RESEARCH

Identification, characterization, and expression profiles of the GASA genes in cotton

QIAO Kaikai, MA Changkai, LV Jiaoyan, ZHANG Chaojun^{*}, MA Qifeng^{*} and FAN Shuli^{*}

Abstract

Background: GASA (Giberellic Acid Stimulated in Arabidopsis) gene family plays a crucial role in the phytohormone signaling pathway, growth and development, and stress responses in plants. Many GASA homologs have been identified in various plants. Nevertheless, little is known about these proteins in cotton.

Results: In the current study, we identified 19, 17, 25, 33, and 38 GASA genes via genome-wide analyses of Gossypium herbaceum, G. arboreum, G. raimondii, G. barbadense, and G. hirsutum, respectively, and performed comprehensive bioinformatics and expression analyses. According to our results, 132 GASA proteins shared similar protein structures and were classified into four groups based on the phylogenetic tree. A synteny analysis suggested that segmental duplication was a key driver in the expansion of the GASA gene family. Meanwhile, the cis-element and protein interaction analyses indicated that GhGASA proteins play a significant role in the hormone responses. Transcriptomic and gRT-PCR (Quantitative real time-polymerase chain reaction) analyses revealed diverse expression profiles of the GhGASA genes in different organs under abiotic stresses, indicating that some GhGASA genes possibly participate in fiber development and abiotic-stress responses.

Conclusions: The GASA genes in cotton were systematically identified and analyzed for the first time in this paper, and it suggested that the GASA genes are important to the development and growth of cotton. These results will support future exploration of the functions of GASA genes in cotton.

Keywords: GASA gene, Genome-wide, Bioinformatics, Expression pattern, Cotton

Introduction

GASA (Giberellic acid stimulated in Arabidopsis) is a CRP (cysteine-rich peptide) protein (Silverstein et al. 2010) and a low-molecular-weight peptide that is widespread in the plant world. Numerous studies have illustrated that GASA proteins are important for plant growth and development, but much remains unknown. The GAST1 gene was the first identified member of the GASA gene family, found in the gib1 tomato mutant (Shi et al. 1992). Previous studies on the GASA gene family

* Correspondence: zcj1999@yeah.net; 13837240176@163.com; fsl427@126.com

Iournal of Cotton Research

© The Author(s). 2021 Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

have been conducted in A. thaliana (Aubert et al. 1998; Roxrud et al. 2007), Moso Bamboo (Hou et al. 2018), apple (Fan et al. 2017), rice (Muhammad et al. 2019), wheat (Lv et al. 2018), Glycine max (Ahmad et al. 2019), Solanum tuberosum (Nahirñak and Rivarola 2016), grapevine (Ahmad et al. 2020), and Sorghum bicolor (Filiz and Kurt 2020). Almost all GASA protein domains have a C-terminal region of approximately 60 amino acids consisting of 12 cysteine residues (Aubert et al. 1998). Studies have shown that missing or alterating crucial peptide residues may lead to a loss of biological function of GASA domain (Sun et al. 2013). This indicates that the evolution of cysteine-rich regions is relatively conservative, and that these regions possesse important biological activities (Sun et al. 2013).







State Key Laboratory of Cotton Biology, Institute of Cotton Research of CAAS, Key Laboratory of Cotton Genetic Improvement, Ministry of Agriculture and Rural Affairs, Anyang 455000, Henan, China

Several studies have shown that GASA proteins influence processes of phytohormone response and plant growth and development, including signal transduction, seed development, floral and root development, and flowering time. For instance, in rice, OsGSR1 can influence brassinosteroid signaling by interacting with DIM/ DWF1 (DIMINUTO/DWARF1), a brassinosteroid synthetase (Wang et al. 2010), and OsGASA1/7 genes are important in the brassinolide (BR) and gibberellin (GA) signaling networks (Lee et al. 2015; Boonpa et al. 2018). The expression levels of several MdGASA genes were upor downregulated under GA and 6-benzylaminopurine (6-BA) treatments in apple (Fan et al. 2017). In Arabidopsis, AtGASA2/3/5 and AtGASA14 are involved in flower induction and participate in the abscisic acid (ABA) signaling response (Roxrud et al. 2007). Moreover, maize ZmGSL genes exert different functions in GA₃-regulated root growth (Zimmermann et al. 2010). Furthermore, the expression of potato GASA genes is affected by GA and ABA hormones, and is highly induced during the seedling stage and floral formation (Nahirñak and Rivarola 2016). Notably, TaGASR7 is associated with grain length in wheat (Dong et al. 2014), and OsGASA genes increase grain size and length in rice (Muhammad et al. 2019). VvGASA2 and VvGASA7 are involved in seed development in grapevines (Vitis vinifera L.) (Ahmad et al. 2020). These results show that GASA genes have a great potential for increasing the crop yield and fruit breeding. However, many reports have indicated that various GASA gene family members serve antagnistic functions. For example, AtGASA4 facilitates flowering while AtGASA5 delays or inhibits flowering in Arabidopsis (Aubert et al. 1998; Roxrud et al. 2007; Zhang et al. 2009), and GEG (Gerbera homolog of GAST1 gene) delays petal development while PRGL (proline-rich and GASA-like) promotes petal development in Gerbera hybrida (Kotilainen et al. 1999; Peng et al. 2010).

In other researches, some GASA proteins have been shown to have important impacts on biotic- and abioticstress resistance. GsGASA1 isolated from Glycine soja was shown to be involved in inhibiting root growth via the accumulation of DELLA proteins under chronic cold stress (Li et al. 2011). Arabidopsis GASA4 (Ko et al. 2007) and GASA5 play a role in response to heat stress (Zhang and Wang 2011), and the overexpression of Beechnut FsGASA4 in Arabidopsis improves tolerance to abiotic stress (Alonso-Ramírez et al. 2009). Another report noted that GASA14 can modulate the accumulation of reactive oxygen species (ROS) to regulate abioticstress resistance (Sun et al. 2013). In disease resistance, HbGASA genes in rubber plants show high expression levels when faced with fungi (An et al. 2018). Indeed, snakin1, -2, and -3 in Solanum tuberosum responded similarly when inoculated with bacterial fungal pathogens (Nahirñak and Rivarola 2016). Meanwhile, Snakin-1 and Snakin-2 proteins exert antibacterial activity in potato (Segura et al. 1999; Berrocal-Lobo et al. 2002; Almasia et al. 2008; Kovalskaya and Hammond 2009; Balaji and Smart 2012). Moreover, CaSn, which is a GASA/snakin protein, is involved in halting the intrusion of root-knot nematodes (*Meloidogyne* spp.) in pepper (Mao et al. 2011).

Cotton is an important fiber crop and is the main raw material used to produce lint, along with cooking oil and other products (Zhang et al. 2008). In recent years, many cotton gene families have been reported and analyzed, including INDETERMINATE DOMAIN genes (Ali et al. 2019), plant U-box genes (Lu et al. 2020), DNA demethylases (Yang et al. 2019b), OSCA (Hyperosmolality-gated calcium-permeable channels) gene (Yang et al. 2019a), and VEFS-box (Ma et al. 2020). However, there has been little research on GASA genes. An earlier study identified GhGASL1-GhGASL7 from the cDNA libraries of upland cotton and suggested that GhGASL genes are involved in fiber development and GA signaling responses (Liu et al. 2013). Overall, most GASA genes in cotton are yet to be identified and researched. Here, we identified the GASA genes in five cotton species using the latest genomic database and bioinformatics methods, and illustrated their chromosomal locations, chemical characterization, phylogenetic relationships, gene structures, synteny, cis-elements, and expression patterns, predicted their potential functions. Our findings will further expand the available information on GASA gene family members and provide a theoretical basis for further studies on the functions of GASA genes in cotton.

