BMC Plant Biology

RESEARCH Open Access

Genome-wide analysis of cotton *SCAMP* genes and functional characterization of *GhSCAMP2* and *GhSCAMP4* in salt tolerance

Zhaojie He[1†], Xiaohu Ma[1†], Qianhao- Zhu[2], Shuaishuai Cheng[1], Feng Liu[1], Tao Zhang[1], Caixia Zhang[1], Jianbin Li[1], Xianpeng Xiong[3*] and Jie Sun[1*]

Abstract

Background Secretory carrier membrane proteins (SCAMPs) form a family of integral membrane proteins and play a crucial role in mediating exocytosis in both animals and plants. While *SCAMP* genes have been studied in several plant species, their functions in cotton, particularly in response to abiotic stress, have not yet been reported.

Results In this study, a total of 53 *SCAMP* genes were identified in *G. arboreum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense*. These genes were classified into five groups based on a phylogenetic analysis with *SCAMPs* from *Arabidopsis thaliana*. The main factor driving the expansion of the *SCAMP* gene family in *G. hirsutum* is tandem and segmental duplication events. Using MEME, in addition to the conserved SCAMP domain, we identified 3–13 other domains in each GhSCAMP. The *cis*-element analysis suggested that *GhSCAMPs* were widely involved in cotton growth and development, and responses to abiotic stresses. RNA sequencing (RNA-Seq) and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) results showed that most *GhSCAMPs* were expressed highly in many tissues and had differential expression responses to drought, cold, and heat stresses. Knock-down of *GhSCAMP2* and *GhSCAMP4* by virus-induced gene silencing (VIGS) lead to a salt-sensitive phenotype and had a lower content of CAT, POD, and SOD.

Conclusions This study identified *SCAMP* genes in four cotton species, enhancing our understanding of the potential biological functions of *SCAMPs*. Additionally, we demonstrated that *GhSCAMP2* and *GhSCAMP4* positively regulate cotton tolerance to salt stress.

Keywords *G. hirsutum*, *SCAMP*, Salt stress, VIGS

†Zhaojie He and Xiaohu Ma contributed equally to this work.

*Correspondence:
Xianpeng Xiong
xiongxianpeng@caas.cn
Jie Sun
sunjie@shzu.edu.cn

[1]The Key Oasis Eco-Agriculture Laboratory of Xinjiang Production and Construction Group, College of Agriculture, Shihezi University, Shihezi, Xinjiang 832000, China
[2]CSIRO Agriculture and Food, Canberra, ACT 2601, Australia
[3]Shenzhen Branch, Guangdong Laboratory of Lingnan Modern Agriculture, Genome Analysis Laboratory of the Ministry of Agriculture and Rural Affairs, Agricultural Genomics Institute at Shenzhen, Chinese Academy of Agricultural Sciences, Shenzhen 518120, China

Introduction

Cotton is the world's most important fiber crop, accounting for approximately 35% of global fiber production, and is an important cash crop in many developing countries [1]. Soil salinization has become a critical factor restricting the sustainable development of cotton production [2], despite cotton has a certain level of salt tolerance compared to other crops and is quite often used as a pioneer crop in salt soil [3]. High concentrations of salt severely impact cotton seed germination, growth and development of cotton plants due to changed physiological-biochemical mechanisms under salt stress, ultimately leading to plant death, seriously affecting cotton yields [4, 5]. Therefore, there is an urgent need to investigate the mechanisms and discover genes related to salt tolerance in cotton, so to provide solutions for developing salt-tolerant varieties.

Secretory carrier membrane proteins (SCAMP) is a highly conserved protein widely distributed in eukaryotic cells, although not found in yeast [6]. Initially identified in secretory vesicles of mammalian exocrine glands, SCAMP was later found to be widespread in membranes, including the trans-Golgi network, synaptic vesicles, secretory granules, and transport vesicles [7]. Structurally, all SCAMPs contain a highly conserved four-transmembrane core known as the SCAMP domain, located centrally, with short cytoplasmic N- and C-terminals on both sides. Between the second and third transmembrane domains (TMD), highly conserved E-peptide sequences are present in SCAMP proteins from animals and plants. The cytoplasmic peptide loop between the second and third transmembrane domains of SCAMPs is conserved in plants and animals. The cytoplasmic N-terminal of SCAMPs typically consists of several Asn-Pro-Phe (NPF) motifs, followed by a region rich in proline (Pro) and charged residues (+). At the cytoplasmic C-terminus, most plant SCAMPs contain a tyrosine sorting motif not found in animal SCAMPs [6]. While SCAMP proteins have been extensively studied in animals, research on SCAMPs in plants is relatively limited, and there are differences in their subcellular localization and transport between animals and plants [7].

Limited research indicates *SCAMP* plays a crucial role in plant growth and development [8–10]. Research findings indicate that SCAMP proteins in *Arabidopsis* and rice are involved in the endocytic pathway, and they play a pivotal role in the plant growth process [8]. In lily, SCAMP and VSR jointly regulate protein transport in secretion and endocytic pathways, coordinately supporting pollen tube elongation [9]. Poplar SCAMP proteins affect the composition of secondary cell walls and the accumulation of polysaccharides and phenolic compounds in the woody tissues of poplar stems [10]. Additionally, SCAMP has been confirmed to play a positive function in salt tolerance in various plants, including *Arabidopsis*, wheat, and soybeans [11]. SCAMP typically interacts with Na^+/H^+ transporters, such as NHX, to move Na^+ into vacuoles, effectively reducing the concentration of Na^+ within the cell [8]. In *Arabidopsis* [12], the positive role of SCAMP in regulation of salt tolerance is achieved through the interaction of *AtSCAMP4* with *AtNHX2*. Recently, *TaSCAMP4* has been shown to interact with the Na^+/H^+ antiporter on vacuolar membranes in wheat. However, the molecular function of *SCAMP* genes has not been reported in cotton.

In this study, we did a genome-wide survey of *SCAMP* genes in cotton with the aim to find *SCAMP* genes with a role in salt tolerance in cotton. We identified 8, 8, 18, and 19 *SCAMP* genes in the genomes of *G. arboretum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense*, respectively, and comprehensively analyzed the characteristics of *G. hirsutum SCAMP* genes, including their chromosome localization, motif distribution and gene structure, and *cis*-elements in promoter regions. On the basis of analysis of the expression profiles of the 18 *G. hirsutum SCAMPs* in different tissues and under salt, drought, heat, and low-temperature stresses, *GhSCAMP2* and *GhSCAMP4* were selected for functional characterization using virus induced gene silencing and were found to be necessary for salt tolerance. The results generated in this study provide the detailed information about cotton *SCAMP* genes and lay a foundation for further study of their function.

Materials and methods

Identification and physicochemical property analysis of the cotton *SCAMP* genes

Utilizing five *Arabidopsis* SCAMP proteins as query sequences, we conducted BLASTP search with an E-value of ≤10^-5 in the CottonGen database (https://www.cottongen.org/). The four cotton species of interest are *Gossypium hirsutum* (Ver. ZJU 2.1) [13], *Gossypium barbadense* (Ver. H1724_ZJU) [13], *Gossypium arboretum* (Ver. HAU.2) [14], and *Gossypium raimondii* (Ver. NSF) [15]. Subsequently, we checked the presence of PF04144, the conserved domain of SCAMP proteins, in the candidate proteins, and manually removed duplicates using databases such as CDD and SMART. Analysis of various physicochemical properties of the SCAMP proteins, including relative molecular weight, isoelectric point, and hydrophilicity coefficient, was accomplished using the online Expasy software (http://web.expasy.org/protparam). The subcellular localization of these proteins was predicted using Cell-PLoc 2.0 (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/).

Chromosomal localization of *SCAMP* genes in four *Gossypium* species

Based on the gff file available in the CottonGen database, we applied the Basic Circos program embedded in TBtools [16] to locate and visualize the identified S*CAMP* genes on the chromosomes of *G. hirsutum*, *G. barbadense*, *G. arboreum*, and *G. raimondii*.

Sequence alignment and phylogenetic analysis

We employed ClustalW [17] to perform multiple sequence alignments on the amino acid sequences of 5 AtSCAMP proteins from TAIR (https://www.arabidopsis.org/) and the 53 cotton SCAMP proteins identified in this study. A phylogenetic tree was constructed using MEGA11 software with the Neighbor-Joining (NJ) method, incorporating 1000 bootstrap replicates [18].

