Genomic analysis of sugar transporter genes in peanut (*Arachis hypogaea*): Characteristic, evolution and expression profiles during development and stress

Zhijun Xu [a,b,*], Lei Xu [a,b], Xiaowen Hu [a,b]

[a] Guangdong Engineering Technology Research Center for Dryland Water-saving Agriculture, Zhanjiang Experiment Station, Chinese Academy of Tropical Agricultural Sciences, Zhanjiang, Guangdong, China
[b] Zhanjiang Experimental and Observation Station for National Long-term Agricultural Green Development, Zhanjiang, Guangdong, China

ARTICLE INFO

Keywords:
Peanut
STP gene Family
Evolution
Expression patterns
Pod development

ABSTRACT

Peanut (*Arachis hypogaea* L.) is an economically significant crop with aerial cleistogamous flowers and subterranean geocarpic fruit (pods). The formation of peanut pod requires movement of the embryo from air to ground and then development in the soil, which is a complex biological process involving transport and accumulation of sugars. Sugar transport proteins (STP) mediate the transport of monosaccharides in various physiological processes, including fertilization, ovary formation, and seed development. In this study, a total of 36 *AhSTP* genes (*AhSTP1–36*) containing the conserved sugar_tr motif were identified in the *A. hypogaea* genome. Phylogenetic analysis revealed that *AhSTP* genes were classified into four clades, and the arrangement of motifs in AhSTP proteins was similar within clades. Synteny analysis revealed that segmental duplication events have played an important role in the expansion of *STP* genes in peanut, and chromosome rearrangements might have facilitated the exchange of *STP* genes between the A and B sub-genomes. Transcriptome analyses revealed that the expression patterns of *AhSTP* genes varied among tissues. Hormone and abiotic stress treatments could up-regulate or down-regulate the expression of *AhSTP* genes, and low temperature had a major effect on the expression of most *AhSTP* genes. Four *AhSTP* genes (*AhSTP3*, *AhSTP9*, *AhSTP19*, and *AhSTP28*) were specifically expressed in the pod, indicating that these genes might be involved in pod formation and development in peanut. The unique expression of these four genes during pod construction and development was confirmed in two different type cultivars using quantitative real-time PCR analysis. Our findings provide new insights into the *STP* gene family in peanut and will aid future functional studies of *AhSTP* genes.

1. Introduction

Sugars are the key source of energy and carbon for plants; they are also important signaling molecules and play an important role in diverse physiological and metabolic processes in plants (Bavnhøj et al., 2021). Three types of transporters facilitate the movement of sugars in plants: monosaccharide transporters (MSTs), sucrose transporters (SUTs), and sugars will eventually be exported transporters(SWEETs) (Zhang et al., 2019). And a two-step process is employed by plants to detect the level of carbon after sucrose from the plant phloem is unloaded by SUTs, and this is important for ensuring that the development of plants proceeds correctly (Slewinski, 2011; Lemoine et al., 2013). Following the enzymatic hydrolysis of apoplastic sucrose, the glucose and fructose products are transported to sink tissues by sugar transport proteins (STPs), which are important members of the MST family (Büttner et al., 2000; Niño-González et al., 2019).

STPs are proton-coupled symporters that mediate the uptake of hexoses from the apoplastic compartment into cells in all plant tissues (Bavnhøj et al., 2021). STPs generally contain 12 transmembrane domains (TMDs); they are localized to the plasma membrane and facilitate the acquisition of various monosaccharide substrates, such as glucose, fructose, galactose, xylose, mannose, and non-metabolized 3-*O*-methyl-glucose (Büttner 2010). STPs play important roles in the development of organs in symplastically isolated tissues such as pollen, fruit, and seeds (Cheng et al., 2015; Rottmann et al., 2018). For example, in cucumber, *CsHT1*, which is a homolog of *AtSTP4, AtSTP9*, and *AtSTP11*, specifically

