Genome-wide identification and analysis of ACP gene family in *Sorghum bicolor* (L.) Moench

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**Abstract**

**Background:** Acyl carrier proteins (ACP) constitute a very conserved carrier protein family. Previous studies have found that ACP not only takes part in the fatty acid synthesis process of almost all organisms, but also participates in the regulation of plant growth, development, and metabolism, and makes plants adaptable to stresses. However, this gene family has not been systematically studied in sorghum.

**Results:** Nine ACP family members were identified in the sorghum genome, which were located on chromosomes 1, 2, 5, 7, 8 and 9, respectively. Evolutionary analysis among different species divided the ACP family into four subfamilies, showing that the SbACPs were more closely related to maize. The prediction results of subcellular localization showed that SbACPs were mainly distributed in chloroplasts and mitochondria, while fluorescence localization showed that SbACPs were mainly localized in chloroplasts in tobacco leaf. The analysis of gene structure revealed a relatively simple genetic structure, that there were 1–3 introns in the sorghum ACP family, and the gene structure within the same subfamily had high similarity. The amplification method of SbACPs was mainly large fragment replication, and SbACPs were more closely related to ACPs in maize and rice. In addition, three-dimensional structure analysis showed that all ACP genes in sorghum contained four α helices, and the second helix structure was more conserved, implying a key role in function. *Cis*-acting element analysis indicated that the *SbACPs* might be involved in light response, plant growth and development regulation, biotic and abiotic stress response, plant hormone regulation, and other physiological processes. What's more, qRT-PCR analysis uncovered that some of SbACPs might be involved in the adaptive regulation of drought and salt stresses, indicating the close relationship between fatty acids and the resistance to abiotic stresses in sorghum.

**Conclusions:** In summary, these results showed a comprehensive overview of the SbACPs and provided a theoretical basis for further studies on the biological functions of SbACPs in sorghum growth, development and abiotic stress responses.

**Keywords:** *Sorghum bicolor* (L.) Moench, Gene family analysis, ACP genes, Bioinformatics

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**Background**

Acyl Carrier Protein (ACP) is a class of acidic proteins with low molecular weight and conserved serine residues [1, 2]; the serine residues on ACP proteins are covalently linked to phosphopantetheine linker, which is connected to the lipoyl group through the -SH group [3, 4]. The site-directed mutation was used to mutate the binding site (Ser38) of spinach acyl carrier protein I (ACP-I) from...
serine to threonine or cysteine residues, which changed the conformation of ACP and led to the loss of ACP function [1], suggesting that this locus had an important biological function. ACP is an important cofactor of fatty acid synthase. There are two different types of type II fatty acid synthases in plant cells. One is the cytoplasmic fatty acid synthase, which is responsible for most fatty acid synthesis, and the other is located in the mitochondria, which produces the fatty acid precursors necessary for the production of lipoic acid. ACPs-loaded lipoic groups shuttle back and forth across various functional sites of fatty acid synthase to perform functions [5–8].

Functionally, ACPs are involved in the metabolism of different types of fatty acids in plants, such as biosynthesis of fatty acids, polyketones and non-ribosomal proteins [9, 10], and also involved in responses to biological and abiotic stresses [11–15]. The unsaturated fatty acids (UFAs) of membrane lipid also adjust to environmental conditions by changing the fluidity of membrane lipids. Besides the production of saturated fatty acids (SFAs), ACPs are also involved in the biosynthesis of UFAs. The Arabidopsis fatty acid desaturase 2, an endoplasmic reticulum (ER)-localized ω-6 desaturase for converting oleic acid to linoleic acid, was necessary for chilling and salt tolerance [16, 17]. Compared with mature leaves, the mRNA level of ACP in young leaves of spinach and soybean was significantly higher, while compared with leaves grown under dark conditions, spinach leaves grown under light also contained higher ACP activity, suggesting that ACP might be related to plant growth and development and light response [18]. Brassica napus could encode multiple copies of ACPs to meet the needs of fatty acid synthesis that occurred during oilseed development [19]. AhACP1 was mostly expressed in peanut seeds, and AhACP4 and AhACP5 showed the same mRNA expression profile in different organs and seeds during development. Two highly expressed mitochondrial ACPs were highly expressed in peanut flower tissues [20]. Besides, combining linkage analysis, whole-genome analysis, candidate gene association analysis and plant transformation, GmACP1, a candidate gene encoding acid phosphatase in hairy roots of soybean, was identified to improve soybean tolerance to low phosphorus stress [21]. In Arabidopsis thaliana, the function of ACP genes had also been extensively studied. AtACP genes could respond to several abiotic stresses, e.g., the expression of AtACP1, AtACP2 and AtACP3 could be induced by drought. The expression of AtACP4 was down-regulated by both iron and nitrogen deficiency. The expression of AtACP5 decreased significantly after salt stress. Overexpression of AtACP5 further led to changes in the composition of fatty acids, mainly including the decrease of oleic acid (C18:1) and increase of palmitic acid (C16:0); in addition, the ratio of sodium to potassium was also significantly lower than that of the wild type [14]. Recently, it was found that wheat chloroplast acyl carrier protein synthase I and chloroplast 20 kDa chaperone proteins significant increased under water stress [15]. All these studies demonstrated the diversity of ACP gene functions.

