/). The color scale represents the expression values, which have been normalized by the online software Omicshare **b** Gene expression of 89 *GhIQD* genes in six different clusters during four developmental stages of cotton fiber cells

elongation, secondary cell wall deposition, and maturation (Tuttle et al. 2015). Based on the heatmap, six clusters of *GhIQD* genes are predominately expressed in cotton fiber cells (Fig. 4a). In detail, the 13 *GhIQD* genes in cluster 2 were highly expressed in fiber cells at 5 DPA; the expression levels of 17 *GhIQD* family members in cluster 5 were up-regulated in the 10 DPA samples; 21 genes in cluster 3 were significantly expressed in fibers at 20 DPA; genes in cluster 1 with 21 members were highly expressed in 25 DPA fiber cells; in cluster 4, the transcripts of seven genes were abundant in fibers at 20 and 25 DPA; and the remaining 10 *GhIQD*s in cluster 6 were highly expressed in the 5 DPA and 25 DPA samples (Fig. 4b). These results imply that *GhIQD* genes may function in fiber cell development in cotton.

Tissue-specific expression analysis of *GhIQD* genes by qRT-PCR

The *GhIQD* gene family has 102 members; according to the expression abundance of *GhIQDs* in transcriptomes, 20 genes were selected to investigate their expression patterns in different tissues, including the calyx, leaf, stigma, stem, root, petal, pollen, and hypocotyl. As shown in Fig. 5, *GhIQDA13.1*, *GhIQDD12.1* and *GhIQDD13.1* were predominantly expressed in pollen (Fig. 5d), indicating that these genes may play pivotal roles in pollen development. *GhIQDD01.3*, *GhIQDD01.2* and *GhIQDD05.2* showed stem-specific expression (Fig. 5b), and *GhIQDA01.1*, *GhIQDA05.2* and *GhIQDA08.1* expressed preferentially in leaves (Fig. 5a). The *GhIQDA06.1*, *GhIQDD06.1* and *GhIQDD09.1* genes