mediates the transport of glucose and galactose and plays an important role in seed development by affecting the growth of the pollen tube (Cheng et al., 2015). Substantial evidences also indicate that STPs participate in the responses of plants to biotic and abiotic stress, including osmotic, salt, drought, and cold stress, wounds, as well as infection by nematodes and pathogens (Truernit et al., 1996; Büttner et al., 2000; Mendgen and Hahn 2002; Büttner, 2007; Yamada et al., 2016). For example, in *Arabidopsis thaliana*, the expression of *AtSTP13* is regulated by several factors, including pathogens, elicitors, chemicals (e.g., ozone and phytoprostanes, which potentially mediate oxidative stress), hormones (e.g., abscisic acid (ABA), methyl jasmonate (MeJA), gibberellic acid (GA), and salicylic acid (SA)), hormone biosynthesis inhibitors (e.g., paclobutrazol (PAC)), nutrients (e.g., phosphorous, potassium, nitrate, and sucrose), and abiotic stress (e.g., cold, heat, hypoxia, osmotic, oxidative, and salt stress) (Lee and Seo, 2021; Urwat et al., 2021).

Peanut is grown in tropical and semi-arid tropical regions of the world, and it is an important source of high-quality vegetable oil and protein. Peanut is an allotetraploid (AABB, 2n = 4x = 40) that formed via hybridization between *Arachis duranensis* (AA, 2n = 2x = 20) and *Arachis ipaënsis* (BB, 2n = 2x = 20) (Favero et al., 2006; Seijo et al., 2007; Grabiele et al., 2012; Samoluk et al., 2015). Peanut has aerial cleistogamous flowers and subterranean geocarpic fruit (pods). Peanut pod formation requires the movement of the embryo from the air to the ground by elongation of the peg (gynophore) and then the embryo is development under dark conditions (Sinha et al., 2020). The main process involved in transferring and development of the embryo include the transportation and accumulation of sugars. However, few studies have examined the expression of *STP* genes during peg elongation and pod development in peanut. Herein, we identified 36 *STP* genes in peanut using genomic and transcriptomic data. And the characteristics of these sequences, including the gene structure, the distribution of motifs in the proteins, and their evolutionary relationships were detailed analyzed. The expression profiles of *AhSTP* genes in different tissues, in response to different hormones, and under different types of abiotic stress revealed substantial variation in the expression patterns of *AhSTP* genes. Four genes that might participate in pod construction and development were identified, and the expression profiles of these four genes in peanut varied. Gene variation analysis was conducted to explore the causes of the different expression patterns of *AhSTP* genes during pod development in different peanut cultivars. The findings of our study provide new insights into the evolution of the *AhSTP* gene family and the functions of *AhSTP* genes during pod development.

2. Materials and methods

2.1. Genome-wide identification of peanut STP genes

The genome sequence and annotated gene models of peanut (*A. hypogaea*, genome assembly version 1) and the genomes of its two wild diploid ancestors (*A. duranensis* and *A. ipaënsis*) were downloaded from Peanutbase (https://peanutbase.org/) (Dash et al., 2016). *STP* genes in the two diploid ancestors were identified to facilitate analyses of the characteristics of peanut *STP* genes. The sugar_tr domain (PF00083) downloaded from Pfam (http://pfam.xfam.org/) was used to conduct searches for *STP* genes in the three peanut species with HMMER (Version 3.0, https://hmmer.janelia.org/), and the best matching sequences were identified using a BLAST search with 14 *AtSTP* and 29 *OsSTP* genes. All protein sequences were checked using the Pfam database and SMART program (http://smart.embl.de). STP sequences of Arabidopsis (AtSTPs), rice (OsSTPs), and soybean (GmSTPs) were obtained from TAIR (http://www.Arabidopsis.org), RGAP7 (http://rice.plantbiology.msu.edu/), and Soybase (https://www.soybase.org/), respectively.

2.2. Properties of STPs and phylogenetic analysis

Various properties of peanut STPs, including protein sequence length, molecular weight (MW), theoretical isoelectric point (pI), aliphatic index (AI), and grand average of hydropathicity (GRAVY), were analyzed using the ProtParam tool (https://web.expasy.org/protparam/). The TMHMM tool (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0) and Plant-mPLoc (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/) were used to predict the TMDs and subcellular localization of STPs, respectively. Multiple alignment of STP sequences of three peanut species, soybean, and Arabidopsis was performed using ClustalW (Table S1 and Table S2). Neighbor-joining (NJ) phylogenetic tree was constructed using MEGA7.0 software with the following parameters: 1000 bootstrap replicates, possion model, uniform rates, and pairwise deletion (Kumar et al., 2016). Furthermore, STP NJ phylogenetic tree of the three peanut species or peanut was constructed using the same method.