At present, agricultural production and food security in developing countries are still facing many threats. Sorghum [Sorghum bicolor (L.) Moench], as the fifth-largest food crop in the world and C4 crops, with high photosynthetic efficiency and a developed root system, is an important crop variety in arid and semi-arid regions with wide uses [22–25]. The importance of drought-tolerant crops like sorghum may increase as some areas unsuitable for rice and maize cultivation due to rising temperatures and decreasing precipitation caused by climate change [26]. Currently, sorghum breeding focuses on improving adaptation to climate change, mainly including biotic and abiotic stresses [27–32]. With the development of high-throughput sequencing, whole genomes of many species have been released. Paterson et al. completed the assembly of the whole sorghum genome and the data are available in the United States Department of Energy (DOE) Joint Genome Institute (JGI) [33]. Meanwhile, genome assembly has been completed in multiple plants including Arabidopsis, soybean, peanut, olive rape, etc., which greatly facilitates the analysis of gene family, and ACP gene families have been characterized in multiple species [19, 20, 34–36]. However, genome-wide analysis of the ACP gene family in sorghum has not been reported. In this study, the ACP gene family was systematically identified and analyzed at the genome level, and the expression patterns of ACPs under two kinds of abiotic stresses including drought and salt stress were also analyzed, which laid a foundation for the further investigation of the molecular mechanism of ACP in response to stress.

Results
Identification and sequence analysis of ACP gene family in sorghum

Nine ACP gene family members were screened and identified from sorghum genome database and named as SbACP1-SbACP9 [37]. The molecular weights of the nine ACP genes ranged from 14,443.48 to 15,727.81 kDa, and the theoretical isoelectric point ranged from 4.68 to 5.67. All nine ACP genes were acidic (PI < 7), so the ACP family in sorghum was a kind of acidic proteins with low molecular weight. Nine ACP family genes were distributed on chromosomes 1, 2, 5, 7, 8 and 9. Of which, SbACP1, SbACP2 and SbACP3 were located on chromosome 1, and SbACP4 and SbACP5 were located on chromosome 2, while SbACP6, SbACP7, SbACP8, and
SbACP9 were distributed on chromosome 5, 7, 8, and 9, respectively (Fig. 1). In addition, the detailed information of these genes was shown in Table 1.

**Subcellular localization of SbACPs**
To figure out where the ACP proteins were expressed, the prediction of subcellular localization was carried out and the results showed that SbACP2, SbACP3, SbACP4 and SbACP9 were highly likely to be expressed in mitochondria or chloroplasts, whose values of reliable index were very close, while the other SbACPs were highly possible to be located in chloroplasts, with the values of reliable index expressed in chloroplasts significantly higher than that in other tissues, indicating that the ACP proteins probably functioned in mitochondria or chloroplasts. (Fig. 2). To verify the reliability of the prediction, five SbACPs with GFP tags were selected to determine their expression localization in tobacco leaf epidermal cells. Confocal fluorescence results showed that all the 5 SbACPs were located in chloroplasts (Fig. 3).

**Evolution analysis of SbACP Proteins**
In order to clarify the evolutionary relationship and obtain more detailed classification of ACP proteins in sorghum, maize, rice and Arabidopsis, the phylogenetic tree including 36 ACP proteins were constructed. Based on the alignment of full-length protein sequences of 9 ACPs in sorghum, 11 ACPs in maize, 8 ACPs in rice and 8 ACPs in Arabidopsis, phylogenetic trees were constructed. The results showed that these ACP proteins divided into 4 main clades (Clade A, Clade B, Clade C and Clade D) with obvious evolutionary divergence. The evolutionary distance was calculated using the p-distance method, and the cladogram was obtained using the Neighbor-joining method (Fig. 2). Within each clade, the ACP proteins were further divided into small groups (Fig. 2). This classification may be partly due to the species-specific evolutionary characteristics of ACP proteins. To verify whether this classification is accurate, the expression localization of these 36 ACP proteins was predicted using TMHMM software and the results showed that most ACP proteins were located in chloroplasts, indicating that the ACP proteins probably played a crucial role in chloroplasts (Fig. 3).

**Table 1**
Protein sequence information of Sorghum ACP family genes in almonds

| Name | gene_ID   | mRNA_ID   | Chromosome location | Length (AA) | Mw (kD)  | Pi  |
|------|-----------|-----------|---------------------|-------------|-----------|-----|
| SbACP1 | SORBI_3001G005700 | EER90483 | Chr1:541,962..544103+ | 146 | 15,727.81 | 4.68 |
| SbACP2 | SORBI_3001G175600 | EER91268 | Chr1:14,742,591..14747705+ | 142 | 15,346.3 | 5.21 |
| SbACP3 | SORBI_3001G370700 | EER92267 | Chr1:65,914,223..65918203+ | 131 | 14,443.48 | 5.37 |
| SbACP4 | SORBI_3002G078300 | EER96074 | Chr2:8,140,737..8141673+ | 134 | 14,526.69 | 5.41 |
| SbACP5 | SORBI_3002G280400 | EER99269 | Chr2:66,176,461..66179180- | 135 | 14,266.39 | 5.06 |
| SbACP6 | SORBI_3005G128100 | EES08540 | Chr5:55,596,128..55599665+ | 143 | 15,127.31 | 5.19 |
| SbACP7 | SORBI_3007G176800 | EES14114 | Chr7:61,061,543..61064169+ | 131 | 13,843.82 | 4.84 |
| SbACP8 | SORBI_3008G116400 | EES17130 | Chr8:52,643,796..52646872- | 142 | 15,143.26 | 5.67 |
| SbACP9 | SORBI_3009G119900 | EES19437 | Chr9:47,006,894..47010121- | 128 | 13,829.76 | 5.54 |
and 8 ACPs in Arabidopsis, these proteins were categorized into 4 different subfamilies named as A, B, C and D (Fig. 4). Cluster A and D contained 13 and 15 ACPs, respectively, while cluster B and C covered 4 ACPs, respectively. Further, SbACPs were distributed in clusters A, C and D, with SbACP1, SbACP5, SbACP7 and SbACP8 in cluster A, SbACP2, SbACP3, SbACP4 and SbACP9 in cluster D, and only SbACP6 in cluster C. Only 4 ACPs of Arabidopsis were contained in cluster B, suggesting a relative distant evolutionary relationship of ACPs between Arabidopsis and other three species, implying the difference of ACP gene sequences between monocotyledon and dicotyledon. Furthermore, ACP proteins in sorghum and maize were always in close branches in clusters A, B and D, implying a closer evolutionary relationship of ACPs in sorghum and maize that are both C4 plants, and followed by sorghum and rice.