Gene structure and conserved motif analyses

We utilized TBtools [16] to retrieve the exon, intron, and untranslated regions of the identified cotton *SCAMP* genes. Structural domain analysis was conducted using the NCBI Conserved Domain Database (CDD) to determine the domain types and positions of all GhSCAMP protein sequences. Subsequently, TBtools [16] was employed for visualization of exons and introns of the genes, as well as the conserved motifs and domains of the corresponding proteins. The parameters were as follows: The number of repetitions was set to zero or one, the maximum number of motifs was set to 20.

Collinearity analysis of *SCAMP* family genes

All cotton protein sequences were compared using the BLAST (E-value of ≤10^-5) one step function in MCScanX within TBtools software. The results were visualized using TBtools software [16].

Cis-acting element analysis

The 2000 bp nucleotide sequences upstream of the start codon of each *GhSCAMP* gene were extracted from the *G. hirsutum* genome and submitted to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) for prediction of *cis*-acting regulatory elements. The predicted *cis*-acting elements were then categorized based on their regulatory functions and visualized in TBtools [16].

Analysis of expression pattern of *GhSCAMP* genes

Cotton variety "Xinluzao33" was obtained from the Key Laboratory of Oasis Eco-Agriculture of Xinjiang Production and Construction Corps, China. Cotton seedlings were cultivated in a growth chamber under a 16/8 h light/dark cycle at 28 °C. During the second true-leaf stage of cotton seedlings, a 200 mM NaCl [19] solution was applied to the bottom of the pots. Root samples were collected at 0 h, 1 h, 3 h, 6 h, 12 h, and 24 h after salt treatment. The collected samples were immediately frozen in liquid nitrogen and stored at -80 °C for future use. Total RNA from the samples was extracted using the RNAiso Reagent Kit from Takara. Subsequently, cDNA was synthesized using the reverse transcription kit from Beijing Tsingke Biological Technology Co., Ltd., following the kit's instructions. *GhUBQ7* (GenBank accession number; DQ116441) was used as the reference gene. Expression levels were quantified using the SYBR Green Master Mix (Takara, China) reagent kit. Each treatment was performed with three independent biological replicates. Furthermore, the expression profile of *GhSCAMP* genes in different cotton tissues and different stresses was obtained from the Cotton Omics database (http://cotton.zju.edu.cn) [13]. The primers used in reverse transcription-quantitative polymerase chain reaction (RT-qPCR) are listed in Supplementary Table S2.

Virus-induced gene silencing (VIGS) of *GhSCAMP2* and *GhSCAMP4*

The TRV-based VIGS experiment in cotton was performed as described previously [20]. Target sequence (300 bp) specific for *GhSCAMP2* or *GhSCAMP4* CDS was determined using the tool available on the Solanaceae Genomics Network website (https://solgenomics.net), and primers were designed for amplifying the target fragments. The target sequences were cloned into the pTRV2 vector using a one-step cloning method. Recombinant plasmids TRV: *GhSCAMP2*, TRV: *GhSCAMP4*, TRV: *GhCHLI* (positive control), and empty vector TRV: 00 (negative control) were transformed into chemically competent cells of *Agrobacterium tumefaciens* strain GV3101 by heat shock. Seedlings of *G. hirsutum* cultivar Xinluzao 33 were used for VIGS and cultivated in a controlled environment inside an incubator, with a temperature of 25 °C, a light schedule of 16 h light/8 h dark cycle, and 60% humidity. The VIGS vectors were injected into the expanded cotton cotyledons and the injected plants were cultivated in the dark for 12 h. Approximately two weeks after injection, when TRV: *GhCHLI* plants exhibited a yellowing phenotype, RNA was extracted from cotton leaves injected with TRV: 00, TRV: *GhSCAMP2*, or TRV: *GhSCAMP4* to measure target gene expression.

Analysis of the salt tolerance function of the *GhSCAMP2* and *GhSCAMP4*

Phenotypic observation was done for TRV: 00, TRV: *GhSCAMP2*, and TRV: *GhSCAMP4* plants at 72 h after treatment with 200 mM NaCl [19] at the two-leaf stage. The activities of catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and the malondialdehyde (MDA) content in cotton leaves were measured using respective assay kits which were obtained from Suzhou

Grace Bio-tech Co., Ltd. The experiment was performed with three independent biological replicates.

Result

Identification of *SCAMP* family members in four *Gossypium* species

Using the conserved domain PF04144 of SCAMP proteins and through comparisons with CDD and SMART databases, we identified 8, 8, 18, and 19 *SCAMP* genes in *G. raimondii*, *G. arboretum*, *G. hirsutum*, and *G. barbadense*, respectively. It is noteworthy that the number of *SCAMP* genes in *G. hirsutum* and *G. barbadense* exceeded the combined total of *G. raimondii* and *G. arboretum* by two and three, respectively, indicating that some *SCAMP* genes had undergone gene duplication after polyploidization. The 18 and 19 *SCAMP* genes identified in *G. hirsutum* and *G. barbadense* were named as *GhSCAMP1 - GhSCAMP18* and *GbSCAMP1 - GbSCAMP19*, respectively, based on their chromosomal positions on A01 to A13 and on D01 to D13 (Table 1, Table S1). Additionally, the *SCAMP* genes of *G. raimondii* (D5) and *G. arboretum* (A2) were named as *GrSCAMP1 - GrSCAMP8* and *GaSCAMP1- GaSCAMP8* according to their chromosome orders (Table S1).

We further analyzed the physical and chemical parameters of the cotton SCAMP proteins, including the number of amino acids (aa), molecular weight, isoelectric point, and subcellular localization. The 53 cotton SCAMP proteins contain 98 to 386 aa, with an average number of 281 aa (Table 1, Table S1). The molecular weight falls within the range of 11.48–43.85 kDa, with an average of 31.70 kDa. Their isoelectric points range from 5.061 to 10.147, with an average of 8.435, suggesting that these proteins are weakly alkaline. Subcellular localization predictions reveal that 45 genes are located on the cell membrane, 6 in the nucleus, 4 in the chloroplast, and 1 in the mitochondrion. These subcellular localization results demonstrate that SCAMP proteins are widely distributed within plant cells and with a predominant presence on the cell membrane.

Chromosomal localization of *SCAMP* senes in four *Gossypium* species

To investigate the chromosomal distribution of *SCAMP* genes in the cotton genomes, we physically mapped these genes onto chromosomes. The 53 genes are randomly distributed on different chromosomes in the four cotton species (Fig. 1). In *G. arboreum*, the 8 genes are distributed on 6 chromosomes (Chr01, Chr04, Chr05, Chr06, Chr09, and Chr13), with the number of genes on each chromosome ranging from 1 to 2 (Fig. 1A). In *G. raimondii*, the 9 genes are distributed across 7 chromosomes (Chr02, Chr03, Chr06, Chr09, Chr10, Chr12, and Chr13), with the number of genes on each chromosome ranging from 1 to 2 (Fig. 1A). Similarly, a tandem duplication was observed on Chr06 in both *G. arboreum* and *G. raimondii* (Fig. 1A-B).

In *G. hirsutum*, the 18 genes are distributed across 14 chromosomes, with 9 genes each on the At and Dt subgenomes (Fig. 1C). The number of *SCAMP* genes on each chromosome ranges from 1 to 2. Tandem repeats were observed on chromosomes A06, A09, D06, and D09. The duplications on A06 and D06 were likely to be inherited from the *G. arboreum* and *G. raimondii* genomes, respectively, and the additional two genes were because of tandem duplication on A09 and D09. In *G. barbadense*, the

Table 1 Physical and chemical characteristics of *G.hirsutum* SCAMP proteins

Gene ID	Gene Name	Protein Length (aa)	MW (kDa)	Charge	PI	Subcellular Location
GH_A01G0169	*GhSCAMP1*	224	25.612	0.5	6.793	Nucleus
GH_A03G0011	*GhSCAMP2*	265	30.028	3	7.053	Cell membrane
GH_A04G1264	*GhSCAMP3*	294	33.175	2.5	7.838	Cell membrane
GH_A05G1646	*GhSCAMP4*	317	35.515	7	9.226	Cell membrane
GH_A06G1460	*GhSCAMP5*	294	33.709	6.5	8.539	Cell membrane
GH_A06G1461	*GhSCAMP6*	318	35.433	4.5	8.431	Cell membrane
GH_A09G0895	*GhSCAMP7*	266	30.445	14	9.696	Cell membrane
GH_A09G0896	*GhSCAMP8*	290	33.078	12.5	9.26	Cell membrane
GH_A13G0584	*GhSCAMP9*	303	33.892	5.5	8.702	Cell membrane
GH_D01G0158	*GhSCAMP10*	279	31.75	3	7.939	Cell membrane
GH_D03G1950	*GhSCAMP11*	265	30.028	3	7.053	Cell membrane
GH_D04G1606	*GhSCAMP12*	295	33.182	3.5	8.17	Cell membrane, Nucleus
GH_D05G1677	*GhSCAMP13*	317	35.454	6	8.996	Cell membrane
GH_D06G1446	*GhSCAMP14*	318	35.504	5.5	8.738	Cell membrane
GH_D06G1447	*GhSCAMP15*	250	28.368	5	8.65	Cell membrane
GH_D09G0855	*GhSCAMP16*	98	11.48	5	9.902	Cell membrane
GH_D09G0856	*GhSCAMP17*	290	32.984	12	9.26	Chloroplast
GH_D13G0678	*GhSCAMP18*	303	33.892	5.5	8.702	Cell membrane