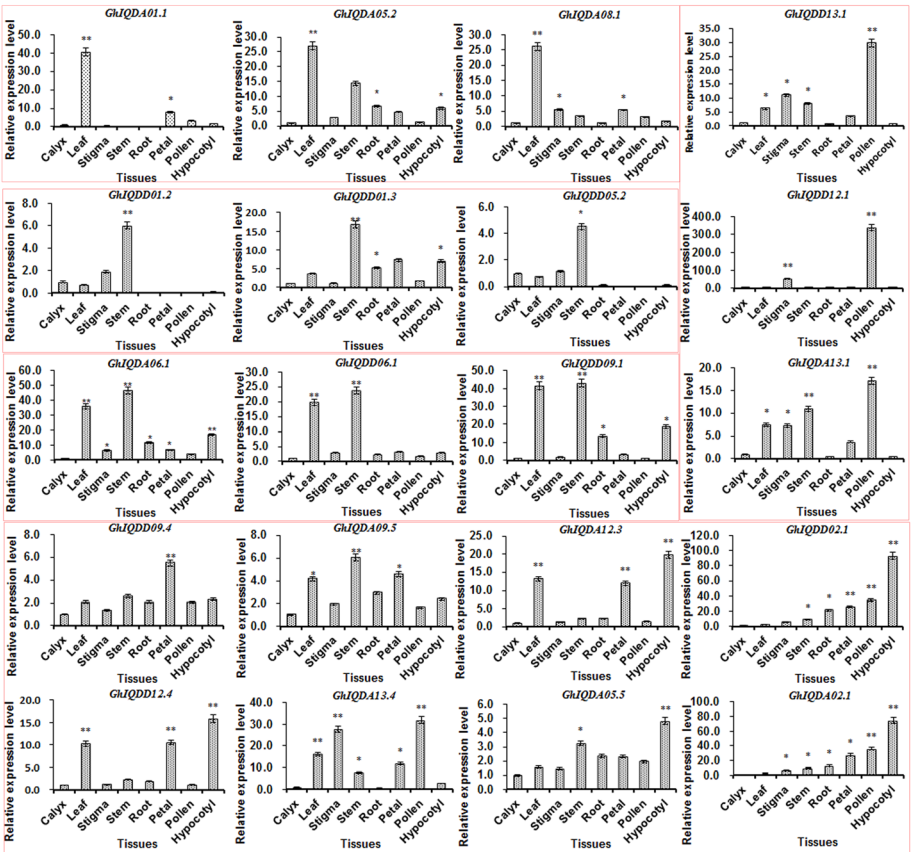

Fig. 5 Tissue-specific expression analysis of 20 selected *GhIQD* genes by qRT-PCR. The relative expression levels of 20 *GhIQD* genes were examined by qRT-PCR assays and normalized to the expression level of the house-keeping gene *GhHis3*. The calyx, leaf, stigma, stem, root, petal, pollen, and hypocotyl tissues are indicated on the X-axis. Relative gene expression levels compared with the calyx are indicated on the Y-axis, * represents a significant difference at the $p < 0.05$ level, ** represents an extremely significant difference at the $p < 0.01$ level

showed higher expression levels in leaves and stems (Fig. 5c). Most genes investigated were abundantly expressed in different tissues (Fig. 5e), as observed for *GhIQDA02.1* and *GhIQDD02.1* that were highly expressed in all tissues with similar expression patterns. The cluster II genes, *GhIQDA12.3* and *GhIQDD12.4*, were highly expressed in the leaf, petal, and hypocotyl (Fig. 5e). The expression patterns in specific tissues are strong evidence for roles of particular *GhIQD* genes in these specific locations and developmental processes.

Expression profiling of *GhIQD* genes in response to MeJA treatment

According to previous studies, the expression of most *IQD* genes can be induced by MeJA stress in plants (Bi et al. 2018). In this study, the expression patterns of *GhIQD* genes in plants exposed to MeJA treatment were examined in a qRT-PCR experiment. The results showed that the expression levels of the 20 selected *GhIQD* genes were significantly increased by MeJA treatment in the leaf (Fig. 6). As the time of treatment increased, the transcript levels for most genes increased significantly. In detail, the expression levels of *GhIQDA09.3*, *GhIQDA13.1*, *GhIQDD01.3*, *GhIQDD02.1*, *GhIQDD05.2*, *GhIQDD09.1*, and *GhIQDD13.1* were induced from 0 h, with the highest expression levels detected at 12 and 24 h after the MeJA treatment. The *GhIQDA06.1*, *GhIQDD06.1*, and *GhIQDD09.4* genes also exhibited the highest expression levels at 24 h after the MeJA treatment. The expression levels of *GhIQDA01.1*, *GhIQDA02.1*, *GhIQDA08.1*, *GhIQDA12.3*, *GhIQDA13.4*, *GhIQDD01.2*, *GhIQDD12.1*, and *GhIQD12.4* peaked at 6 h after the treatment. Compared with the other genes, the maximum expression of *GhIQDA05.5* occurred at 72 h. These results showed that the *GhIQD* genes are widely induced by MeJA treatment.

Discussion

Calcium is one of the most important cytosolic second messengers, and calcium levels can be induced by intracellular and extracellular stimuli. CaM is one of the most important Ca^{2+} signaling receptors regulating diverse