Materials and methods

Identification of the GASA proteins in Gossypium spp.

First, we used the AtGASA protein sequences as queries to search in the cotton genome database (https:// cottonfgd.org/; https://www.cottongen.org/) (Zhu et al. 2017; Yu et al. 2014), using the BLAST Similarity Search tool. Second, proteins containing the GASA domain (PF02704) in the *Gossypium* spp. database were detected via the hidden Markov model (HMM). The complete GASA domains of the protein sequences were examined using Batch CD-Search tools (https://www.ncbi.nlm.nih. gov/Structure/cdd/cdd.shtml), SMART (http://smart. embl.de/smart/set_mode.cgi?NORMAL=1#), and Pfam (http://pfam.xfam.org/). Finally, conserved domains of all candidate protein sequences were verified via manually removing incomplete domain sequences.

Physicochemical characterization, chromosomal location, and sequence alignments

The ExPASy program was used to predict the characteristics of the GASA protein (Artimo et al. 2012). The subcellular locations of the GASA proteins were predicted using an online tool (http://cello.life.nctu.edu.tw/) (Yu et al. 2010). Information on the chromosomal locations of the *GASA*s was acquired from the Cottongen database (https://www.cottongen.org/), and the gene locations were plotted on the chromosomes using MapChart software (Voorrips 2002) and further embellished using Adobe Illustrator CC 2018. ClustalW in the SeaView software was used to align the GASA protein sequences, while the sequence logos were further created using the WebLogo platform (Crooks et al. 2004), and GeneDoc software was used to color the conserved amino acids. The 3D structures of the GASA proteins were predicted using PHYRE server v2.0 (http://www.sbg.bio.ic.ac.uk/phyre2/html/page. cgi?id=index, which is an online tool.

Phylogenetic tree, gene structure, and promoter region analyses

Phylogenetic trees were constructed using the neighborjoining method under 1 000 replicates bootstrap test by MEGA 7.0 software (Kumar et al. 2016). The online GSDS tool (http://gsds.cbi.pku.edu.cn) was used to visualize the exon-intron structures of the *GASA* genes. Prediction of the *cis*-acting elements was performed using the PlantCARE online tool on the upstream sequence of each gene (1.5 KB) (Lescot et al. 2002), and the image were ultimately created using TBtools software (Chen et al. 2020).

Synteny analysis and protein interactions of GASA genes in Gossypium hirsutum

The synteny and collinearity of GASA genes were determined using the Multiple Collinearity Scan toolkit (MCScanX) (Wang et al. 2012a). We then used the Circos software to visualize the syntenic relationship of the duplicated genes (Krzywinski et al. 2009). The protein interaction database is only available for *G. raimondii*, which was obtained from the String website (https:// string-db.org/), then the CDS sequence of the *GASA* genes in upland cotton was blast against CDS sequence of *G. raimondii* to obtain corresponding protein interactions of upland cotton. Related protein information was based on an available database (https://www.uniprot.org/). The Cytoscape software was used to map the proteinprotein interactions (Shannon et al. 2003).

Plant materials and treatments

Gossypium hirsutum cv. TM-1 was planted in field in Anyang, Henan, China. The roots, stems, leaves, anthers, and petals of the plants were harvested for RNA extraction. The ovules of the flowers were collected in liquid nitrogen for RNA extraction 3 days before flowering, as well as at 0 days, 1 day, 3 days, and 5 days after flowering. Seedlings with two cotyledons were moved to a light incubator at 4 °C. The whole plants were collected at 1 h, 3 h, 6 h, 12 h, and 24 h in the 4 °C treatment. The control seedlings were planted under consistent natural conditions. We immediately froze all samples in the liquid nitrogen and stored them at - 80 °C. Three independent biological experiments were conducted for each sample.

Transcriptome data analysis and quantitative RT-PCR (qRT-PCR) for GASA genes in Gossypium hirsutum

To analyze the expression profiles of *GhGASA* genes in different tissues and at different developmental stages, we retrieved the FPKM (fragments-per-kilobase-per-million) expression data from CottonFGD (https://www.cottonfgd. org) (Zhu et al. 2017). The TBtools software (Chen et al. 2020) was used to assemble transcripts for each RNA-seq analysis. Heatmap charts were generated based on the FPKM values. The gene-specific primers used in qRT-PCR (Quantitative real time-polymerase chain reaction) were listed in Additional file 1: Table S1. The PCR conditions were as follows: preheating for 30 s at 95 °C; 35 cycles for 30 s at 95 °C, 60 s at 60 °C, and 10 s at 72 °C for 10 s, and, finally, 2 min at 72 °C for extension. The relative gene expression levels were calculated according to the $2^{-\Delta\Delta C}$ method (Schmittgen and Livak 2008).

Results

Identification and protein features of GASA genes in Gossypium spp.

To identify the GASA genes in Gossypium spp., we used the Blast by Similarity Search Tool and the Hidden Markov Model (HMM) alignment search on the protein sequence databases of G. herbaceum, G. arboreum, G. raimondii, G. barbadense, and G. hirsutum, respectively. Then, the Batch CD-Search tools, Pfam and SMART were used to verify the obtained sequences. Finally, the numbers of GASA genes confirmed in G. herbaceum (GheGASAs), G. arboreum (GarGASAs), G. raimondii (GrGASAs), G. barbadense (GbGASAs), and G. hirsutum (GhGASAs) were 19, 17, 25, 33, and 38, respectively. All genes were named based on their locations on the chromosomes. Meanwhile, previously reported GhGASL genes were renamed according to their homologs. The predicted sequences of GASA genes were deposited in GenBank (accession numbers MW429289-MW429326, MW450702-MW450776).

Detailed information about cotton *GASA* genes and proteins characteristics are listed in Table 1 and Additional file 2: Table S2, including the length of gene, molecular weight (MW) of the protein, isoelectric point (pI) of the protein, subcellular localization of the protein, length of the CDS sequence. The analysis results showed that the GarGASA17 was the shortest protein, in *G. arboreum*, while GbGASA19 was the longest, in *G. barbadense*. The average MW of GhGASAs was