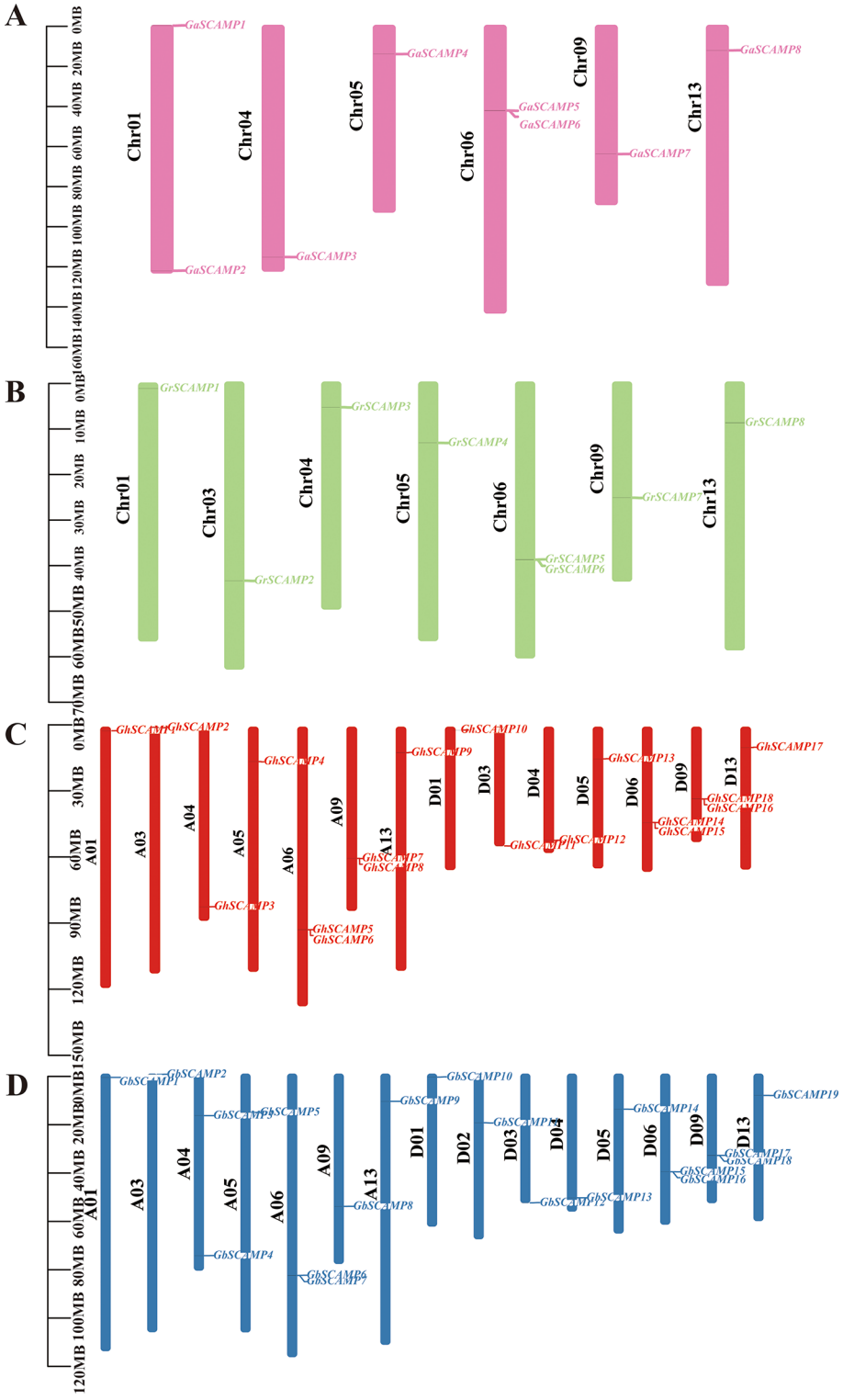

Fig. 1 Chromosomal positions of *SCAMP* genes in the four cotton species. **(A)** *G. arboreum*, **(B)** *G.raimondii*, **(C)** *G. hirsutum*, **(D)** *G. barbadense*. Chromosomes are represented by vertical bars and the chromosome number is positioned directly to the left of each vertical bar

19 genes are randomly distributed across 14 chromosomes, with 1–2 genes on each chromosome and one more gene in the Dt subgenome compared to the At subgenome (Fig. 1D). Consistent with *G. hirsutum*, we detected tandem duplications of the *SCAMP* genes on chromosomes A06, D06, and D09, but no tandem duplications appeared on A09. In comparison to *G. hirsutum*, *G. barbadense* exhibits an additional *SCAMP* gene on chromosome D04. The findings suggest that the primary factor driving the increased number of *SCAMP* genes following polyploidization is tandem duplication.

Phylogenetic analysis of *SCAMP* genes from four *Gossypium* species

To better understand the evolutionary relationships among the members of the *SCAMP* gene family in *Arabidopsis* and the four cotton species, we generated a phylogenetic tree utilizing the 53 cotton *SCAMPs* and the 5 *SCAMPs* from *Arabidopsis*. The results revealed that the 58 *SCAMP* genes could be classified into five subgroups (Fig. 2). The largest subgroup, Group I, encompasses 40 *SCAMP* genes, with all five *Arabidopsis SCAMP* genes belonging to this group. Groups II, III, IV, and V comprise three, three, six, and six cotton *SCAMP* genes, respectively. We observed that most *SCAMP* genes from the two allotetraploid cottons consistently cluster together with diploid cotton *G. arboreum* or *G. raimondii*, supporting the previously established hybrid origin of *G. hirsutum* and *G. barbadense* from the interspecific cross between *G. arboreum* and *G. raimondii*. Except *GbSCAMP2*, *GbSCAMP3*, and *GbSCAMP17* in *G. barbadense* and *GhSCAMP7*, *GhSCAMP11*, and *GhSCAMP18* in *G. hirsutum*, all *SCAMP* genes of *G. barbadense* and *G. hirsutum* cluster together in pairs, indicating that the homoeologous *SCAMP* genes were faithfully retained in the allotetraploid cotton species. Notably, *GbSCAMP3* exhibits high homology with *AtSCAMP3*, suggesting a potentially analogous function to *AtSCAMP3*.