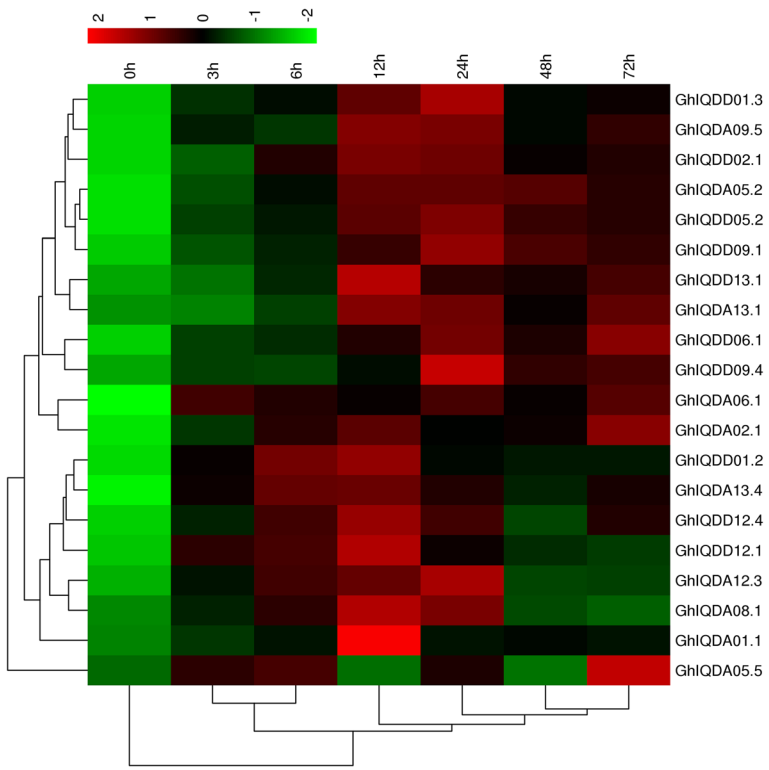

Fig. 6 The expression profiles of 20 selected *GhIQD* genes in response to MeJA treatment at 0, 3, 6, 12, 24, 48, and 72 h. The gene expression values were determined by qRT-PCR and calculated using the $2^{-\Delta\Delta C}{}_T$ method. The online software Omicshare (https://www.omicshare.com/tools/Home/Soft/heatmap) was used to normalize the expression values and draw the heatmap. The color scale on the top of the figure represents expression values computed by the $2^{-\Delta\Delta C}{}_T$ method

physiological and biochemical reactions. CaM functions by interacting with CaM-binding proteins (CaMBPs) to modulate cellular physiology. IQD proteins are plant-specific CaM/CML CaMBPs that are characterized by 67-amino acid domains. In this study, we identified 102 *GhIQD* genes in *G. hirsutum* and analyzed their chromosomal locations, protein physicochemical properties, gene duplication events, phylogenetic relationships, and expression patterns during the development of fiber cells. Twenty selected *GhIQD* genes were used for the analysis of tissue-specific expression patterns and their response to MeJA treatment.

The *GhIQD* gene family expanded by segmental duplication

A number of *IQD* genes have been reported in different plants; there are 33 *AtIQD* genes in *A. thaliana*, 28 *OsIQD* genes in *O. sativa* (Abel et al. 2005), 38 *PtIQD* genes in *P. trichocarpa* (Ma et al. 2014), 29 *PeIQD* genes in *P. edulis* (moso bamboo) (Wu et al. 2016), 67 *GmIQD* genes in *Glycine max* (Feng et al. 2014), and 34 *SlSUN/IQD* genes in *Solanum lycopersicum* (Huang et al. 2013). In this study, a total of 102 *GhIQD* genes were identified in *G. hirsutum* L. The number of *IQD* genes in *G. hirsutum* L. is greater than that found in other plant species, possibly because *G. hirsutum* L. is an allotetraploid cotton species that originated from the hybridization of *G. arboreum* and *G. raimondii* and subsequent polyploidization 1~2 million years ago (Wang et al. 2019). A whole-genome duplication analysis showed that 100 of the *GhIQD* genes arose from segmental duplication, and other two genes, *GhIQDA13.3* and *GhIQDD13.3*, originated from proximal duplications. Additionally, *Ka*/*Ks* analysis indicated that most of the *GhIQD* genes were under purifying selection, which indicates that the segmentally duplicated *GhIQD* genes were subjected to strong purifying constraints during evolution. The divergence time of *GhIQDs* was earlier than 1.81 MYA, which indicates that the duplication events of *GhIQDs* occurred before the polyploidization events in *G. hirsutum*.

GhIQD genes participate widely in the regulation of growth in *G. hirsutum* L.