Table 1 Information of the GASA genes in G. hirsutum

Gene name	Sequence ID	Chromosome	Protein Length /aa	CDS Length /bp	Gene position	MW /Da	pl	Subcellular prediction	Accession number
GhGASA1	GH_ A02G0648.1	A02(-)	103	312	9 191 049– 9 191 443	11 234.2	8.56	Extracellular	MW429289
GhGASA2	GH_ A02G1291.1	A02(+)	119	360	55 425 153– 55 426 696	13 079.3	8.69	Extracellular	MW429290
GhGASA3	GH_ A04G1429.1	A04(+)	88	267	84 442 850– 84 443 222	9 863.7	8.44	Extracellular	MW429291
GhGASA4	GH_ A05G1862.1	A05(+)	102	309	17 547 630– 17 548 124	10 775.6	8.7	Extracellular	MW429292
GhGASA5	GH_ A05G2086.1	A05(-)	88	267	19 997 476– 19 997 844	10 086.9	8.55	Extracellular	MW429293
GhGASA6	GH_ A05G2637.1	A05(-)	86	261	28 125 670– 28 126 046	9 466.4	9.5	Extracellular	MW429294
GhGASA7	GH_ A05G2768.1	A05(-)	90	273	30 654 351– 30 654 812	9 759.6	8.06	Extracellular	MW429295
GhGASA8	GH_ A05G3865.1	A05(+)	106	321	101 587 855– 101 588 525	11 895	9.22	Extracellular	MW429296
GhGASA9/ GhGASL1	GH_ A06G0035.1	A06(+)	102	309	250 147–250 553	10 668.5	9.18	Extracellular	MW429297
GhGASA10	GH_ A06G0037.1	A06(+)	104	315	254 660–255 177	11 326	6.44	Extracellular	MW429298
GhGASA11	GH_ A06G0225.1	A06(-)	88	267	2 296 267– 2 296 753	9 646.4	8.25	Extracellular	MW429299
GhGASA12	GH_ A07G0862.1	A07(-)	105	318	11 202 685– 11 204 347	11 867.9	9.14	Extracellular	MW429300
GhGASA13/ GhGASL4	GH_ A07G2469.1	A07(-)	264	795	94 606 844– 94 608 048	27 763.7	10.44	Nuclear, Plasma Membrane	MW429301
GhGASA14/ GhGASL2	GH_ A09G0176.1	A09(-)	106	321	3 985 530– 3 986 221	11 945	9.08	Extracellular	MW429302
GhGASA15	GH_ A09G0286.1	A09(-)	213	642	7 457 030– 7 458 016	23 064	9.02	Nuclear, Extracellular, Plasma Membrane	MW429303
GhGASA16	GH_ A09G1863.1	A09(-)	112	339	75 508 595– 75 509 153	11 903	8.75	Extracellular	MW429304
GhGASA17	GH_ A10G1353.1	A10(-)	76	231	68 911 818– 68912048	8 982.4	8.81	Extracellular	MW429305
GhGASA18	GH_ A11G2325.1	A11(+)	97	294	62 945 933– 62 946 385	10 450.2	8.61	Extracellular	MW429306
GhGASA19	GH_ A12G0598.1	A12(+)	103	312	13 285 723– 13 286 200	11 079.8	8.44	Extracellular	MW429307
GhGASA20	GH_ D02G0660.1	D02(-)	101	306	8 705 229– 8 705 622	11 010.9	8.56	Extracellular	MW429308
GhGASA21	GH_ D02G2258.1	D02(+)	90	273	67 179 221– 67 179 588	10 071.1	9.04	Extracellular	MW429309
GhGASA22	GH_ D03G0268.1	D03(-)	112	339	2 569 723– 2 570 204	12 224.4	9.3	Extracellular	MW429310
GhGASA23/ GhGASL5	GH_ D04G0496.1	D04(-)	106	321	7 607 854– 7 608 525	11 825.9	9.07	Extracellular	MW429311
GhGASA24/ GhGASL3	GH_ D04G1773.1	D04(+)	90	273	53 548 230– 53 548 599	10 064.9	8.55	Extracellular	MW429312
GhGASA25	GH_ D05G1900.1	D05(+)	102	309	16 130 361– 16 130 855	10 715.6	8.84	Extracellular	MW429313
GhGASA26	GH_ D05G1901.1	D05(+)	103	312	16 132 289– 16 132 796	11 548.3	8.15	Extracellular	MW429314
GhGASA27	GH_	D05(-)	88	267	18 147 149-	10 086.9	8.55	Extracellular	MW429315

Gene name	Sequence ID	Chromosome	Protein Length /aa	CDS Length /bp	Gene position	MW /Da	pl	Subcellular prediction	Accession number
	D05G2117.1				18 147 531				
GhGASA28	GH_ D05G2661.1	D05(-)	86	261	24 666 254– 24 666 629	9 452.4	9.5	Extracellular	MW429316
GhGASA29	GH_ D05G2783.1	D05(-)	90	273	26 448 762– 26 449 224	9 758.7	8.32	Extracellular	MW429317
GhGASA30	GH_ D05G2784.1	D05(-)	90	273	26 470 771– 26 471 233	9 758.7	8.32	Extracellular	MW429318
GhGASA31	GH_ D06G0022.1	D06(+)	102	309	134 310–134 720	10 591.5	9.19	Extracellular	MW429319
GhGASA32	GH_ D06G0024.1	D06(+)	104	315	141 982–142 488	11 342	6.44	Extracellular	MW429320
GhGASA33/ GhGASL6	GH_ D06G0209.1	D06(-)	88	267	2 047 165– 2 047 637	9 678.4	8.25	Extracellular	MW429321
GhGASA34	GH_ D07G0864.1	D07(-)	96	291	9 661 192– 9 662 681	10 563.4	8.97	Extracellular	MW429322
GhGASA35	GH_ D09G0292.1	D09(-)	190	573	7 330 104– 7 331 019	20 669.2	9.29	Extracellular, Nuclear, Plasma Membrane	MW429323
GhGASA36	GH_ D09G1817.1	D09(-)	112	339	45 112 430– 45 112 988	11 933.9	8.38	Extracellular	MW429324
GhGASA37	GH_ D11G2128.1	D11(-)	97	294	26 171 794– 26 172 246	10 406.1	8.68	Extracellular	MW429325
GhGASA38	GH_ D12G0617.1	D12(+)	103	312	10 310 554– 10 311 031	10 957.7	8.43	Extracellular	MW429326

Table 1 Information of the GASA genes in G. hirsutum (Continued)

11 776.78 Da, and the average MW of GheGASAs, Gar-GASAs, GrGASAs, and GbGASAs were 15 972.93 Da, 11 349.69 Da, 12 350.80 Da, and 14 071.10 Da, respectively. The average isoelectric point (pI) of GheGASAs, GarGASAs, GrGASAs GhGASAs and GbGASAs were 8.65, 8.65, 8.85, 8.68, and 8.70, respectively, and they showed similar statistical results. The pI values of the five cotton species' proteins ranged from 6.44 to 10.60. The pI of GarGASA11, GheGASA12, and GhGASA32 were the lowest, 6.44, while that of GrGASA3 was the highest, 10.60. The function of a protein is usually closely linked to its subcellular positions. The predicted subcellular locations suggested that most of the GASA proteins in cotton are located in the extracellular. Only a few proteins are located in nuclear and plasma membrane.

Based on the predicted 3D protein structures, α helices, β sheets, extended strands, and random coils were found in GhGASA proteins. According to our analysis, all GhGASA proteins have coils which is a flexible structure, at least three α helices, while β sheets are uncommon (Additional file 3: Fig. S1).