The structures of peanut *STP* genes were analyzed using TBtools software (Chen et al., 2020) by comparison of cDNA and gene sequences. Conserved motifs in peanut STP proteins were analyzed using the MEME program (http://meme-suite.org/tools/meme) with the following parameters: optimum width, 20–60; number of repetitions, any; and maximum number of motifs, 12.

2.3. Synteny analysis

The Multiple Collinearity Scan toolkit (MCScanX) (Wang et al., 2008) was used to identify duplication events of *STP* genes with default parameters. A circos map of peanut *STP* genes was built to identify segmental duplications using TBtools software (Chen et al., 2020). Syntenic maps were built using TBtools software (Chen et al., 2020) to characterize syntenic relationships of *STP* genes between peanut and other plants. The rate of non-synonymous substitutions (Ka) and synonymous substitutions (Ks) of each duplicated *STP* gene pair was determined using DnaSP version 6.0 (Rozas et al., 2017). Divergence time was estimated using the following formula: $T = Ks/2 \times m$, where m indicates the molecular clock (Bertioli et al., 2016; Yin et al., 2020). The average mutation rate for *Arachis* was assumed to be 8.12×10^{-9} mutations per base per year based on previous studies (Bertioli et al., 2016; Yin et al., 2020).

2.4. Gene expression and qRT-PCR analyses

To characterize the expression profiles of *AhSTP* genes, the RNA-seq read count data of 22 different peanut tissues were downloaded from the peanut developmental transcriptome map dataset in Peanutbase (https://peanutbase.org/) (BioProject ID: PRJNA291488) submitted by Clevenger et al. (2016), and the data were transformed into fragments per kilobase per million fragments mapped (FPKM) values. The 22 peanut tissues, including leaf, root, floral organ, peg, and pod tissues, are described in Table S3. The heatmap of *AhSTP* gene expression profiles was constructed using TBtools software (Chen et al., 2020) with Z-score standardization. The gene expression atlas for subsp. *fastigiata* cultivar ICGV 91114, submitted by Sinha et al. (2020), was downloaded from the National Center for Biotechnology Information Sequence Read Archive database (BioProject ID: PRJNA484860) to characterize the expression patterns of genes in different cultivars. The transcriptome data of *AhSTP* genes under different hormonal and abiotic stress treatments were downloaded from the Peanut Genome Resource (Zhuang et al., 2019). The data was from peanut seedlings treated with drought (8 days), low temperature (10 °C), 3 mmol/L salicylic acid (SA), 10 mmol/L ethylene, 10 μg/mL abscisic acid (ABA), 0.1 mg/L brassinosteroid (BR), 150 mg/L PAC paclobutrazol (PAC) and their control plants were treated with clean water in a controlled green house (Zhuang et al., 2019). The detailed

procedure for sample harvesting and data analysis is presented in the article of Zhuang et al. (2019).

The cultivar 'Xiaobaisha' of the subsp. *fastigiata* and 'ZPG13' (a breeding line derived from the hybrid of a Costa Rica cultivar and M51) of the subsp. *hypogaea* were used to detect the expression patterns of genes in different varieties. Peg, pod shell, seed_5, seed_15, and seed_25 tissues were collected, and these tissues corresponded to the "peg tip below soil," "Pericarp Pattee 5," "peg tip to fruit Pattee 1," "Seed Pattee 6," and "Seed Pattee 10" development stages in Clevenger et al. (2016). Tissues were collected from five different plants (one biological replicate), and each experiment was conducted using three biological replicates (Wang et al., 2021). Total RNA was extracted using a Quick RNA Isolation Kit (Huayueyang, Beijing, China) following the manufacturer's instructions. First-strand cDNA sequences were synthesized using a Hifair® II1st Strand cDNA Synthesis Kit (Yeasen, Shanghai, China). Quantitative real-time PCR (qRT-PCR) assays were performed using TransStart® Green qPCR SuperMix (Transgen, Beijing, China). The thermal cycling conditions for the qRT-PCR reactions were as follows: 94 °C for 30 s, followed by 40 cycles of 94 °C for 5 s, 55 °C for 15 s, and 72 °C for 10 s. The actin gene *EF1b* was used as an internal control (Chi et al., 2012), and the gene-specific primers for the *STP* genes used in qRT-PCR reactions are listed in Table S4. The relative expression levels of genes were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.5. Gene variation analysis