**Gene structure and conserved motif analysis of SbACP proteins**

In order to study the exon–intron organization of individual SbACP gene, the GSDS website was used to predict the gene structure of SbACPs. Based on the phylogenetic tree, the SbACP genes were clearly classified into three major subfamilies (cluster A, C and D). The analysis of gene structure suggested that the members within the same family possessed the similar exon–intron structure (Fig. 5C). Of all 9 SbACP genes, 4 genes belonging to cluster A, including SbACP2, SbACP3, SbACP4 and SbACP9 were relatively simple in structure, each had 2 exons and 1 intron. For another 4 genes in cluster D, namely SbACP1, SbACP5, SbACP7 and SbACP8, each of them contained 4 exons and 3 introns. The remaining SbACP6 contained three exons and two introns, which belonged to cluster C. These results further verified the reliability of phylogenetic tree and indicated that genes in the same clusters tended to be more similar in genetic structure including the number of exon and intron, but might be with different intron length. Meanwhile, the diversity of gene structure among SbACP genes indicated that different selection events might have taken place during gene evolution.

The conserved motifs were often closely related to protein function involved in protein–protein interactions, nuclear localization, and transcriptional activities [38]. Therefore, to uncover the characteristic regions of ACP proteins, MEME software was used to analyze the conserved motif of ACP proteins. Totally 10 motifs were
Fig. 3  Subcellular localizations of SbACPs in tobacco leaf epidermal cells by confocal laser-scanning microscopy. The 5 selected SbACP-GFP fusion proteins were predominantly localized to the chloroplast. GFP, green fluorescent protein.
identified and designated as Motif 1–10 (Fig. 5B). Among them, Motif 1 and Motif 2 existed in all ACP family members, Motif 3 and Motif 7 existed in cluster B, cluster C and cluster D; Motif 4, Motif 5 and Motif 8 existed in cluster A; Motif 9 and Motif 10 existed only in cluster A and cluster B, respectively (Fig. 5B). Combining the analysis of conserved motifs, evolutionary tree, and gene structure, we found that genes within the same subfamily tended to share similar gene structure and motif, and meanwhile, we inferred that the proteins within the same cluster containing the similar composition of conserved motifs might share the similar function. Taken together, all these results further confirmed the classification of SbACP proteins in sorghum.

Duplication and syntenic analyses in sorghum ACP genes

Genome replication events have long been considered the origin of evolutionary novelty. During evolution, gene duplication, tandem duplication and large fragment duplication tend to initiate the generation of gene families. Therefore, we analyzed the gene duplication events of the SbACP genes in sorghum. All nine SbACPs were so far apart from each other on the genome that no tandem repetition occurred (Fig. 1). However, we found that a pair of ACP genes were segmented repeats (SbACP7/SbACP5) (Fig. 6). These results indicated that most of the SbACP genes might evolve independently, and segment repeats play a slightly role in contributing to the expansion of the SbACP gene family.

In order to further explore the evolutionary information of ACP genes between sorghum and other species, collinearity analysis was respectively performed between the genome of sorghum and three other plants, including one dicotyledons (Arabidopsis) and two monocotyledons (rice and maize). From the results, we found 0, 6, and 8 collinear gene pairs in sorghum with Arabidopsis, rice, and maize, respectively (Fig. 7). Five SbACPs (SbACP4, SbACP6, SbACP7, SbACP8, SbACP9) have

![Fig. 4 Clustering of SbACPs in three species. Sb: sorghum; At: Arabidopsis; Zm: maize. All protein members were divided into four clusters: cluster A on pink background, cluster B on blue background, cluster C on orange background and cluster D on green background. The genes marked with purple asterisk were the members of sorghum ACP family](image)
collinear pairs in all two monocot species and no collinearity of ACPs existed between sorghum and Arabidopsis, indicating evolution divergence of ACP gene family between monocotyledons and dicotyledons. Simultaneously, we speculated that SbACPs might have evolved from homologous genes of other monocotyledons.

Multiple sequence alignment and three-dimensional structure prediction of SbACP proteins

In order to further identify the sequence feature of ACP conserved domain, the protein sequences of 9 SbACP genes were aligned and analyzed. The results of alignment suggested that 4 domains of SbACP including Helix A-D were relatively conserved in sorghum (Fig. 8), especially the sequences around the Asp-Ser-Leu (DSL) motif (Helix B), which was the binding site of phosphopantetheine. This indicated that the DSL motif might have an important biological function.

Protein conformation was often related to their function. In order to further understand the function of the SbACP proteins, their three-dimensional structures were predicted through SWISS-MODEL website (Fig. 9). The results showed that more than 50% of the secondary structure of SbACP proteins was α-helix. SbACPs contained 4 α-helix structures, with A helix parallel to B helix and D helix, and C helix connecting B helix and D helix was relatively short. The results of multiple sequence alignments showed that B helix was the most conserved, while A, C and D helix were relatively less conserved. The binding site of phosphopantetheine was found at the nitrogen end of B helix, which might be the reason why the second helix was so conserved.