Sequence analysis, chromosomal localization, and phylogenetic tree of cotton GASA genes

To verify the GASA domain, the full GASA protein sequences were aligned using SeaView and GeneDoc software. All the GASA proteins shared similar structures: an N-terminal signal peptide containing 18 to 30 amino acids, a variable peptide region, and a highly conserved C-terminal region consisting of about 60 amino acids with 12 cysteine residues (Fig. 1, Additional file 4: Fig. S2, Additional file 5: Fig. S3). Based on the publicly available information on the cotton genome, chromosomal maps were drawn for the 132 GASA genes from G. herbaceum, G. arboretum, G. barbadense, G. hirsutum, and G. raimondii using MapChart software (Additional file 6: Fig. S4, Additional file 7: Fig. S5). In total, 131 GASAs were dispersed across the chromosomes, except for one GASA gene of G. arboretum that was located in the unmapped scaffold. Notably, longer chromosomes did not necessarily contain more members of the GASA gene family, which indicates that the number of GASA genes on each chromosome is independent of its length. This result showed that the GASA protein-coding genes of G. hirsutum, as in other plants (Fan et al. 2017; Muhammad et al. 2019; Ahmad et al. 2020), are not evenly distributed on 26 chromosomes. To study the evolutionary relationships of the GASA gene family in cotton, a phylogenetic tree was constructed using the neighbor-joining method for 173 GASA proteins from G. arboreum (17 GarGASAs), G. herbaceum (19 GheGASAs), G. raimondii (25 GrGASAs), G. hirsutum (38 GhGASAs), G. barbadense (33 GbGASAs),

Malus domestica (26 MdGASAs), and A. thaliana of e (15 AtGASAs) (Fig. 2). These results showed that all GASA genes could be divided into four groups (G1, G2, G3, and G4) based on topological consistency.

Gene structure analysis of GASA members in G. hirsutum

To further study the evolutionary relationships of GhGASA proteins, a phylogenetic tree was reconstructed (Additional file 8: Fig. S6A). The coding sequences within the corresponding genomic sequences were aligned for a comparative analysis of the intron– exon structures (Additional file 8: Fig. S6B). The structures of *GASA* genes were divided into four groups. Each member of G4 contained two exons, while the number of exons in G1, G2, and G3 ranged from 2 to 4, 2 to 3, and 1 to 4, showing that the structure of G4 group members is more conserved than that of other subgroups.

Synteny analysis of GASA genes in G. hirsutum

BLAST and MCScanX were used for genomic synteny analysis to further investigate the expansion mechanism of the *GASA* gene family in *G. hirsutum*. As shown in Additional file 9: Fig. S7, two genes were tandemly duplicated (*GhGASA5* and *GhGASA6*), while 20 pairs of segmental duplication were observed in the 25 genes. Thus, compared with tandem duplication, segmental duplication has played a dominant





role in the evolution of *GASA* gene family. To explain the evolutionary relationship of *GASA* genes, the synteny analysis was conducted using the genomes of different cotton species (Fig. 3). *G. herbaceum* (A_1) and *G. arboreum* (A_2), *G. arboreum* (A_2) and *G. hirsutum* A-subgenomes (GH_At) had 15, 15 collinear gene pairs, respectively (Fig. 3a). We found that most chromosomes showed evidence of whole-genome duplica-

tion during evolution, while a few chromosomes

(A01, A02) underwent a segmental swap. This indi-

cates that the A-genomes were similar and stable

across different cotton species. As shown in Fig. 3b, there were 12 and 16 collinear gene pairs in the *G. hirsutum* D-subgenome (GH_Dt) and *G. raimondii* (D₁), and *G. barbadense* D-subgenome (Gb_Dt) and *G. raimondii* (D₁). In addition, the collinearity relationships of D-genome of between tetraploid cotton and *G. raimondii* were irregular, except for the chromosome D03 and D13, which experienced genome duplication. However, the D-genome of different cotton species remained highly consistent in the collinearity blocks, which is consistent with the fact that



the ancestor of the D-genome of tetraploid cotton was originated from *G. raimondii* (Wang et al. 2012b; Paterson et al. 2012; Li et al. 2015).

Promoter region analysis of GhGASA genes

Cis-element contribute to the regulation of gene expression. To study the regulatory mechanisms of *GhGASA* genes, we selected the 1.5 kb upstream of the start codon according to the *G. hirsutum* genome sequence and analyzed the potential *cis*-elements (Additional file 10: Fig. S8, Additional file 11: Fig. S9). *Cis*-elements related to phytohormones were detected in 38 *GhGASA* gene promoters, including TGACG-motif, TATC-box. P-box, ABRE, TGA-element, AuxRR-core, ERE and TCA-element. Among these, most *cis*-elements were related to abscisic acid, gibberellin, and salicylic acid. In most genes, *cis*-elements associated with different types of stress response (LTR and STRE) and disease resistance

(TC-rich repeats, W-box, and WUN-motif) were found. The *cis*-elements involved in zein metabolism regulation (O2-site) and seed-specific regulation (RY-element) were also found in these parts of the promoters. Moreover, *cis*-elements associated with anaerobic respiration (ARE) and light response (GT1-motif, TCT-motif, Box 4, GATA, G-BOX, and AE-box) were detected in the promoters of most of the *GhGASA* genes. The results for these *cis*-acting regulatory elements indicated that *GhGASA* genes may be regulated by a variety of plant hormones and respond to various stresses in upland cotton.

Expression profiles of *GhGASAs* in different tissues of upland cotton

To explore the possible biological functions of *GhGA-SAs*, published transcriptomic data were downloaded from CottonFGD (https://www.cottonfgd.org) to analyze

the expression of 38 GhGASA genes in different tissues of cotton (Additional file 12: Fig. S10). Most of the GASA genes in G. hirsutum were found to have no expression or extremely low expression in multiple tissues, including anther, filament, pistil, bract, sepal, petal, torus, root, leaf, stem, fiber, and ovule. GhGASA9 and GhGASA31 were similar, with expression observed in almost all tissues and showed higher transcriptional levels than those in other tissues in pistil, petal, and torus. And the transcriptional levels of GhGASA14 in leaf, GhGASA4, GhGASA25, and GhGASA26 in stem; and GhGASA8 and GhGASA23 in root and stem were significantly higher than respective expression levels in other tissues. Moreover, the GhGASA38 gene were only expressed in the root. These results show that the above-mentioned GhGASA genes may have special functions in different tissues. According to Additional file 12: Fig. S10, the expression levels of GhGASA3, GhGASA23, and GhGASA24 continuously increased from -3 to 5 days post anthesis (DPA) fiber, and the corresponding expression levels of 3 and 5 DPA fiber were far higher than -3 and 1 DPA fiber, suggesting that *GhGASA3*, GhGASA23, and GhGASA24 play an important role in the early elongation of cotton fiber cell. Moreover, GhGASA3, GhGASA24 in 10 DPA_fiber, 15 DPA_fiber, and 20 DPA_fiber was highly expressed, GhGASA19 and GhGASA23 in 10 DPA_fiber, 15 DPA_fiber were highly expressed, suggesting that the above-mentioned genes have an important influence on the development of fiber.