Gene sequence variation was characterized using 37 diverse peanut cultivars, with 14 cultivars of subsp. *hypogaea* and 23 cultivars of subsp. *fastigiata*. Information of the 37 peanut cultivars is provided in Table S5. Genomic DNA was extracted from the young leaves of these cultivars using a modified cetyltrimethyl ammonium bromide method. The full-length sequences of *AhSTP3*, *AhSTP9*, *AhSTP19*, and *AhSTP28* were downloaded from Peanutbase (https://peanutbase.org/) (Dash et al., 2016). Genic simple sequence repeats (genic SSRs) of these full-length sequences were identified using the MISA tool with default parameters, and the primers of the identified SSRs were designed using Primer 3 software (Zhao et al., 2017). PCR reactions were conducted following the procedures of Huang et al. (2016). All SSR primers are listed in Table S6.

Sequences of *AhSTP3*, *AhSTP9*, *AhSTP19*, and *AhSTP28* were cloned from 'ZPG13' and 'Xiaobaisha' to analyze sequence variation in these genes. Pairwise alignment of gene sequences was performed using ClustalW. The secondary structure of proteins was predicted using SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and PredictProtein (https://predictprotein.org/home). Gene promoters and *cis*-acting regulatory elements were predicted using PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and NEW PLACE (https://www.dna.affrc.go.jp/PLACE/?action=newplace).

3. Results

3.1. Genome-wide identification of STP genes and characteristics of STP proteins in peanut

A total of 36 *STP* genes were identified in the peanut genome, including 17 in the AA sub-genome and 19 in the BB sub-genome (Table 1). In addition, 15 and 16 *STP* genes were identified in the possible diploid ancestors *A. duranensis* and *A. ipaënsis*, respectively. The distribution of *STP* genes among chromosomes was uneven in all three species. No *STP* genes were present on chromosome A07 of *A. duranensis* and chromosome B08 of *A. ipaënsis* and *A. hypogaea*. The length of STP proteins ranged from 397 to 1208 amino acids (aa), and the predicted MW ranged from 44.01 to 135.36 kD. pI, AI, and GRAVY ranged from 5.92 to 9.72, 93.7 to 111.67, and 0.14 to 0.67, respectively. Sixty of the 67 STPs contained 10–12 conserved TMDs, six STPs contained nine conserved TMDs, and AhSTP26 contained only eight TMDs. The conserved STP domain of STPs ranged from 376 to 476 aa. All STP proteins in peanut were predicted to be localized in the cell membrane.

3.2. Phylogenetic analysis of STP genes from different species

A phylogenetic tree was constructed using the NJ method and 14 AtSTP, 15 AdSTP, 16 AiSTP, 36 AhSTP, and 30 GmSTP full-length protein sequences to clarify the evolutionary relationships among *STP* genes in peanut (Fig. 1). Phylogenetic analysis revealed that the *STP* genes were divided into four groups, each of which contained AtSTP, AdSTP, AiSTP, AhSTP, and GmSTP proteins. Most of the AhSTP proteins were grouped in pairs with their corresponding orthologs in *A. duranensis* or *A. ipaënsis*. For example, in Group I, AhSTP23 was clustered with the *A. ipaënsis* ortholog AiSTP6, and AhSTP2 was highly homologous with its *A. duranensis* counterpart AdSTP2. However, patterns of homology among AhSTPs and their corresponding orthologs were more complex. For example, in Group II, AhSTP11 and AhSTP30 were grouped in the same clade with AdSTP10 and AiSTP10.

3.3. Structure of STP genes and motif composition of STP proteins in peanut

Conserved motif analysis of all peanut STP proteins was performed using the MEME program and Pfam database. A total of 12 putative motifs ranging in length from 21 to 49 aa were identified, and motifs 1, 2, 3, 5, 6, 7, and 8 were parts of the putative sugar_tr domain (Table 2, Fig. 2). Motifs 1, 2, 6, and 8 were highly conserved among all 67 STPs. The motif composition of *STP* genes within the same clade was similar. For example, in Group II, AhSTP8, AhSTP 17, AhSTP34, AdSTP15, and AiSTP15 in the same clade had similar motif composition.