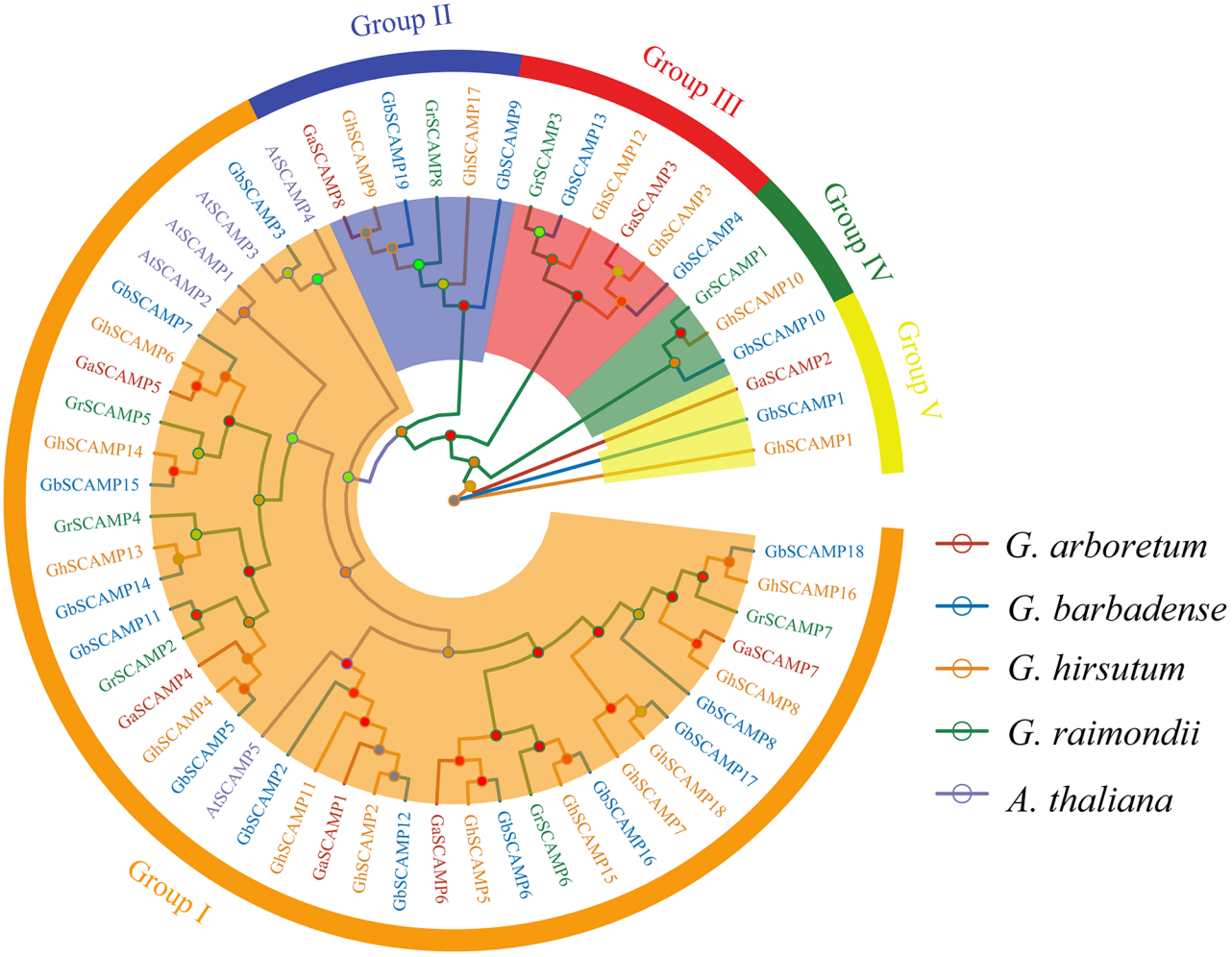

Fig. 2 Phylogenetic analysis of the 58 SCAMP proteins from *G. hirsutum*, *G. barbadense*, *G. arboreum*, *G. raimondii*, and *Arabidopsis*. The SCAMP proteins from the five species are indicated by different colors

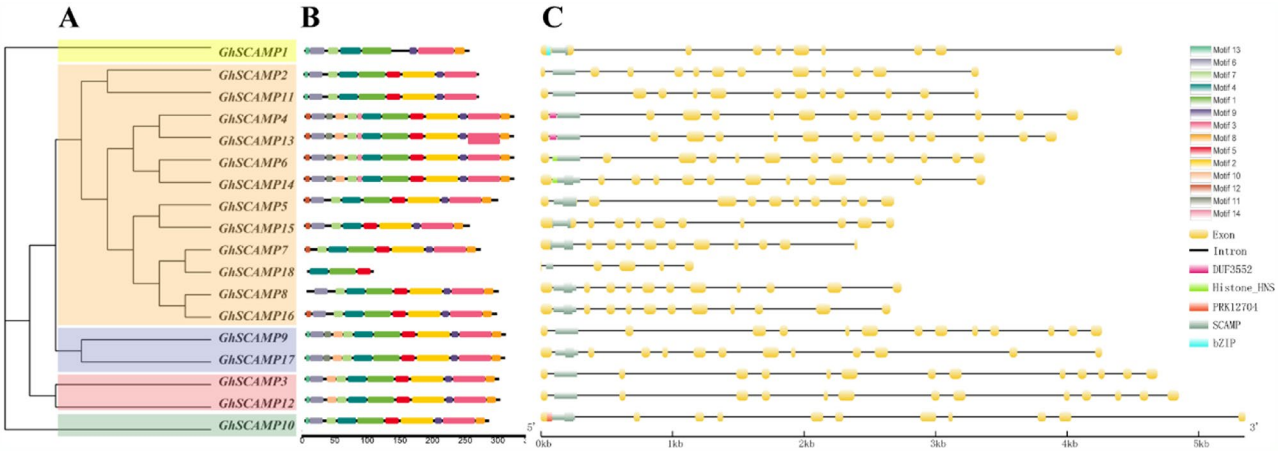

Fig. 3 Gene structure, conserved protein motifs, and domains of the *SCAMP* genes in *G. hirsutum*. **(A)** The phylogenetic tree of *GhSCAMPs*. **(B)** Conserved domains of the GhSCAMP proteins. **(C)** Gene structure of the *GhSCAMP* genes

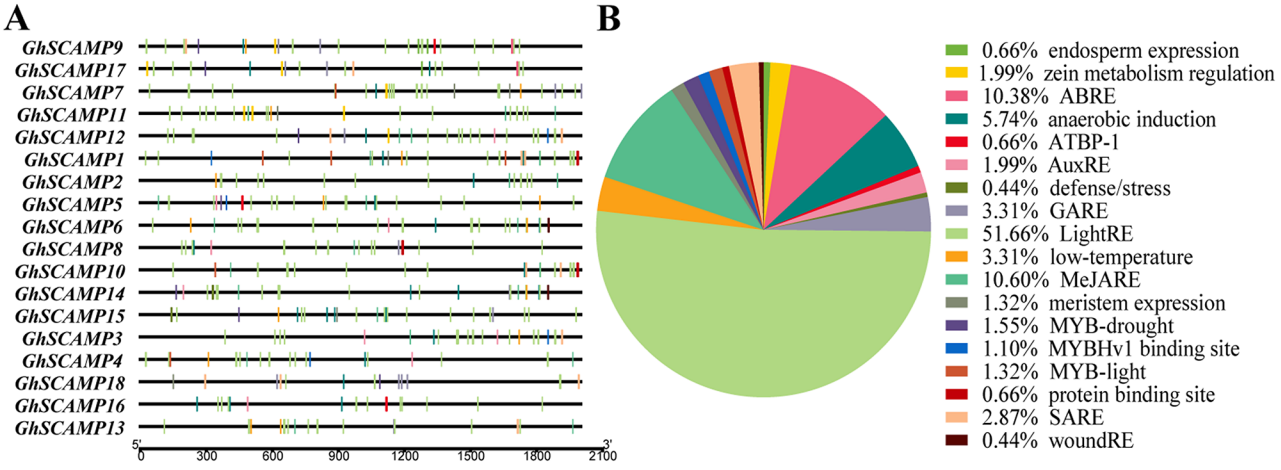

Fig. 4 *Cis*-element analysis of the *GhSCAMP* genes in *G. hirsutum*. **(A)** Predicted *cis*-elements in *GhSCAMP* genes. Each element is indicated by a specific color. **(B)** The proportion of different types of *cis*-elements. AuxRE (auxin responsive element), ABRE (ABA responsive element), GARE (Gibberellin responsive element), LightRE (Light responsive element), MeJARE (methyl jasmonate responsive element), SARE (salicylic acid responsive element), woundRE (wound responsive element)

Analysis of GhSCAMP protein motif structure

To gain further insights into the potential structural evolution of the *GhSCAMP* family members, we conducted gene structure and conserved motif analysis. Gene structure analysis revealed that *GhSCAMP* genes possess both exons and introns, with the number of exons ranging from 5 to 13. Genes belonging to the same evolutionary branch exhibit a conserved gene structure pattern in terms of the number and length of exons (Fig. 3A). A total of 14 potential motifs were identified within the GhSCAMP members, with varying numbers of motifs for each family member, ranging from 3 to 13, and the same subgroup of GhSCAMP proteins exhibits similar motif compositions (Fig. 3B). Except GhSCAMP8 and GhSCAMP18, the N-termini of all other family members start with motif 13 or motif 12, and they all contain motifs 1, 4, and 3. Additionally, apart from the SCAMP domain, some cotton SCAMPs contain additional domains, such as GhSCAMP1 with a bZIP domain, GhSCAMP4 and GhSCAMP13 with a DUF3552 domain, GhSCAMP6 and GhSCAMP14 with a Histone_HNS domain, and GhSCAMP10 with a PRK12704 domain (Fig. 3C). In addition, through the comparison of the full-length amino acids of GhSCAMP1-18 by using DNAMAN software, it was found that the SCAMP domain and bZIP superfamily domain of these 18 members are relatively conserved (Fig. 1).