The cis-acting element of SbACPs

In order to explore the potential molecular function of SbACP gene family, the 1.5 kb promoter sequences
upstream of SbACP genes were analyzed to detect the cis-acting elements on Plant CARE website. The results suggested that a variety of cis-acting elements involved in physiological processes were revealed, including some basic elements like TATA-box and CAAT-box, (Fig. 10). For example, the cis-acting elements AE-box, GA-Motif, G-box, TCT-motif, GATA-motif, and GT1-motif were involved in light reaction; the binding sites of cis-acting elements ABRE and MYB were involved in abscisic acid response; MBS was involved in drought induction, and cis-regulatory element CAT-box was associated with meristem expression. These results clearly suggested that SbACP genes might participate in regulating biotic or abiotic stress, light response, plant growth and development, and signaling transduction pathways, as well as other physiological processes.
Expression patterns of SbACPs gene family under drought and salt stress

Previous studies have proved that ACP genes played critical roles in response to abiotic stresses in Arabidopsis [14], and the identification of cis-acting elements related to abiotic stress response in SbACPs promoter regions also indicated their potential function involved in different abiotic stress response pathways. Meanwhile, gene expression patterns could provide important clues to gene function. Consequently, to further confirm molecular function of SbACP genes in response to abiotic stress, the expression levels of SbACP genes in sorghum leaves were analyzed by qRT-PCR at 0, 6, 12 and 24 h after drought and salt treatment (Figs. 11 and 12). The results showed that seven of the nine SbACP genes were up-regulated or down-regulated within 24 h of drought and salt treatment, implying that these genes might play a role in response to drought and salt stress. Still, no significant expression differences of SbACP4 and SbACP8 were observed when compared with the control under drought and salt stress, indicating that these two genes were not directly involved in stress responses. Furthermore, we found that different genes showed different expression patterns under stresses. Under salt stress, SbACP1,
**ShbACP3**, ShbACP6 and ShbACP7 were significantly down-regulated at 24 h after treatment, whereas ShbACP2 was significantly up-regulated. ShbACP5 and ShbACP9 were up-regulated at 12 h. Under drought stress, ShbACP3, ShbACP5 and ShbACP9 were significantly up-regulated at 12 h, ShbACP1, ShbACP2 and ShbACP9 were significantly up-regulated at 24 h, whereas ShbACP7 was significantly down-regulated at 24 h. It’s worth noting that, of all genes with expression difference, ShbACP1 showed the greatest fold of expression difference at 24 h after salt treatment, while under drought treatment, the expression of ShbACP1 at 24 h was up-regulated up to five times. Interestingly, ShbACP9 was continuously up-regulated after 6 h of drought treatment. In a word, these results revealed that most of ShbACP genes could take part in response to the two abiotic stresses, but might be with different mechanisms due to their different expression patterns.

**Discussion**

With the development of sequencing technology, large numbers of plant genomes have been successfully assembled and released, which has greatly improved the research of genomes, including analysis of gene families and identification of important functional genes. Acyl carrier proteins are important members of the carrier protein family and play a key role in the direct synthesis pathway of long-chain fatty acids [13, 39].

Gene family is a group of genes that originate from the same ancestor and produce two or more copies of one gene through gene duplication [40]. They have obvious similarities in structure and function and encode similar protein products. There were 9 ACPs in sorghum, 8 ACPs in rice, 11 ACPs in maize and 8 ACPs in Arabidopsis. During gene evolution, gene duplication or loss may lead to differences in the number of gene family members [41]. In this study, nine ShbACPs were identified from sorghum genome by bioinformatics methods, and their physicochemical properties were predicted to be a class of acidic small molecule proteins, which was consistent with previous reports [42]. The protein length, molecular weight, and isoelectric point of ShbACPs were highly conservative, whereas the number of exons varied from 2 to 4. Multi-sequence alignment of the nine ShbACP proteins found that the amino acid sequences around the DLS motif were very conserved, and these sequences were the typical feature of the ShbACP family, which was the binding site of phosphopantetheine [43]. These results showed a variation in the sequence structure of the ShbACPs family, despite a high degree of conservation in key regions. ShbACPs might have undergone evolutionary events that resulted in changes in gene structure and function [44, 45].

Genes with similar gene structure and protein structure often have similar functions. In phylogenetic tree
In the analysis, all identified genes in four species were roughly divided into four branches. We found that there was almost always an ACP gene of maize and rice adjacent to a SbACP gene, while the branches of Arabidopsis ACP genes were farther from the SbACPs, suggesting that sorghum was more closely related in evolutionary
relationship with maize and rice than Arabidopsis. Generally, the genes under the same branch had high homology and similar functions [46]. We also studied the collinearity of SbACPs in sorghum genome and Arabidopsis, rice and maize genome, respectively. Collinearity analysis showed that there were more homologous ACPs between monocotyledons. These findings suggested that the SbACP genes might function in similar way with ACP genes of maize, which might be the result of the further differentiation of ACP genes on different branches after the differentiation of monocotyledons [47]. SbACP5 and SbACP7 shared a large fragment replication, so they were in the same branch of the evolutionary tree, and their gene structure and conserved motif were basically same. The segmented copies of genes tend to have the same function and expression patterns [48]. In this study, different from previous studies, SbACP5 and SbACP7 had different response patterns to drought and salt treatment, which might be caused by redundancy of function. Another alternative reason for the different response patterns of the two genes may be related to the differences in their cis-acting elements, which endowed them with different functions [49, 50]. Although about 10% of the colinear genes have been found throughout the genome, no tandem duplication existed in SbACPs family, and fragment replication also played only a minor role in the evolution and replication of the SbACPs family, hinting relative independent evolution events of most of SbACPs [49].