In order to verify the transcriptome data, the GhGASA4, GhGASA8, GhGASA9, GhGASA13, GhGASA14, and GhGASA26 genes were selected to analyze the expression patterns in anther, stem, root, leaf, and petal by qRT-PCR (Fig. 4a). The results showed that four genes (GhGASA4, GhGASA8, GhGASA13, and GhGASA26) displayed higher expression levels in stem and lower expression levels in anther. GhGASA9 in petal, GhGASA13 in root, GhGASA14 in leaf showed higher transcription levels. In addition, the GhGASA3, GhGASA8, GhGASA13, GhGASA16, GhGASA23, and GhGASA24 genes were used to analyze the expression patterns in the ovule (-3, 0, 1, 3, 5 DPA) by qRT-PCR (Fig. 4b). *GhGASA8* and *GhGASA23* showed continuous increasing expression levels from -3 to 3 DPA ovule. GhGASA16 showed continuous increasing expression from - 3 to 1 DPA ovule, and then decreased from 1 to 5 DPA ovule. The expression level of GhGASA3 and GhGASA24 in 3 DPA ovule showed higher expression. GhGASA13 displayed differential expression levels from - 3 to 5 DPA ovules, whereas the highest expression level was in 1 DPA ovule. These results were consistent with the RNA-seq data (Zhu et al. 2017), indicating that the data from CottonFGD are reliable.

Expression profiles of *GhGASAs* under abiotic stress in upland cotton

Transcriptomic data downloaded from CottonFGD (https://www.cottonfgd.org) were used to analyze the expression profiles of GhGASA genes under abiotic stress to study their potential functions in G. hirsutum. As shown in Fig. 5a, more than half of the genes had relatively no or very low expression levels under different stress conditions in cotton leaves. The GhGASA9 and GhGASA31 genes were similar in response to multiple stress treatments, especially the 6 h cold treatment, 6 h heat treatment, 6 h polyethylene glycol (PEG) treatment, and 1 h and 6 h salt treatments, in which presenting higher expression levels of GhGASA9 and GhGASA31 thanin the control treatment. The expression of GhGASA25 was downregulated under 3h cold, 3h PEG, and 3h salt treatment and upregulated under 1h PEG and 1h salt treatment. The expression levels of GhGASA4 and GhGASA26 were similar, both showed high expression under 3 h and 12 h cold treatments, and had significantly increased expression levels from 3 h to 6 h under salt treatments. Moreover, the GhGASA12 under 12h PEG treatment and GhGASA18 and GhGASA37 under 3h cold and 6h salt treatments presented higher expression levels than the control treatment. These results indicate that these genes may play roles in plant resistance to abiotic stress and are potential candidate genes for further research.

Based on previous researches and transcriptome data, GhGASA4, GhGASA9, GhGASA18, and GhGASA25 were selected for qRT-PCR (Fig. 5b). As shown in Fig. 5b, the expression levels of GhGASA4 and GhGASA18 significantly increased after 3 h of cold treatment and significantly decreased over the next three hours. GhGASA25 was poorly expressed under the cold treatment, showing a significant downregulation level at 3 h. GhGASA9 showed differential expression levels under cold treatment, whereas downregulated at 1 h, 3 h, and 12 h and upregulated at 6 h and 24 h.

Protein interactions of GhGASA genes

To predict the functions of *GhGASA* genes, we mapped the protein interaction network. Meanwhile, the available database (https://www.uniprot.org/) was subcellular localizations, utilized. Based on GhGASA13 and GhGASA35 were selected to the analysis of protein interaction by Cytoscape software (Fig. 6). Our results suggest that the GhGASA proteins may interact with several other proteins, including the TPR repeat-containing thioredoxin TTL1, the ATPase ASNA1 homolog, the ATPase GET3, the DnaJ homolog subfamily C member 7,





GASA genes were shown to be involved in plant growth, development, and physiological processes, including seed development, floral organ development, flowering time, phytohormone signaling transduction, stress response, defense responses against pathogens, and plant reproductive processes. Although some *GASA* genes have been identified and analyzed, *GASA* genes have not been systematically studied in cotton. The genomes of two allotetraploid species (Hu et al. 2019) and three diploid species (Du et al. 2018; Huang et al. 2020) were improved upon or published, which provided more precise information for the whole-genome analysis of cotton gene family. In this study, 19, 17, 25, 33, and 38 candidate *GASA* genes were systematically identified in *G. herbaceum*, *G. arboretum*, *G. raimondii*, *G. barbadense*, and *G. hirsutum*, respectively.

Expansion analysis of GASA genes in cotton

According to previous reports, allotetraploid cotton was originated from the intergenomic hybridization of the A-genome diploid species and the D-genome







diploid species, followed by chromosomal doubling (Paterson et al. 2012). The number of most gene family members in the tetraploid species is close to the sum of the gene family members in the diploid progenitor. However, in this study, the number of GASA genes in tetraploid and diploid cotton did not present the expected relationship. Recent studies showed that all existing A-genomes may have originated from a common ancestor, A₀ (Huang et al. 2020). The number of GASA genes of G. herbaceum (A_1) , G. arboreum (A_2) , G. barbadense A-subgenome and G. hirsutum A-subgenome hasis 19, 17, 17 and 18, respectively. Moreover, most chromosomes of the A-genome in the cotton species had undergone whole-genome duplication, according to the synteny analysis, indicating tha GASA genes in A-genomes have been highly conserved during evolution. This demonstrated, again, that an unknown A_0 is the possible common origin of the A-genomes. Past research has shown that the D-genomes of the allotetraploid cotton species are derived from the genome of G. raimondii (Wang et al. 2012b; Paterson et al. 2012; Li et al. 2015). However, the number of GASA genes in the G. raimondii (D_1) and G. hirsutum D-subgenome and the G. barbadense Dsubgenome was 25, 20, and 16, respectively. Moreover, the collinear relationships of the D-genome in cotton species were shown to be unordered. Hence, we argue that the loss of some GASA genes in the D-subgenomes during evolution may be the reason

why the *GASA* genes of tetraploid cotton are not the sum of the *GASA* genes of two diploid cotton.

GhGASA genes are possibly involved in plant hormone responses

GA is an important phytohormone that influences multiple developmental and physiological processes (Sun and Gubler 2004; Li et al. 2010), as well as stress responses (Alonso-Ramírez et al. 2009; Qin et al. 2011; Magome et al. 2008; Zhu et al. 2012). As a class of low-molecularweight signal peptides, GASA proteins play an important role in the GA signaling pathways (Silverstein et al. 2010), thereby affecting plant growth. The functions of many of GASA proteins have been illustrated in plants. In this study, the cis-acting elements of GhGASA genes were classified and analyzed to explore their potential functions (Additional file 10: Fig. S8, Additional file 11: Fig. S9). The results of the *cis*-acting element analysis indicated that GhGASA genes might be related to many plant hormone responses (abscisic acid, GA, salicylic acid, MeJA, and auxin), as well as stress responses. Meanwhile, we constructed a protein-protein interaction network to predict the functions of the GhGASA proteins (Fig. 6). The results showed that GhGASA13 and GhGASA35 proteins could interact with the TPR repeat-containing thioredoxin TTL1 that participates in osmotic stress and abscisic acid (ABA) responses and be a positive regulator of ABA signals during germination and seedling development under stresses (Rosado et al. 2006; Lakhssassi et al. 2012). Previous studies have demonstrated that GASA proteins can



respond to plant hormone signaling. In *Arabidopsis*, *AtGASA2/3/5* and *AtGASA14* respond to ABA signaling (Roxrud et al. 2007). In rice, *OsGSR1* can influence brassinosteroid signaling (Wang et al. 2010). Moreover, OsGASA1/7 proteins have been suggested to play a role in BR and GA signaling (Lee et al. 2015; Boonpa et al. 2018). Our analysis suggest that GhGASA proteins may be involved in phytohormone responses.