Cis-element analysis of *GhSCAMPs*

To investigate the *cis*-acting regulatory elements within the promoter region of *GhSCAMPs*, we searched for *cis*-acting elements in the 2000 bp promoter sequence of each gene. As depicted in Fig. 4B, a total of 18 predominant *cis*-acting elements were identified within the

promoter region of *GhSCAMPs*. Among these elements, light-responsive *cis*-regulatory elements constitute a substantial proportion, accounting for 51.66% of the total. Subsequently, hormone-related elements (e.g., auxin, ABA, gibberellin, methyl jasmonate) comprise 26.28% of all elements, constituting the second-largest category (Fig. 4A-B). The third major category includes defense/stress-related *cis*-acting elements, such as salicylic acid, cold responsiveness, and defense/stress elements, representing 12.8% of the total. Lastly, metabolism-related *cis*-acting elements constitute 9.26% of the total. These findings underscore the potentially significant roles of *GhSCAMPs* in the growth and development of upland cotton. It is noteworthy that each *GhSCAMP* gene possesses at least one *cis*-acting element associated with hormones, particularly those related to methyl jasmonate. Among the 18 *GhSCAMPs*, only *GhSCAMP7*, *GhSCAMP9*, *GhSCAMP16*, *GhSCAMP17*, and *GhSCAMP18* lack elements related to methyl jasmonate. In conclusion, the results suggest a crucial role of *GhSCAMPs* in conferring resistance to adverse environmental conditions in upland cotton.

Collinearity analysis of *GhSCAMP* family member

According to previous reports, gene duplication is considered as one of the primary driving forces in genome evolution, with segmental and tandem duplication being the two major mechanisms contributing to the expansion of plant gene families [21]. Segmental duplication, involving chromosome rearrangements after polyploidization, leads to a multiplication of multiple genes [22]. Tandem duplication is defined as the occurrence of multiple members of a gene family within the same or neighboring genomic regions [23]. In this study, the expansion mechanism of the cotton *SCAMP* gene family was investigated using blast and MCScanX. The Circos plot (Fig. 5A) revealed that eight of the 18 *GhSCAMPs*, namely *GhSCAMP5/6*, *GhSCAMP7/8*, *GhSCAMP14/15*, and *GhSCAMP16/17*, forms four tandem clusters on chromosomes A06, A09, D06, and D09, respectively. Of the four pairs, the tandem duplicates of *GhSCAMP5/6* and *GhSCAMP14/15* were derived from the two diploid ancestral species, the *GhSCAMP16/17* pair was generated after the polyploidization event but before the divergence of *G. hirsutum* and *G. barbadense*, and the *GhSCAMP7/8* pair was generated in *G. hirsutum* after its divergence from *G. barbadense* as it is absent in *G. barbadense* (Fig. 5B).

Tissue-specific expression patterns of *GhSCAMP* genes

To deduce the potential biological functions of *GhSCAMPs*, we utilized publicly available RNA-seq data [13] to examine the expression patterns of all *GhSCAMP* genes in different tissues ((root, stem, leaves, flower, ovule, and fibers at 5, 10, 15, and 20 days-post-anthesis (DPA)) of *G. hirsutum*. Of the 18 *GhSCAMP* genes, *GhSCAMP8*, *GhSCAMP16*, and *GhSCAMP17* exhibited no expression in any tissue, *GhSCAMP1* and *GhSCAMP7* showed high expression in the 10 dpa fiber and root, respectively, and the remaining 13 *GhSCAMP* genes were highly expressed in at least two different tissues (Fig. 6A). Specifically, *GhSCAMP4*, *GhSCAMP9*, *GhSCAMP11*, *GhSCAMP13*, and *GhSCAMP14* exhibited high expression levels in nearly all tissues. More than half of the *GhSCAMP* genes (*GhSCAMP1*, *GhSCAMP2*, *GhSCAMP4*, *GhSCAMP5*, *GhSCAMP9*, *GhSCAMP10*, *GhSCAMP11*, *GhSCAMP13*, *GhSCAMP18*) were expressed during fiber development, implying the potential involvement of these genes in the development of cotton fibers. The diverse expression patterns of *GhSCAMP* genes suggest their broader biological functions in cotton growth and development. The RNA-seq-based results of six genes were verified by qPCR analysis (Fig. 6B-G).

Expression analysis of *GhSCAMP* genes under different abiotic stress conditions

To investigate the functions of the *GhSCAMP* genes under abiotic stress conditions, we analyzed the expression patterns of all *GhSCAMP* genes in response to cold, heat, salt, and drought treatments using publicly available RNA-seq data [13]. As shown in Fig. 7A, except *GhSCAMP8*, *GhSCAMP16*, and *GhSCAMP17*, the remaining 15 *GhSCAMP* genes exhibited differential expression under different abiotic stress, suggesting their involvement in response to heat, cold, salt, or drought stress.

Under heat stress, we observed that after 1 h of treatment, *GhSCAMP2*, *GhSCAMP5*, *GhSCAMP6*, *GhSCAMP11*, and *GhSCAMP14* were highly expressed, but their expression levels showed a decreasing trend over time. In contrast, *GhSCAMP7* exhibited high expression after 3 h of heat stress, with no significant elevation at other time points.

Under low-temperature stress, only *GhSCAMP7* and *GhSCAMP11* exhibited high expression levels at 24 h and 3 h after exposure to 4 °C stress. The expression of the remaining genes was not obviously induced under low-temperature stress.

In response to salt stress, except *GhSCAMP1*, *GhSCAMP5*, *GhSCAMP7*, *GhSCAMP*8, *GhSCAMP16*, and *GhSCAMP17*, the remaining genes showed an upregulation trend after salt treatment, especially *GhSCAMP2*, *GhSCAMP12*, and *GhSCAMP18*, which displayed high expression levels at 6 and 12 h post-treatment. This suggests a crucial role for these *GhSCAMP* genes in salt tolerance.

In response to drought stress, except *GhSCAMP1* (which exhibited high expression after 6 h of drought stress), the expression patterns of the remaining genes