In Arabidopsis, ACP genes were divided into two types: plastidial ACP and mitochondrial ACP [35, 42]. In sorghum, ACP genes were also predicted to express in chloroplast and mitochondria. Fluorescence localization verified that all the 5 selected SbACP genes

![Fig. 11 qRT-PCR analysis of SbACPs under salt treatment. *, **, *** and ****, represent significant differences at P<0.05, P<0.01, P<0.001 and P<0.0001, respectively. One-way ANOVA was used to analyze the significance of relative expression difference of SbACP1-9 at 0, 6, 12 and 24 h under salt treatment and control.](image-url)
were expressed in chloroplasts, including SbACP2 and SbACP4, which were predicted to localize in chloroplast or mitochondria, indicating the reliability and accuracy of prediction results. But we still could not rule out the possibility of double-localizing for several SbACP genes. On the other hand, Yang et al. systematically analyzed ACP genes of 20 plant species and found that protein sequences (motifs and length) were highly conserved in different ACP branches [42]. Coincidently, Farmer et al. proposed the same opinions and thought that the three-dimensional structure of the acyl carrier proteins was highly conserved, which were folded into a flexible α-helical bundle [42]. In this study, the three-dimensional structure prediction showed the same results with the former researches. Rich α-helix of SbACPs formed α-helical bundle, and further constructed the hydrophobic cavities with structural plasticity, that allowed the accommodation of different lengths of thioester-bound acyl chains [39, 51].

The number and type of cis-acting elements in gene promoter region have different regulation effects on regulation of gene expression. Analysis of the 1.5 kb promoter region upstream of SbACPs revealed many TATA-box elements binding to RNA polymerase, which determined the transcription initiation of SbACPs. In addition, the promoter region of SbACPs contained many elements related to ABA, light response and abiotic stress, suggesting that the function of SbACP genes might be diversified. Some previous studies have proven this opinion. For example, seven motifs related to ABA and light-mediated gene regulation in the promoter of ACP (CACTFTP-PCA1, DOFCOREZM, GT1CONSENSUS, CAATBOX1, ARRI1AT, POLLEN1LELAT52 and GATABOX) in different species were found [42], indicating that ACPs might participate in several biological processes.

In Arabidopsis, the expression level of AtACP4 (At4g25050), a key gene to the synthesis of fatty acids in chloroplast membrane lipids, was positively correlated
with the photosynthetic system, which demonstrated that *AtACP4* might play an important role in light, nitrogen, and iron deficiency [14]. *SbACP6*, located in the same cluster as *AtACP4*, had many cis-acting elements related to light response in the promoter region, such as AE-box, G-box, GATA-Motif and GT1-motif, which implied that *SbACP6* and *AtACP4* might have similar response patterns. Meanwhile, the identification of MBS, MYB and MYB-like stress-related elements suggested that *SbACP6* may be involved in abiotic stress, which was verified by qRT-PCR [34]. Additionally, previous studies showed that the overexpression of *AtACP5* (*At5g27200*), a plastid localized protein, could increase the content of saturated fatty acids, reduce the Na⁺/K⁺ ratio, and significantly increase the salt tolerance [14], indicating that fatty acids in plants were associated with abiotic stress. Plastids can be divided into three types: chloroplasts, chromoplasts (or chromatids), and leucoplasts. Most SbACPs proteins displayed obvious expression signals in chloroplasts and they contained many stress-related elements in promoter sequences. Consequently, we inferred that when plants were subjected to abiotic stress, ACP was regulated by cis-acting elements (such as MYB, etc.) to increase spatio-temporal expression, and increase the proportion of saturated fatty acids on chloroplast membrane, thus reducing the dissolution of thylakoid grana lamellae [17, 52].

Fatty acid is one of the main components of membrane, and also an important substance for cells to adapt to environmental changes because of the important barrier function of cell membrane [53]. The change of fatty acids in membrane is also a response mechanism to various abiotic stresses [16, 17, 54, 55]. ACPs could react to the external environment stimulation, and further lead to the changes in fatty acid composition and content, implying that ACPs might play a crucial part in response to the adverse environment [16, 17], which was identified by the expression differences of several SbACPs under salt and drought treatments in the current study. Seven of all the nine SbACP genes could response to salt or drought stress with different expression patterns, except SbACP4 and SbACP8. Remarkably, the expression of *SbACP9* was up-regulated from 6 to 24 h after treatment, and showed a rapid and continuous response to drought stress, which suggested that *SbACP9* might function in the drought resistance in a direct way. All these results suggested that ACP genes in sorghum might play an important role in response to various biological process, including light and abiotic stresses, which could provide valuable information for the subsequent functional studies of SbACP genes, and would benefit the breeding of sorghum resistant germplasm.

### Conclusions

In summary, a total of 36 genes encoding SbACP proteins with PP-binding domain were identified in sorghum, maize, rice, and Arabidopsis, which were mainly divided into four families according to the analysis of phylogenetic of ACPs. These ACP family genes were unevenly distributed on six chromosomes. The gene structure and motif composition were also analyzed. The phylogenetic relationship among ZmACP and SbACP proteins were closer, providing a clue to its possible function. It was found that fragment replication might lead to the production of SbACPs genes. Phylogenetic comparison and synteny analysis of ACP genes from 4 typical plant species provided valuable clues about the evolutionary characteristics of ACP gene family members in sorghum. Colinear analysis of ACP in sorghum and other three plants provided information for the evolution of SbACP gene family in sorghum. Besides, these identified SbACP genes were analyzed by cis-acting element for a further investigation on their performance in plant growth and stress responses. Gene expression patterns of SbACP genes in leaves under drought and salt stresses were also analyzed. This study of the SbACP family in structure, evolution and expression profiling facilitated the analysis of the SbACP gene function and built a foundation for a better research of the mechanism of abiotic stress tolerance in sorghum.