In *Arabidopsis*, the AtIQD proteins were reported to widely link calcium signaling to microtubules,

membrane subdomains, and the nucleus (Bürstenbinder et al. 2017a). From the results of the transcriptome analysis, the *GhIQD* gene expression patterns could be clustered into six groups. In the fiber development process, the 5 DPA ovule stage is the primary cell wall synthesis stage of fiber cells; 10 DPA corresponds to the elongation stage of fiber development; and 20~25 DPA is the transition stage of fiber development from elongation to secondary wall synthesis, which is important for fiber strength (Hu et al. 2013). *PdIQD10* gene was found to be involved in the secondary cell wall biosynthesis and biomass formation in *Populus* (Badmi et al. 2018). Therefore, the genes in cluster 6 might participate in primary cell wall synthesis, the *GhIQD* genes in cluster 5 may contribute to fiber elongation, and the *GhIQD* genes in clusters 1, 3, and 4 may be involved in fiber strength development.

In *Arabidopsis*, *AtIQD* genes function as hubs in Ca^{2+} signaling to regulate growth and development tissue-specifically (Bürstenbinder et al. 2017b). To further elucidate the possible functions of the *GhIQD* genes, their expression patterns were investigated in various tissues (Fig. 5). The results show that some *GhIQD* genes are predominantly expressed in specific tissues, and the paralogous gene pairs exhibit similar expression patterns, such as *GhIQDA13.1* and *GhIQDD13.1,* which are predominantly expressed in pollen, indicating that *GhIQD* gene pairs are functionally redundant.

MeJA is an ester of jasmonic acid and is widely present in plants (Yan et al. 2018). MeJA triggers the biosynthesis of plant defensive compounds and initiates the expression of pathogenesis-related genes involved in systemic acquired resistances and local resistances (Pei 2017). In this study, the expression levels of all 20 selected *GhIQD* genes were increased (Fig. 6) in response to MeJA treatment, which is consistent with previous studies showing that most of the *IQD* genes in *P. trichocarpa* and moso bamboo are induced by MeJA treatment (Ma et al. 2014; Wu et al. 2016). These results imply that the *IQD* gene family plays an important role in tolerance to MeJA abiotic stress in plants.

Conclusions

In conclusion, we identified 102 *GhIQD* genes in *G. hirsutum* L. Segmental duplication was the main driving force behind the expansion of the *GhIQD* family. *Ka*/*Ks* analysis showed that *GhIQD* genes were under purifying selection. Based on the expression analysis, 89 genes could be detected during the stages of fiber development, tissue-specific expression analysis showed that some of the *GhIQD* genes were specifically expressed, and all 20 selected *GhIQD* genes could be induced by MeJA treatment. These preliminary results provide the foundation for further research on the physiological and biochemical functions of IQD proteins in *G. hirsutum* L.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-021-00079-3.

Additional file 1 : **Table S1**. Gene-specific primer pairs used in the qRT-PCR experiments. **Table S2**. Predicted calmodulin-binding sites of IQD proteins in *G. hirsutum* L. The calmodulin-binding sites were predicted with the online Calmodulin Target Database software, and the table shows strings of amino acid residues with a score of at least 7. Residues with a score of 9 are highlighted in bold. The numbers before the strings and after the strings indicate the locations of the first and the last amino acid residues of the strings in the GhIQD protein, respectively. **Table S3.** List of segmentally duplicated *IQD* gene pairs in the *G. hirsutum* L. genome along with their e-values identified from MCScanX. **Table S4.** List of tandem and segmentally duplicated *IQD* genes in the *G. hirsutum* L. genome identified with MCScanX software. **Table S5**. Divergence between paralogous *IQD* gene pairs in *G. hirsutum* L.

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Authors' contributions

Formal analysis, Kang YY and Zou CS; Investigation, Tian RJ. Resources, Li HZ; Software, Pang CY and Shang HH; Validation, Li SY, Liu FP, Cao LY, Jin YH, Li JY, Huang DQ and Liu Y; Visualization, Lv LM and Wang WB; Original draft, Dou LL; Reviewing and editing, Song GL and Xiao GH. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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