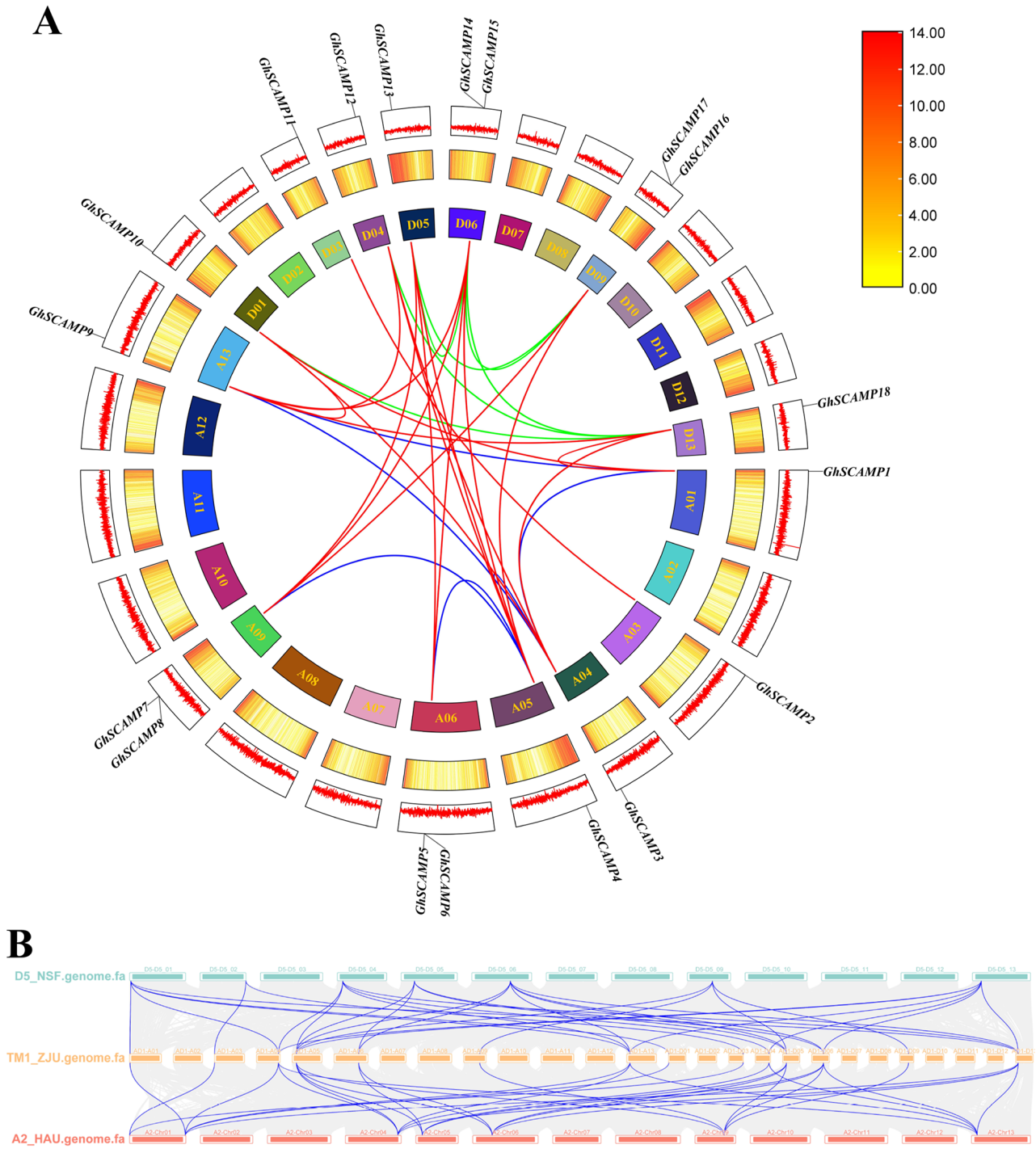

Fig. 5 Intraspecific and interspecific collinearity analysis of *SCAMPs*. **(A)** Synteny analysis between the At and Dt subgenome of *G. hirsutum*. **(B)** Collinearity of repeated gene pairs in three cotton species (*G. hirsutum*, *G. arboreum*, and *G. raimondii*)

had similar trends to those under salt stress. We then selected six genes for RT-qPCR validation and found that, except *GhSCAMP4*, the RNA-seq-based expression patterns of all other genes under salt and drought treatments were verified (Fig. 7B-G). *GhSCAMP4* reached its highest expression level after 3 h of salt stress, followed by a subsequent decrease at 6–12 h and an increase at 24 h. *GhSCAMP4* was also induced by drought stress and showed the highest expression at 3 h after the treatment and then exhibited a decreasing trend over time (Fig. 7C).

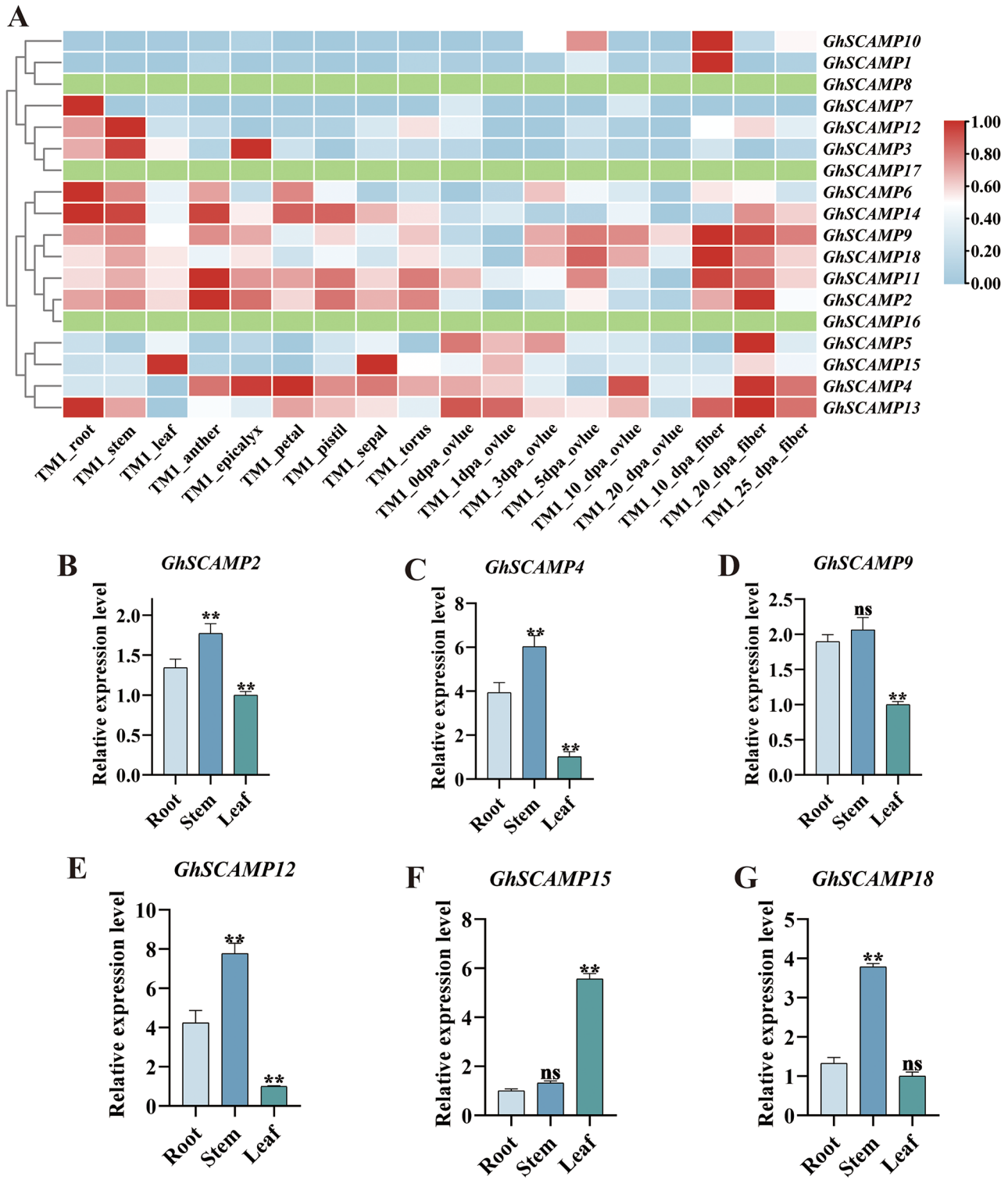

Fig. 6 Analysis of *GhSCAMP* expression in different tissues. **(A)** Expression heatmap of the *GhSCAMP* genes in different tissues of TM-1 based on RNA-seq data. **(B-G)** Expression levels of six selected *GhSCAMPs* in various (root, stem, and leaf) tissues. *GhUBQ7* was selected as the internal reference for the expression analysis of cotton genes. Error bars represent the standard deviation (SD) calculated from three independent experiments. "ns" indicates that there is no statistically significant difference in stem and leaf compared to the root. Asterisks indicate statistically significant differences in stem and leaf compared to root (Student's t-test, *and ** represent $P < 0.05$ and $P < 0.01$, respectively)