### Materials and methods

#### Identification of ACP gene family in sorghum

The sorghum BTx623 genome dataset, including the CDSs and protein sequences, was downloaded from the Ensembl database (http://plants.ensembl.org/index.html). The hidden Markov model (HMM) was used to query the SbACP protein sequences according to the PP-binding domain (PF00550) in Pfam (http://pfam.xfam.org/) throughout the whole genome using the default parameters in HMMER software. The Hmmssearch program in the Linux system was used to search for proteins containing the conserved domain [56], and these sequences were confirmed on SMART, NCBI CDD, and Pfam, respectively. Finally, nine SbACP genes were identified and named according to their position on chromosomes. The ExPASy Proteomics Server software (http://web.expasy.org/protparam/) was used to analyze the protein sequence, protein molecular weight (Mw), and calculate isoelectric point (Pl) [57].

#### Chromosome localization analysis of ACP family in sorghum

The location of 9 SbACP genes and chromosome length information were extracted through the annotation
information of the sorghum genome, and the chromosome location map of genes was drawn by Mapchart software [58].

Subcellular localization of ACP proteins in sorghum
SbACP protein sequences were submitted to CELLO (http://cello.life.nctu.edu.tw/) to predict the subcellular localization of proteins [59] and then the prediction results were verified by experiments. Specific primers (Table S1) were designed according to the sequence of SbACPs CDS which were used to amplify the whole length of CDS of SbACPs by KOD One TM PCR Master Mix (TOYOBO, Osaka, Japan). The Vector was linearized using HindIII and BamHI (NEB, Nebraska, USA). The full length CDSs of 5 selected SbACPs were subcloned into the destination vector with GFP using the ClonExpress® II One Step Cloning Kit (Vazyme, Nanjing, China). The target gene containing the GFP fusion proteins were transferred into Agrobacterium GV3101 and infecting tobacco leaves. After cultured for 48 h of tobacco plants in dark condition at 28°C, the fluorescence images were observed using Leica TCS SP8 (Mannheim, Germany) and A1R HD25 (Nikon, Japan) confocal microscope image system.

Evolution analysis of ACPs in sorghum
The phylogenetic tree of ACP family proteins in sorghum, maize, and Arabidopsis was constructed by the adjacency joining algorithm in MEGA X software [60] and a bootstrap test (replications) with 1,000 iterations was performed. The obtained evolutionary tree was further modified using the Evolveweb site (http://www.omicsclass.com/article/671).

Analysis of ACP family gene structure and motif in sorghum
To analyze the structural information of SbACPs, the annotation information of 9 SbACP genes was extracted. The GSDS2.0 website (http://gsds.gao-lab.org/) were used to analyze the exon and intron structure and draw the gene structures. The MEME (https://meme-suite.org/meme/) was utilized to carry out the motif analysis based on the protein sequences of nine SbACPs.

Duplication and syntenic analyses of ACPs between sorghum and other species
Multiple Collinearity Scan toolkit (MCScanX) [61] with the default parameters was used to analyze the gene duplication events. To investigate the homology of the ACPs gene family between sorghum and three other species, the Dual Systeny Plotter software (https://github.com/CJ-Chen/TBtools) was used to map the intergenomic collinearity analysis.

Multi-sequence alignment and three-dimensional structure prediction of sorghum ACP family protein structure
To analyze the conserved domains of the SbACP proteins, clustalw from MEGA X [62] was used for multi-sequence alignment of 9 protein sequences, and then the geneDoc software [63] was used to calculate and analyze the conserved sequences of the SbACP family. To further analyze the protein structure of the SbACP family, the 3D structure of SbACP proteins was predicted according to the protein sequence of SbACPs, and 3D protein models were constructed on the Swiss-Model website (https://swissmodel.expasy.org) by the homologous protein modeling method.

Cis-acting element analysis
To explore the regulation of gene expression, 1.5 kb sequences upstream of the initiation codon of SbACP genes were extracted to analyze the cis-acting elements of these genes. Plant CARE (Cis-Acting Regulatory Element, http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to further analyze the cis-acting elements, and the results were mapped using the GSDS online website (http://gsds.gao-lab.org/).

Plant materials and treatment
In this study, the sorghum BTx623 was grown in a climate chamber to the two-leaf stage and then transferred to Hoagland nutrient solution. The temperature and light cycle were set at 24 °C with a 14 h/10 h (light and dark) photoperiod. After two days of culture, the seedlings were treated with 400 mM PEG6000 [64] and 150 mM NaCl treatment [65], respectively. The control groups were set for each treatment. The leaves from both the blank control and salt or drought (PEG) treatment with three biological replicates were collected at 0, 6, 12 and 24 h after treatments, then immediately frozen in liquid nitrogen and stored in an ultralow temperature refrigerator at -80 °C for subsequent experiments.

RNA extraction and qRT-PCR
The RNA was extracted using the ultrapure RNA extraction kit (CWIBIO, Taizhou, China), and the HiScript III RT SuperMix for qPCR (+gDNA Wiper) kit (Vazyme, Nanjing, China) was used for reverse transcription. ChamQ SYBR qPCR Master Mix (LowROX Premixed) kit (Vazyme, Nanjing, China) was used for real-time quantitative PCR analysis. Primer 5.0 was used to design qPCR primers for the ACP gene family, and the sequences of primers were shown in Table S2. The reaction volume was 20µL, and the amplification procedures were: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 10 s, annealing at 60°C for 30 s, and 40 cycles [66]. Each gene
was replicated three times biologically and three times technically. Actin was used as a control and related gene expression levels were quantified by \(2^{-\Delta \Delta Ct}\) [67].