Expression of *GhGASA* genes in different tissues provide the reference for further research

The expression profiles of genes in different tissues of plants can provide important clues for the identification of their functional properties. To reveal these expression patterns more intuitively, we drew an summary chart to present the expression locations of *GhGASA* genes in cotton based on the transcriptome data (Fig. 7). As shown, *GhGASA23* and *GhGASA24* showed high expression levels during the development of the fiber stages. In practice, *GhGASL3*, the orthologous gene of *GhGASA23*, may be involved in early stage of fiber elongation, according to previous findings (Liu et al. 2013). Actually, qRT-PCR analysis of *GhGASA23* and *GhGASA23* and *GhGASA24* again hinted

that GhGASA23 and GhGASA24 play a positive role in the early elongation of cotton fiber cell. These results indicate that GhGASA23 and GhGASA24 genes may be involved in cotton fiber development, provide the reference for the in-depth analysis of cotton fiber development related genes. Analysis of transcriptome data and qRT-PCR also found other fiber-related genes, including GhGASA3, GhGASA8, GhGASA14, and GhGASA19. Previous studies confirmed that GASA proteins are important for regulating floral development in other species (Kotilainen et al. 1999; Peng et al. 2010). However, little is known about their potential roles in floral development in cotton. Here, we investigated the GhGASA genes expression patterns in floral organs and found that GhGASA9, GhGASA31, and GhGASA18 are highly expressed in most floral organs, including the anther, filament, pistil, bract, sepal, petal, and torus, with the highest expression levels observed in the petal. These suggests GhGASA genes play an important role in the floral development in cotton. Meanwhile, we found that GhGASA38 was only expressed in the root, showing that it may have special functions in root development. GhGASA4, GhGASA8 and GhGASA26 in stem, GhGASA14 in leaf with high expression level by qRT-PCR analysis, suggesting theroles in stem or leaf development.

GhGASA genes are involved in abiotic stress response

Research has confirmed that GASA genes are closely related to abiotic-stress responses. For example, the overexpression of GASA4 in Arabidopsis enhanced resistance to heat stress (Ko et al. 2007), abiotic stress affects the expression of the GASA5 gene (Zhang and Wang 2011), and the different expression levels of OsGASA1/8/10 show a response to salt stress (Muhammad et al. 2019). In our study, GhGASA9 and GhGASA31 presented high expression levels under abiotic stress, especially under heat treatment. GhGASA4 and GhGASA26 expression was also increased under salt stress. The expression of GhGASA25 was significantly downregulated under cold stress but upregulated under salt treatment. These results show that GhGASAs (GhGASA4, GhGASA9, GhGASA25, GhGASA26 and GhGASA31) may be involved in stress response. Meanwhile, qRT-PCR analysis of GhGASA4, GhGASA9, GhGASA18, and GhGASA25 confirmed GhGASA genes involved in cold stress response. Especially, GhGASA4 and GhGASA18 were induced intensely in reply to cold environment. Xinjiang province is the primary cottongrowing areas in China. Cold damage on cotton happens widely due to the late spring coldness of Xinjiang, resulting in yield decline. So, it is important to improve the cold-resistance of cotton. This study provides reference for further research on cotton's defense against low temperature.

Conclusion

Cotton is the most important crop for the production of renewable fiber. In this paper, a systematic wholegenome analysis of the GASA gene family was performed for the first time in five cotton species. As a result, 132 GASA genes were identified and subjected to protein structural, chromosomal localization, and phylogenetic characterizations. Further focusing on the gene structures, synteny analyses, tissue-specific expression patterns, expression profiles under abiotic stress, and protein interactions of GhGASA genes enriched our understanding of the GASA gene family of cotton. Finally, we found several GhGASA genes that may be related to fiber development and stress response. The results also suggest that the GhGASA proteins are closely related to phytohormone signaling. These findings provide valuable information for further study into the functions of these candidate GASA proteins.

Supplementary Information

The online version contains supplementary material available at https://doi. org/10.1186/s42397-021-00081-9.

Additional file 1: Table S1. The primers designed for qRT-PCR.

Additional file 2: Table S2. Information on the GASA genes in G. arboretum, G. barbadense, G. herbaceum, G. raimondii, and A. thaliana. Additional file 3: Fig. S1. Predicted 3D structures of the GhGASA proteins.

Additional file 4: Fig. S2. Multiple alignments of the GarGASA, GheGASA, GbGASA, and GrGASA conserved domain protein sequences.

Additional file 5: Fig. S3. Sequence logo analysis of the conserved AtGASA, GarGASA, GbGASA, GheGASA, and GrGASA domains.

Additional file 6: Fig. S4. Chromosomal localization of the GASA genes in G. hirsutum and G. barbadense.

Additional file 7: Fig. S5. Chromosomal localization of the GASA genes in G. arboretum, G. raimondii, and G. herbaceum.

Additional file 8: Fig. S6. The phylogenetic relationships and exonintron structures of GhGASAs. (A) Phylogenetic tree of the 38 GhGASA protein sequences. (B) Exon-intron structures of the *GhGASAs*.

Additional file 9: Fig. S7. Schematic diagram of the chromosomal distribution and inter-chromosomal relationship of the *GASA* genes in *G. hirsutum.* Red lines represent segmental duplication, while the green line represents tandem duplication.

Additional file 10: Fig. S8. Predicted *cis*-elements in the 38 *GhGASA* gene promoters.

Additional file 11: Fig. S9. Distribution of *cis*-elements classified according to their function. Different colored boxes indicated different functional responses.

Additional file 12: Fig. S10. Expression patterns of *GhGASA* genes in different tissues of cotton.

Acknowledgments

We would like to express gratitude to Robert McKenzie, PhD, from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Authors' contributions

Qiao KK wrote the manuscript. Ma CK analyzed data. Lv JY designed the experiments. Ma QF and Zhang CJ conceived and revised the manuscript. Fan SL provided funding. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (grant no. 31701474).

Availability of data and materials

All data that support the findings of this study are available from the first author upon request.

Consent for publication

We accept publication.

Competing interests

The authors declare no conflicts of interest.

Received: 13 September 2020 Accepted: 14 February 2021 Published online: 28 March 2021

References

- Ahmad B, Yao J, Zhang S, et al. Genome-wide characterization and expression profiling of GASA genes during different stages of seed development in Grapevine (*Vitis vinifera* L.) predict their involvement in seed development. Int. J Mol. Sci. 2020;21(3):1088. https://doi.org/10.3390/ijms21031088.
- Ahmad MZ, Sana A, Jamil A, et al. A genome-wide approach to the comprehensive analysis of *GASA* gene family in *Glycine max*. Plant Mol Biol. 2019;100(6):607–20. https://doi.org/10.1007/s11103-019-00883-1.
- Ali F, Qanmber G, Li Y, et al. Genome-wide identification of Gossypium INDETE RMINATE DOMAIN genes and their expression profiles in ovule development and abiotic stress responses. J Cotton Res. 2019;2:3. https://doi.org/10.1186/ s42397-019-0021-6.