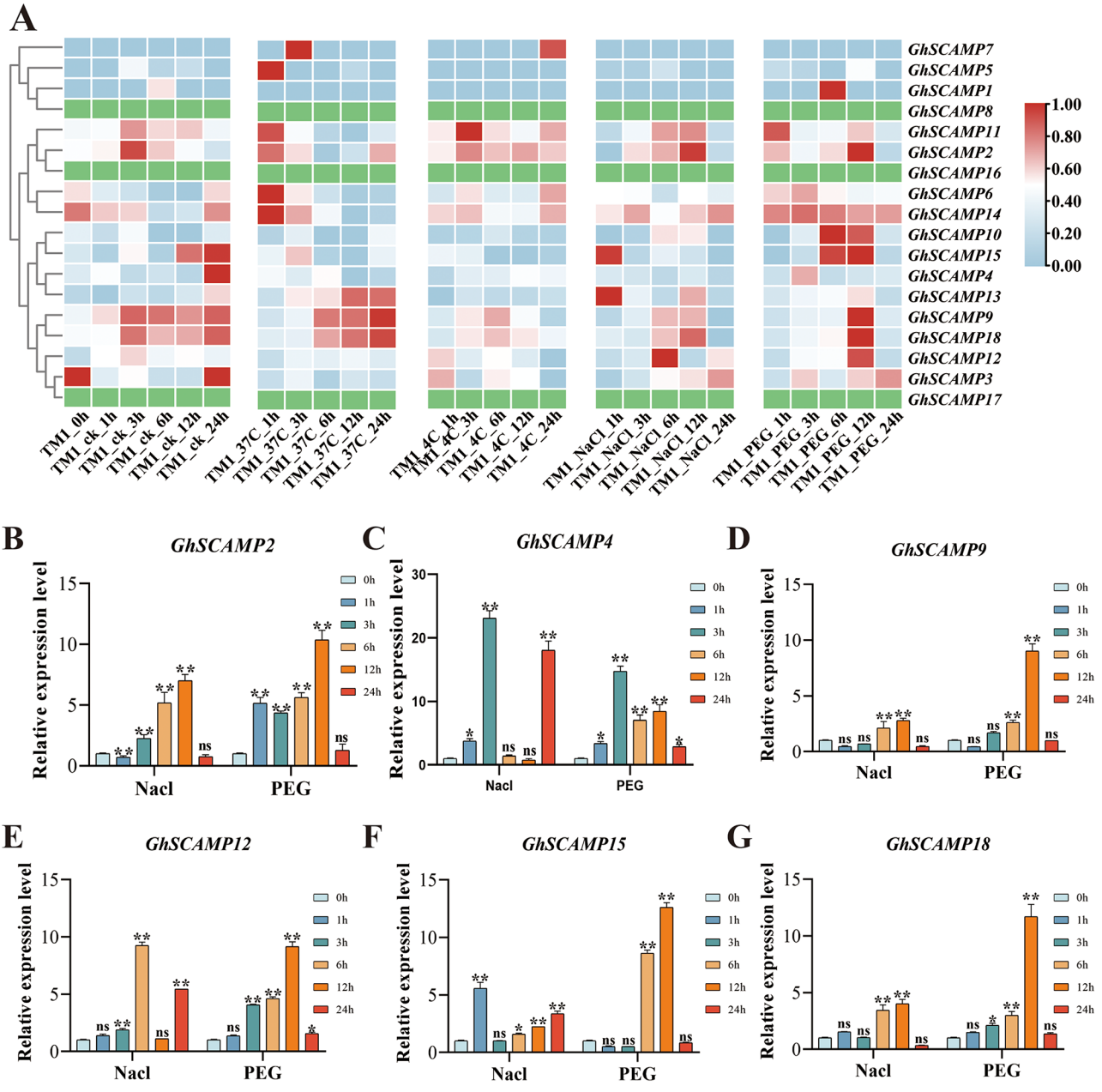

Fig. 7 Analysis of the expression pattern of *GhSCAMP* genes under abiotic stresses. **(A)** Expression heatmap of the *GhSCAMP* genes under salt, drought, heat, or low-temperature stress in *G. hirsutum* based on RNA-seq data. **(B-G)** Expression levels of six selected *GhSCAMPs* after salt (NaCl) or drought (PEG) treatments. *GhUBQ7* was selected as an internal reference for the expression analysis of cotton genes. Error bars represent the standard deviation (SD) calculated from three independent experiments. "ns" indicates that there is no statistically significant differences between groups. Asterisks indicating statistically significant differences between groups (Student's t-test, *and ** represent $P < 0.05$ and $P < 0.01$, respectively)

Silencing of *GhSCAMP2* and *GhSCAMP4* reduces salt tolerance in cotton

Considering that *GhSCAMP2* and *GhSCAMP4* exhibit high expression levels in the root, stem, and leaf tissues of cotton (Fig. 6B-C) and were significantly upregulated under salt stress (Fig. 7B-C), we used the VIGS method to investigate their roles in response to salt stress. Ten days post-VIGS treatment, we observed the expected leaf yellowing phenotype in TRV: *GhCHLI* plants, indicating the success of the VIGS experiment (Fig. 8A). RT-qPCR analysis revealed a significant reduction in the expression levels of *GhSCAMP2* and *GhSCAMP4* in TRV: *GhSCAMP2* and TRV: *GhSCAMP4* cotton plants, respectively (Fig. 8B-C). TRV: *GhSCAMP2* and TRV: *GhSCAMP4* plants displayed more severe wilting phenotypes compared to TRV: 00 plants at 7 days after 200 mM NaCl treatment (Fig. 8A). These results suggest that

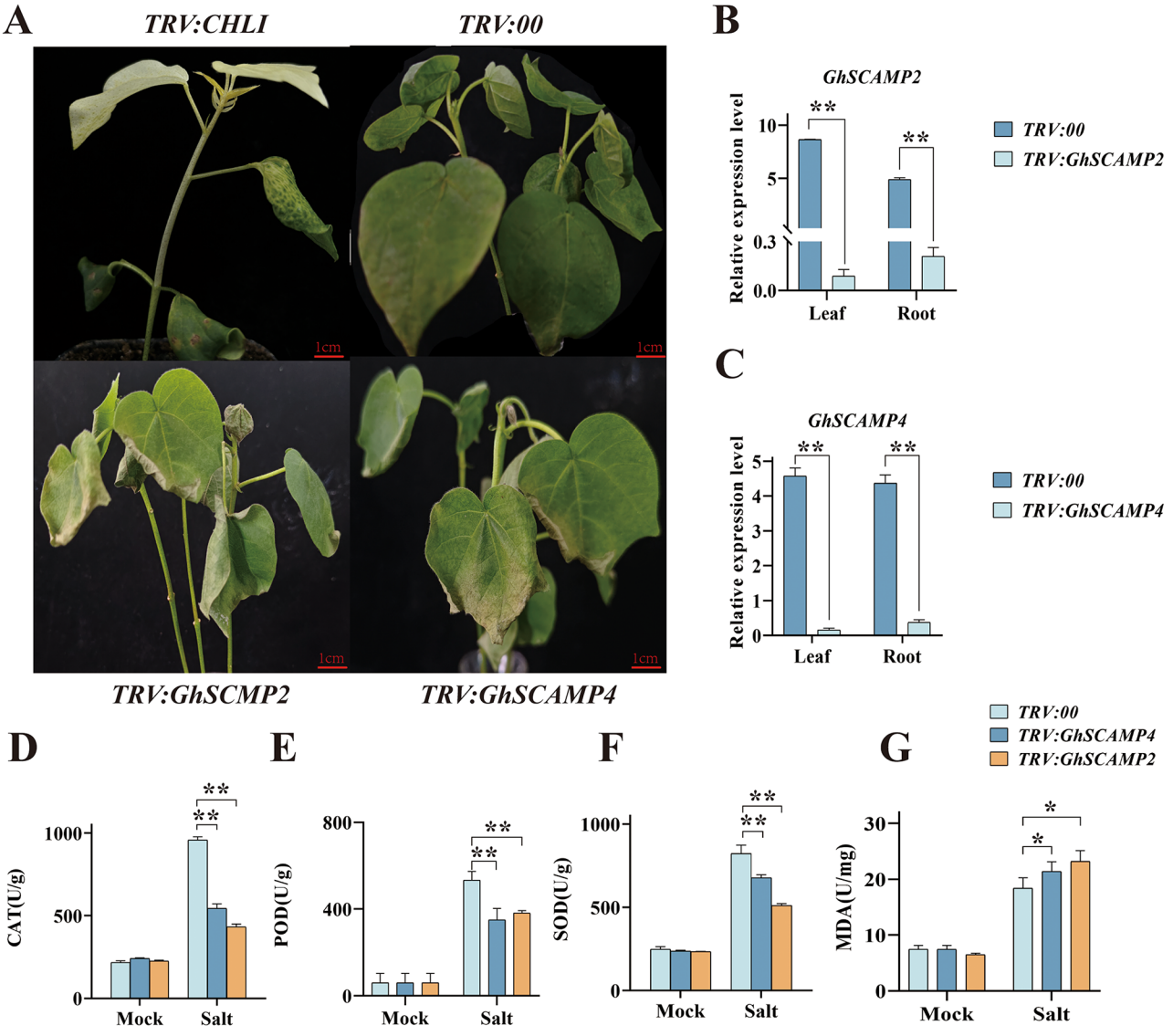

Fig. 8 Silencing of *GhSCAMP2* and *GhSCAMP4* reduces cotton salt tolerance. **(A)** Phenotype of *GhSCAMP2*- or *GhSCAMP4*-silenced plants at 7 days after salt stress. **(B-C)** The expression level of *GhSCAMP2* and *GhSCAMP4* in *GhSCAMP2*- or *GhSCAMP4*-silenced cotton plants. The activity of CAT **(D)**, POD **(E)**, SOD **(F)** and MDA **(G)** in TRV: *00*, and *GhSCAMP2*- or *GhSCAMP4*-silenced plants under the control conditions (Mock) and at 3 days after salt stress. The value represents the mean ± SD of three independent experiments. Asterisks indicate significant differences between TRV: 00, and *GhSCAMP2*- or *GhSCAMP4*-silenced plants (Student's t-test, *and ** represent $P < 0.05$ and $P < 0.01$, respectively)

GhSCAMP2 and *GhSCAMP4* have a positive role in regulating salt stress in cotton.