**Abbreviations**

ACPs: Acyl Carrier Protein; (s): qRT-PCR; Quantitative real-time PCR; GSDDS: Gene Structure Display Server; MW: Molecular weight; PI: Theoretical isoelectric point; PEG: Polyethylene glycol; Ser: Serine; FAs: Fatty acids; UFAs: Unsaturated FAs; SPAs: Saturated FAs; ER: Endoplasmic reticulum; JGI: The United States Department of Energy joint Genome Research Institute; DSL: Asp-Ser-Leu; HIMM: Hidden Markov model; PP-binding: Phosphopantetheine, or pantethine; 4phosphate-binding, MCScanX: Multiple Collinearity Scan toolkit.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08776-2.

**Additional file 1:** Table S1. The primers for subcellular localization.

**Additional file 2:** Table S2. The primers for qRT-PCR.

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**Authors’ contributions**

This study was designed by BHW, PL and HF. HQG, JJX, MZH, WWA and JPW carried out all the experimental analyses and prepared all figures and tables. The manuscript was drafted by HQG. BHW and PL assisted in explaining the results and BHW and HF revised the final manuscript. All authors have reviewed and approved the final manuscript.

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**Availability of data and materials**

The datasets generated during the current study are available in the Ensemble, https://asia.ensembl.org/index.html and their public access to these databases are open. All data generated or analyzed during this study are included in this article and its supplementary information files.

**Declarations**

**Ethics approval and consent to participate**

This article does not contain any studies with human participants or animals performed by the authors. These methods were carried out in accordance with relevant guidelines and regulations including the IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora. We confirm that all experimental protocols were approved by Nantong University.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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**References**

1. Majerus PW, Alberts AW, Vogelos PR. The acyl carrier protein of fatty acid synthesis: purification, physical properties, and substrate binding site. Proc Natl Acad Sci USA. 1964;51(6):1231–8.

2. Javorski JG, Post-Bettenmillera MA, Ohlrogge JB. Site-directed mutagenesis of the spinach acyl carrier protein-I prosthetic group attachment site. Eur J Biochem. 1989;184(3):603–9.

3. Lambalot RH, Gehring AM, Flugel RS, Zuber P, LaCelle M, Marahiel MA, Reid R, Khosla C, Walsh CT. A new enzyme superfamily—the phosphopantetheinyl transferases. Chem Biol. 1996;3(11):923–36.

4. Joshi AK, Zhang L, Ragan V, Smith S, Cloning, expression, and characterization of a human 4′-phosphopantetheinyl transferase with broad substrate specificity. J Biol Chem. 2003;278(55):53142–9.

5. Arthur CJ, Szafrańska A, Evans SE, Findlow SC, Burston SG, Owen P, Clark-Lewis I, Simpson TJ, Crosby J, Crump MP. Self-malonylation is an intrinsic property of a chemically synthesized type II polyketide synthase acyl carrier protein. Biochemistry. 2005;44(46):15414–21.

6. Arthur CJ, Szafrańska AE, Long J, Mills J, Cox RJ, Findlow SC, Simpson TJ, Crump MP, Crosby J. The malonyl transferase activity of type II polyketide synthase acyl carrier proteins. Chem Biol. 2006;13(6):587–96.

7. Misra A, Sharma SK, Suroila N, Suroila A. Self-acetylation properties of type II fatty acid biosynthesis acyl carrier protein. Chem Biol. 2007;14(7):775–83.

8. Misra A, Suroila N, Suroila A. Catalysis and mechanism of malonyl transferase activity in type II fatty acid biosynthesis acyl carrier proteins. Mol Biosyst. 2009;5(6):651–9.

9. Gokhale RS, Sankaranarayanan R, Mohanty D. Versatility of polyketide synthases in generating metabolic diversity. Curr Opin Struct Biol. 2007;17(6):736–43.

10. Koglin A, Walsh CT. Structural insights into nonribosomal peptide enzymatic assembly lines. Nat Prod Rep. 2009;26(8):987–1000.

11. Weissman KJ. Polyketide biosynthesis: understanding and exploiting modularity. Philos Trans Royal Soc A. 1825;304(1354):2671–90.

12. Finking R, Marahiel MA. Biosynthesis of nonribosomal peptides1. Annu Rev Microbiol. 2004;58:453–88.

13. Chan DL, Vogel HJ. Current understanding of fatty acid biosynthesis and the acyl carrier protein. Biochem J. 2010;430(1):1–19.

14. Huang JX, Xue CW, Wang H, Wang LS, Schmidt W, Shen RF, Lan P. Genes of acyl carrier protein family show different expression profiles and overexpression of acyl carrier protein 5 modulates fatty acid composition and enhances salt stress tolerance in Arabidopsis. Front in Plant Sci. 2017;8:987.

15. Nazari M, Moosavi SS, Maleki M, Goharrizi KJ. Chloroplastic acyl carrier protein synthase I and chloroplastic 20 kDa chaperonin proteins are involved in wheat (Triticum aestivum) in response to moisture stress. J Plant Interact. 2020;15(1):180–7.

16. Miquel M, James D, Dooner H. Arabidopsis requires polyunsaturated lipids for low-temperature survival. Proc Natl Acad Sci USA. 1993;90(13):6208–12.

17. Zhang J, Liu H, Sun J, Li B, Zhu Q, Chen S, Zhang H. Arabidopsis fatty acid desaturase FA2D is required for salt tolerance during seed germination and early seedling growth. PLoS ONE. 2012;7(1):e30355.

18. Hanappel DJ, Ohlrogge JB. Regulation of acyl carrier protein messenger RNA levels during seed and leaf development. Plant Physiol. 1988;86(4):1174–8.

19. de Silva J, Loader NM, Jarman C, Windust JH, Hughes SG, Safford R. The isolation and sequence analysis of two seed-expressed acyl carrier protein genes from Brassica napus. Plant Mol Biol. 1990;14(4):362:2671–90.