- Alonso-Ramírez A, Rodríguez D, Reyes D, et al. Evidence for a role of gibberellins in salicylic acid-modulated early plant responses to abiotic stress in *Arabidopsis* seeds. Plant Physiol. 2009;150(3):1335–44. https://doi.org/10.1104/ pp.109.139352.
- An B, Wang Q, Zhang X, et al. Comprehensive transcriptional and functional analyses of *HbGASA* genes reveal their roles in fungal pathogen resistance in *Hevea brasiliensis*. Tree Genet Genomes. 2018;14(3):1–13. https://doi.org/10.1 007/s11295-018-1256-y.
- Artimo P, Jonnalagedda M, Arnold K, et al. ExPASy: SIB bioinformatics resource portal. Nucleic Acids Res. 2012;40(1):597–603. https://doi.org/10.1093/nar/ gks400.
- Aubert D, Chevillard MC, Dorne AM, et al. Expression patterns of GASA genes in Arabidopsis thaliana: the GASA4 gene is up-regulated by gibberellins in meristematic regions. Plant Mol Biol. 1998;36(6):871–83. https://doi.org/10.1 023/a:1005938624418.
- Balaji V, Smart CD. Over-expression of snakin-2 and extensin-like protein genes restricts pathogen invasiveness and enhances tolerance to *Clavibacter* michiganensis subsp. michiganensis in transgenic tomato (Solanum lycopersicum). Transgenic Res. 2012;21(1):23–37. https://doi.org/10.1007/s1124 8-011-9506-x.
- Berrocal-Lobo M, Segura A, Moreno M, et al. Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. Plant Physiol. 2002;128(3):951–61. https://doi.org/10.11 04/pp.010685.
- Boonpa K, Tantong S, Weerawanich K, et al. Heterologous expression and antimicrobial activity of OsGASR3 from rice (Oryza sativa L). J Plant Physiol. 2018;224–225:95–102. https://doi.org/10.1016/j.jplph.2018.03.013.
- Chen C, Chen H, Zhang Y, et al. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol Plant. 2020;13(8):1194–202. https://doi.org/10.1016/j.molp.2020.06.009.
- Crooks GE, Hon G, Chandonia JM, et al. WebLogo: a sequence logo generator. Genome Res. 2004;14(6):1188–90. https://doi.org/10.1101/gr.849004.
- Dong L, Wang F, Liu T, et al. Natural variation of *TaGASR7-A1* affects grain length in common wheat under multiple cultivation conditions. Mol Breeding. 2014; 34(3):937–47. https://doi.org/10.1007/s11032-014-0087-2.
- Du X, Huang G, Gong W, et al. Resequencing of 243 diploid cotton accessions based on an updated a genome identifies the genetic basis of key agronomic traits. Nat Genet. 2018;50(6):796–802. https://doi.org/10.1038/s41 588-018-0116-x.
- Fan S, Zhang D, Zhang L, et al. Comprehensive analysis of GASA family members in the Malus domestica genome: identification, characterization, and their expressions in response to apple flower induction. BMC Genomics. 2017; 18(1):827. https://doi.org/10.1186/s12864-017-4213-5.
- Filiz E, Kurt F. Antimicrobial peptides *Snakin/GASA* gene family in sorghum (*Sorghum bicolor*): genome-wide identification and bioinformatics analyses. Gene Reports. 2020;20:100766. https://doi.org/10.1016/j.genrep.2020.100766.
- Hou D, Bai Q, Li J, et al. The gibberellic acid-stimulated transcript gene family in Moso bamboo: a genome-wide survey and expression profiling during development and abiotic stresses. J Plant Growth Regul. 2018;37(4):1135–47. https://doi.org/10.1007/s00344-018-9805-z.
- Hu Y, Chen J, Fang L, et al. Gossypium barbadense and Gossypium hirsutum genomes provide insights into the origin and evolution of allotetraploid cotton. Nat Genet. 2019;51(4):739–48. https://doi.org/10.1038/s41588-019-0371-5.
- Huang G, Wu Z, Percy RG, et al. Genome sequence of *Gossypium herbaceum* and genome updates of *Gossypium arboreum* and *Gossypium hirsutum* provide insights into cotton A-genome evolution. Nat Genet. 2020;52(5):516–24. https://doi.org/10.1038/s41588-020-0607-4.
- Ko CB, Woo YM, Dong JL, et al. Enhanced tolerance to heat stress in transgenic plants expressing the GASA4 gene. Plant Physiol Bioch. 2007;45(9):722–8. https://doi.org/10.1016/j.plaphy.2007.07.010.
- Kotilainen M, Helariutta Y, Mehto M, et al. GEG participates in the regulation of cell and organ shape during corolla and carpel development in *Gerbera hybrida*. Plant Cell. 1999;11(6):1093–104. https://doi.org/10.1105/ tpc.11.6.1093.
- Kovalskaya N, Hammond RW. Expression and functional characterization of the plant antimicrobial snakin-1 and defensin recombinant proteins. Protn Expres Purif. 2009;63(1):12–7. https://doi.org/10.1016/j.pep.2008.08.013.