To further validate the role of *GhSCAMP2* and *GhSCAMP4* in enhancing cotton salt resistance, we measured the following physiological indicators under salt treatment, including MDA content, CAT activity, POD activity, and SOD activity. As shown in Fig. 8D-G, the activities of CAT, POD, and SOD were significantly reduced, while the MDA content was significantly increased in TRV: *GhSCAMP2* and TRV: *GhSCAMP4* plants compared to TRV: 00 plants under the salt stress conditions.

Discussion

SCAMPs are widely distributed in animals and plants, participating in both exocytic and endocytic pathways, and vesicle budding/fusion [7]. In plants, apart from *Arabidopsis*, in which 5 *SCAMP* genes were identified [7], genome-wide identification of the *SCAMP* genes has only been conducted in soybean, maize, and rice, with 10, 11, and 8 *SCAMP* genes identified in the respective species [11]. In this study, we identified 8, 8, 18 and 19 *SCAMP* genes in *G. arboretum*, *G. raimondii*, *G. hirsutum* and *G. barbadense*, respectively (Table 1). In the common ancestors of allotetraploid *G. barbadense* and *G. hirsutum*, i.e., *G. arboreum* and *G. raimondii* (Wendel

and Cronn, 2003), 8 and 9 *SCAMP* genes were identified, respectively (Table 1, Table S1), while *G. hirsutum* and *G. barbadense* had 18 and 19 *SCAMP* genes, respectively. Most *SCAMP* genes identified in the two diploids were retained in the two tetraploid cotton species during the process of evolution. However, there are instances where the genes identified in the diploid did not pass onto the tetraploids or the genes identified in the tetraploids did not have counterparts in their diploid progenitors. For example, *GaSCAMP2* has been lost during evolution, while *GhSCAMP8* and *GhSCAMP16* represent newly generated copies during the evolutionary process. Gene duplication is a crucial mechanism for generating new genes and functions, which can occur through four major mechanisms: whole-genome duplication, segmental duplication, tandem duplication, and transposon-mediated duplication [24]. Both tandem and segmental duplication seem to play a role in amplification of the *SCAMP* gene in *G. hirsutum* and *G. barbadense* (Fig. 5).

The expression pattern of genes is closely related to their function [25]. We found that most *GhSCAMP* genes exhibited high expression levels in both roots and stems (Fig. 5A-B). Similarly, high expression of *GmSCAMP* genes was observed in soybean roots and root nodule tissues [11]. Notably, more than half of the *GhSCAMP* genes showed high expression levels in 10 and 20 dpa fiber (Fig. 6A). Previous studies have shown that poplar *SCAMP* regulates secondary cell wall synthesis in poplar [10]. Cotton fiber development includes the processes of primary and secondary wall formation [26]. Therefore, we hypothesize that cotton *SCAMP* genes may play a crucial role in cotton fiber development. We observed that six *GhSCAMP* genes, including *GhSCAMP*2, *GhSCAMP*6, *GhSCAMP*9, *GhSCAMP*11, *GhSCAMP*14, and *GhSCAMP*15, showed high expression in anthers (Fig. 5A). In lily, *SCAMP* genes have also been observed to be highly expressed in anthers [9]. We observed a large number of growth and development-related *cis*-acting elements in the promoter of *GhSCAMP* genes (Fig. 4). Based on the expression pattern and *cis*-acting elements of *GhSCAMP*, further research is needed to determine the roles of *GhSCAMP* genes in development of various tissues in cotton.

In the promoter of *SCAMP* family genes, we identified numerous *cis*-elements related to key stress and hormone responses in plants (Fig. 4B-C). Subsequently, we analyzed the expression patterns of *GhSCAMP* genes under different stress conditions. Under NaCl stress, most *GhSCAMP* genes showed an upregulation trend with prolonged salt stress time, indicating that these *GhSCAMP* genes are responsive to salt stress (Fig. 7A-B), consistent with previous studies in soybeans [11]. Based on the results of gene expression patterns and *cis*-element analysis, we speculate that *GhSCAMP2* and *GhSCAMP4* are candidate genes that may play a role in cotton salt tolerance. Therefore, we used VIGS experiments to silence *GhSCAMP2* and *GhSCAMP4* to study the functions of these two genes. After salt stress treatment, we observed that TRV: *GhSCAMP2* and TRV: *GhSCAMP4* plants exhibited more withered leaves compared to TRV: *00* plants (Fig. 8A). Excessive salt stress induces the generation of reactive oxygen species (ROS), typically alleviated by enzymes such as superoxide dismutase (SOD) or catalase (CAT) [27]. The activities of POD, SOD, and CAT in TRV: *GhSCAMP2* and TRV: *GhSCAMP4* were significantly lower than those in TRV: 00, while MDA activity was significantly higher (Fig. 8D-G), indicating that silencing *GhSCAMP2* and *GhSCAMP4* led to a decrease in cotton's ability to remove reactive oxygen. Therefore, these results indicate that *GhSCAMP2* and *GhSCAMP4* genes regulate cotton salt tolerance by modulating the plant's ability to clear reactive oxygen species.

Previous studies have demonstrated that SCAMP interacts with Na^+/H^+ transport proteins in *Arabidopsis* and wheat [12]. A recent study showed overexpression and suppression of *GmSCAMP5* expression can decrease and increase sodium and potassium content in soybean leaves under salt stress, respectively, thereby improving or reducing soybean salt tolerance [11]. Whether *GhSCAMP*2 and *GhSCAMP*4 interact with Na^+/H^+ transporters and enhance cotton salt tolerance by expelling sodium ions requires further in-depth research. Furthermore, our expression data revealed that, in addition to responding to salt stress, *GhSCAMP* genes also respond to drought, heat, and low-temperature stresses (Fig. 7A-B), particularly, most *GhSCAMP* genes showed upregulation under PEG stress, and whether these genes are involved in cotton's tolerance to other abiotic stresses need further study.

Conclusion

In this study, 53 *SCAMP* members were identified in *G. arboreum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense*, and their protein characteristics, gene structure, and evolution were investigated. Both *cis*-element analysis and gene expression results demonstrated that *GhSCAMPs* could be widely involved in cotton growth and development, and response to various abiotic stresses. VIGS experiment results indicate that the downregulation of *GhSCAMP2* and *GhSCAMP4* expression reduced the salt tolerance of cotton plants, and the SOD, POD, and CAT activities were higher in *GhSCAMP2*- or *GhSCAMP4*-silenced cotton plants than in wild-type cotton. Although the exact mechanism underlying *GhSCAMP2*- and *GhSCAMP4*-mediated salt tolerance was still yet to be uncovered, our study has laid the foundation for a deeper comprehension of the *SCAMP* genes in cotton

and provided functional candidate genes responding to salt stress.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-024-05571-x.

Supplementary Material 1: Table S1. Physical and chemical characteristics of *G. arboreum*, *G. raimondii*, and *G. barbadense* SCAMP genes. Table S2. The primers used in this study. Table S3. The FPKM values of the *SCAMP* gene under different treatments. Table S4. The CDS (Coding Sequence) of the *SCAMP* gene *in G.hirsutum*. Table S5. The protein sequence of *SCAMP* gene in *Arabidopsis thaliana*, *G.hirsutum*, *G.barbadense*, *G.arboreum* and *G.raimonaii*.

Supplementary Material 2: Fig S1. Multiple sequence alignment of SCAMP proteins.

Acknowledgements
Not applicable.

Author contributions
QZ, XX, and JS designed the research and modified the manuscript. ZH and XM performed the bioinformatics analysis, and VIGS experiment and wrote the original manuscript. SC, FL revised the manuscript. TZ, CZ, and JL performed RNA extraction, reverse transcription, and qPCR analysis. All authors reviewed the manuscript.

Funding
This project was supported by the Science and Technology Major Program of XINJIANG (2023A02003), the Science and Technology Major Program of BINGTUAN (2023AA008), the Guangdong Basic and Applied Basic Research Foundation (2022A1515110758), and the Henan Province Joint Research Project on High-Quality Seed Development (2022010301).

Data availability
RNA-seq data used in this study were downloaded from the Cotton Omics database (http://cotton.zju.edu.cn). Sequence data from this article can be found in the CottonGen database (https://www.cottongen.org/).

Declarations

Ethics approval and consent to participate
The plant materials used in this study were grown in an artificial climate chamber at Shihezi University. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 4 April 2024 / Accepted: 4 September 2024
Published online: 18 September 2024

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