20. Li MJ, Wang XJ, Su L, Bi YP, Wan SB. Characterization of five putative acyl carrier protein (ACP) isoforms from developing seeds of Arabis hypogaea L. Plant Mol Biol Rep. 2010;28(3):365–72.

21. Zhang D, Song H, Cheng H, Hao D, Wang H, Kan GZ, Jin HK, Yu DY. The acyl phosphatase-encoding gene GmACP1 contributes to soybean tolerance to low-phosphorus stress. PLoS Genet. 2014;10(1):e1004061.

22. Ludlow MM, Muchow RC. A critical evaluation of traits for improving crop yields in water-limited environments. Adv Agron. 1990;43:107–53.
23. Zheng LY, Guo XS, He B, Sun LJ, Peng Y, Dong SS, Liu TF, Jiang SY, Ramachandran S, Chun- Li M, Jing HC. Genome-wide patterns of genetic variation in sweet and grain sorghum (Sorghum bicolor). Genome Biol. 2011;12(1):R114.

24. Mace ES, Tai S, Gilling EK. Whole-genome sequencing reveals untapped genetic potential in Africa’s indigenous cereal crop sorghum. Nat Commun. 2013;4:2320.

25. Ighbird MA. Agronomic management strategies elevate forage sorghum yield: A review. J Adv Bot Zool. 2015;31:6–1.

26. Morris GP, Ramu P, Deshpande SP, Hash CT, Shah T, Upadhyaya HD, Riera-Lizarazu O, Brown PJ, Achariya CB, Mitchell SE, Harriman J, Glaubitz JC, Buckler ES, Kresovich S. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. Proc Natl Acad Sci USA. 2013;110(2):453–8.

27. Kebede H, Subudhi PK, Rosenow DT, Nguyen HT. Quantitative trait loci influencing drought tolerance in grain sorghum (Sorghum bicolor L. Moench). Theor Appl Genet. 2001;103:266–76.

28. Netondo GW, Onyango JC, Beck E. Response of growth, water relations, and ion accumulation to NaCl salinity. Crop Sci. 2004;44(3):797–805.

29. Krishnamurthy L, Seraj R, Hash CT, Dakhele AJ, Reydy BVs. Screening sorghum genotypes for salinity tolerant biomass production. Euphytica. 2007;156:15–24.

30. Ashraf M. Inducing drought tolerance in plants: Recent advances. Biotech. 2010;28(1):169–83.

31. Tari I, Laskay G, Takács Z, Poor P. Response of Sorghum to abiotic stresses: a review. J Agron Crop Sci. 2013;199(4):264–74.

32. Harris-Shultz KR, Hayes CM, Knoll JE. Mapping QTLs and identification of genes associated with drought resistance in Sorghum. Methods Mol Biol. 2019;1931:1–10.

33. Paterson AH, Bowers JE, Bruggmann R, Dubchak I, Gre agreed P, Sandor T, Poliakov A, et al. The Sorghum bicolor genome and the diversification of grasses. Nature. 2009;457(7229):551–6.

34. Bonaventure G, Ohlrogge JB. Differential regulation of mRNAs levels of acyl carrier protein isoforms in Arabidopsis. Plant Physiol. 2002;128(1):225–35.

35. Meyer EH, Heazlewood JL, Millar AH. Mitochondrial acyl carrier proteins in Arabidopsis thaliana are predominantly soluble matrix proteins and none can be confirmed as components of respiratory Complex I. Plant Mol Biol. 2007;64(3):319–27.

36. Wang J, Toth K, Bowers JE, Bruggmann R, Dubchak I, Grimwood J, Zhang A. Genome-wide analysis of the NF-Y transcription factor family in Sorghum bicolor. BMC Genomics. 2014;15(1):281.

37. Li M, Li G, Liu W, Dong X, Zhang A. Genome-wide analysis of the TIFY gene family in Sorghum bicolor. BMC Genomics. 2021;22(1):1–17.

38. Li X, Zhang X, Shi T, Shi T, Chen M, Jia C, Wang J, Hou Z, Han J, Jian S. Identification of LFAR family in blueberry and its potential involvement of fruit development and pH stress response. BMC Genomics. 2022;23(1):329.

39. Kim Y, Prestegard JH. A dynamic model for the structure of acyl carrier protein in solution. Biochemistry. 1989;28(2):8792–97.

40. Xu G, Guo C, Shan H, Kong H. Divergence of duplicate genes in exon-intron structure. Proc Natl Acad Sci USA. 2012;109(4):1187–92.

41. Tang C, Zhu X, Qiao X, Gao H, Li Q, Wang P, Wu J, Zhang S. Characterization of the pectin methyl-esterase gene family and its function in controlling pollen tube growth in pear (Pyrus pyrifolia). J Exp Bot. 2012;73(5):413–23.

42. Li M, Li G, Liu W, Dong X, Zhang A. Genome-wide analysis of the NF-Y gene family in peach (Prunus persica L.). BMC Genom. 2019;20(1):612.

43. Liu Y, Belachew A, Ma S, Young M, Ade J, Shen Y, Marion CM, Holtan HE, Bailey A, Stone JK. The EDLDE motif, a potent plant transcriptional activation domain from AP2/ERF transcription factors. Plant J. 2012;70(5):685–95.

44. Byers DM, Gong H. Acyl carrier protein: structure-function relationships in a conserved multifunctional protein family. Biochem Cell Biol. 2007;85(6):649–62.

45. Xu G, Guo C, Shan H, Kong H. Divergence of duplicate genes in exon-intron structure. Proc Natl Acad Sci USA. 2012;109(4):1187–92.

46. Cantu DC, Forrester MJ, Charov K, Reilly PJ. Acyl carrier protein structural classification and normal mode analysis. Protein Sci. 2012;21(5):665–66.