- Krzywinski M, Schein J, Birol I, et al. Circos: an information aesthetic for comparative genomics. Genome Res. 2009;19(9):1639–45. https://doi.org/1 0.1101/gr.092759.109.
- Kumar S, Glen S, Koichiro T. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–4. https://doi. org/10.1093/molbev/msw054.
- Lakhssassi N, Doblas VG, Rosado A, et al. The Arabidopsis tetratricopeptide thioredoxin-like gene family is required for osmotic stress tolerance and male sporogenesis. Plant Physiol. 2012;158(3):1252–66. https://doi.org/10.1104/ pp.111.188920.
- Lee SC, Han SK, Kim SR. Salt- and ABA-inducible OsGASR1 is involved in salt tolerance. J Plant Biol. 2015;58(2):96–101. https://doi.org/10.1007/s12374-014-0497-z.
- Lescot M, Déhais P, Thijs G, et al. PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res. 2002;30(1):325–7. https://doi.org/10.1093/nar/3 0.1.325.
- Li F, Fan G, Lu C, et al. Genome sequence of cultivated upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution. Nat Biotechnol. 2015;33(5):524–30. https://doi.org/10.1038/nbt.3208.
- Li H, Wang Y, Li X, et al. GA-insensitive dwarf mutant of Brassica napus L. correlated with mutation in pyrimidine box in the promoter of GID1. Mol Biol Rep. 2010;38(1):191–7. https://doi.org/10.1007/s11033-010-0094-2.
- Li KL, Bai X, Li Y, et al. GsGASA1 mediated root growth inhibition in response to chronic cold stress is marked by the accumulation of DELLAs. J Plant Physiol. 2011;168(18):2153–60. https://doi.org/10.1016/j.jplph.2011.07.006.
- Liu ZH, Zhu L, Shi HY, et al. Cotton *GASL* genes encoding putative gibberellinregulated proteins are involved in response to GA signaling in fiber development. Mol Biol Rep. 2013;40(7):4561–70. https://doi.org/10.1007/s11 033-013-2543-1.
- Lu X, Shu N, Wang D, et al. Genome-wide identification and expression analysis of *PUB* genes in cotton. BMC Genomics. 2020;21(1):213. https://doi.org/10.11 86/s12864-020-6638-5.
- Lv LJ, Chen XY, Zhang YL, et al. Bioinformatics analysis of GASA gene family in wheat. Crops. 2018;6:58–67.
- Ma Q, Qu Z, Wang X, et al. *EMBRYONIC FLOWER2B*, coming from a stable QTL, represses the floral transition in cotton. Int J Biol Macromol. 2020;163:1087– 96. https://doi.org/10.1016/j.ijbiomac.2020.07.116.
- Magome H, Yamaguchi S, Hanada A, et al. The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA20x7, under high-salinity stress in Arabidopsis. Plant J. 2008;56(4):613–26. https://doi.org/1 0.1111/j.1365-313X.2008.03627.x.
- Mao Z, Zheng J, Wang Y, et al. The new CaSn gene belonging to the snakin family induces resistance against root-knot nematode infection in pepper. Phytoparasitica. 2011;39(2):151–64. https://doi.org/10.1007/s12600-011-0149-5.
- Muhammad I, Li WQ, Jing XQ, et al. A systematic in silico prediction of gibberellic acid stimulated GASA family members: a novel small peptide contributes to floral architecture and transcriptomic changes induced by external stimuli in rice. J Plant Physiol. 2019;234-235:117–32. https://doi.org/10.1016/j.jplph.2019. 02.005.
- Nahirñak V, Rivarola M, de Urreta MG, et al. genome-wide analysis of the Snakin/ GASA gene family in Solanum tuberosum cv. Kennebec. Am J Potato Res. 2016;93(2):172–88. https://doi.org/10.1007/s12230-016-9494-8.
- Paterson AH, Wendel JF, Gundlach H, et al. Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres. Nature. 2012;492(7429):423–7. https://doi.org/10.1038/nature11798.
- Peng J, Lai L, Wang X. Temporal and spatial expression analysis of PRGL in Gerbera hybrida. Mol Biol Rep. 2010;37(7):3311–7. https://doi.org/10.1007/s11 033-009-9917-4.
- Qin F, Kodaira KS, Maruyama K, et al. SPINDLY, a negative regulator of gibberellic acid signaling, is involved in the plant abiotic stress response. Plant Physiol. 2011;157(4):1900–13. https://doi.org/10.1104/pp.111.187302.
- Rosado A, Schapire AL, Bressan RA, et al. The Arabidopsis tetratricopeptide repeatcontaining protein TTL1 is required for osmotic stress responses and abscisic acid sensitivity. Plant Physiol. 2006;142(3):1113–26. https://doi.org/10.1104/ pp.106.085191.
- Roxrud I, Lid SE, Fletcher JC, et al. GASA4, one of the 14-member Arabidopsis GASA family of small polypeptides, regulates flowering and seed development. Plant Cell Physiol. 2007;48(3):471–83. https://doi.org/10.1093/pcp/pcm016.
- Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C method. Nat Protoc. 2008;3(6):1101–8. https://doi.org/10.1038/nprot.2008.73.

- Segura A, Moreno M, Madueño F, et al. Snakin-1, a peptide from potato that is active against plant pathogens. Mol Plant Microbe. 1999;12(1):16–23. https:// doi.org/10.1094/MPMI.1999.12.1.16.
- Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003; 13(11):2498–504. https://doi.org/10.1101/gr.1239303.
- Shi L, Gast RT, Gopalraj M, et al. Characterization of a shoot-specific, GA3- and ABA-regulated gene from tomato. Plant J. 1992;2(2):153–9. https://doi.org/1 0.1111/j.1365-313X.1992.00153.x.
- Silverstein KAT, Moskal WA, Wu HC, et al. Small cysteine-rich peptides resembling antimicrobial peptides have been under-predicted in plants. Plant J. 2010; 51(2):262–80. https://doi.org/10.1111/j.1365-313X.2007.03136.x.
- Sun S, Wang H, Yu H, et al. GASA14 regulates leaf expansion and abiotic stress resistance by modulating reactive oxygen species accumulation. J Exp Bot. 2013;64(6):1637–47. https://doi.org/10.1093/jxb/ert021.
- Sun TP, Gubler F. Molecular mechanism of gibberellin signaling in plants. Annu Rev Plant Biol. 2004;55:197–223. https://doi.org/10.1146/annurev.arplant.55. 031903.141753.
- Voorrips RE. MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered. 2002;93(1):77–8. https://doi.org/10.1093/ihered/93.1.77.
- Wang Y, Tang H, Debarry JD, et al. MCScanX: a toolkit for detection and evolutionary analysis of gene synteny and collinearity. Nucleic Acids Res. 2012a;40(7):e49. https://doi.org/10.1093/nar/gkr1293.
- Wang L, Wang Z, Xu Y, et al. OsGSR1 is involved in crosstalk between gibberellins and brassinosteroids in rice. Plant J. 2010;57(3):498–510. https://doi.org/1 0.1111/j.1365-313X.2008.03707.x.
- Wang K, Wang Z, Li F, et al. The draft genome of a diploid cotton Gossypium raimondii. Nat Genet. 2012b;44(10):1098–103. https://doi.org/10.1038/ng.2371.
- Yang X, Xu YC, Yang FF, et al. Genome-wide identification of OSCA gene family and their potential function in the regulation of dehydration and salt stress in Gossypium hirsutum. J Cotton Res. 2019a;2:11. https://doi.org/10.1186/s423 97-019-0028-z.
- Yang XM, Lu XK, Chen XG, et al. Genome-wide identification and expression analysis of DNA demethylase family in cotton. J Cotton Res. 2019b;2:16. https://doi.org/10.1186/s42397-019-0033-2.
- Yu CS, Chen YC, Lu CH, et al. Prediction of protein subcellular localization. Proteins. 2010;64(3):643–51. https://doi.org/10.1002/prot.21018.
- Yu J, Sook J, Cheng CH, et al. CottonGen: a genomics, genetics and breeding database for cotton research. Nucleic Acids Res. 2014;D1:1229–36. https://doi. org/10.1093/nar/gkt1064.
- Zhang HB, Li Y, Wang B, et al. Recent advances in cotton genomics. Int J Plant Genomics. 2008;2008:742304. https://doi.org/10.1155/2008/742304.
- Zhang S, Wang X. Overexpression of GASA5 increases the sensitivity of Arabidopsis to heat stress. J Plant Physiol. 2011;168(17):2093–101. https://doi. org/10.1016/[.jplph.2011.06.010.
- Zhang S, Yang C, Peng J, et al. GASA5, a regulator of flowering time and stem growth in Arabidopsis thaliana. Plant Mol Biol. 2009;69(6):745–59. https://doi. org/10.1007/s11103-009-9452-7.
- Zhu T, Liang C, Meng Z, et al. CottonFGD: an integrated functional genomics database for cotton. BMC Plant Biol. 2017;17(1):101. https://doi.org/10.1186/ s12870-017-1039-x.
- Zhu W, Miao Q, Sun D, et al. The mitochondrial phosphate transporters modulate plant responses to salt stress via affecting ATP and gibberellin metabolism in *Arabidopsis thaliana*. PLoS One. 2012;7(8):e43530. https://doi.org/10.1371/journal.pone.0043530.
- Zimmermann R, Sakai H, Hochholdinger F. The gibberellic acid stimulated-like gene family in maize and its role in lateral root development. Plant Physiol. 2010;152(1):356–65. https://doi.org/10.1104/pp.109.149054.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