The exon-intron organization of all *STP* genes was analyzed to clarify diversity in the structure of *STP* genes. There was substantial variation in the structure of *STP* genes in peanut (Fig. 2). The number of exons of *STP* genes ranged from 1 to 11. For example, *AhSTP20* and *AhSTP24* each had 11 exons and 10 introns, and AhSTP2, AdSTP2, and AiSTP2 had only one exon. Most *STP* genes had three to four exons, and the lengths and positions of exons were generally similar within the same clade.

3.4. Synteny analysis of STP genes in peanut

According to the previous studies, segmental duplications were result of multiple genes through polyploidy followed by chromosome rearrangement, and tandem duplications were characterized as multiple members of one family occurring within the same intergenic region or in neighboring intergenic regions (Yu et al., 2005; Zhu et al., 2014). In peanut, a total of 24 *AhSTP* genes were identified as the products of 18 segmental duplication events on 18 chromosomes, with the exception of chromosomes A02 and B08 (Fig. 3A). For example, segmental duplications of *AhSTP9*, *AhSTP15*, *AhSTP28*, and *AhSTP33* were observed on chromosomes A05, A09, B05, and B09. Segmental duplications were also observed for *AhSTP13*, *AhSTP14*, and *AhSTP32* on chromosomes A07, A08, and B07 (Fig. 3A). A tandem duplication event with two genes (*AhSTP35/36*) was observed on chromosome B10.

Two comparative syntenic maps of peanut with four species, including its two diploid ancestors (*A. duranensis* and *A. ipaënsis*) and two other dicot species (Arabidopsis and soybean), were built to clarify the syntenic relationships of *STP* genes in peanut (Fig. 3B and C). Syntenic relationships were observed between 28 *AhSTP* genes and 14 *AdSTP* genes in *A. duranensis*, 28 *AhSTP* genes and 15 *AiSTP* genes in *A. ipaënsis*, 6 *AhSTP* genes and 7 *AtSTP* genes in *A. thaliana*, and 17 *AhSTP* genes and 17 *GmSTP* genes in *G. max*. The number of orthologous pairs between peanut and these four species (*A. duranensis*, *A. ipaënsis*, *A. thaliana*, and *G. max*) was 28, 28, 7, and 44, respectively. Some *AhSTP* genes were associated with more than one gene pair, and this was especially common between peanut and soybean *STP* genes. For example, both *AhSTP14* and *AhSTP32* were associated with four *GmSTP* genes (*GmSTP5/9/23/24*).

Table 1
Information on the *STP* genes of peanut (*Arachis hypogaea*) and its two diploid ancestors (*Arachis duranensis* and *Arachis ipaënsis*).

Species	Name	Gene ID	Chromosome	AA	MW (kDa)	pI	AI	GRAVY	STP domain location	TMD
Arachis hypogaea	*AhSTP1*	*UYDT2Y*	A01	702	77.9	9.72	98.26	0.29	231–688	11
	AhSTP2	*N9JVQC*	A02	516	56.84	9.23	94.88	0.51	28–491	11
	AhSTP3	*8TG862*	A03	518	57.26	8.95	102.57	0.46	27–489	12
	AhSTP4	*C2XN8V*	A03	516	56.77	9.33	95.62	0.5	28–491	11
	AhSTP5	*HY7M04*	A03	868	95.03	8.31	97.56	0.23	379–842	11
	AhSTP6	*X11R4G*	A03	511	56.63	9.1	105.73	0.48	29–488	12
	AhSTP7	*255V2J*	A04	507	55.33	9.14	103.67	0.54	27–487	10
	AhSTP8	*A4MVXX*	A04	513	56.75	8.53	110.99	0.64	27–489	12
	AhSTP9	*3GMY5M*	A05	524	57.45	9.22	107.54	0.51	28–490	12
	AhSTP10	*95WRW2*	A05	488	53.95	9.22	103.07	0.54	25–466	11
	AhSTP11	*S5JADZ*	A06	494	54.5	6.45	103.24	0.55	26–464	11
	AhSTP12	*T4RTS6*	A06	489	53.93	6.46	101.7	0.53	26–459	11
	AhSTP13	*N55S1W*	A07	544	59.8	9.51	111.32	0.59	68–527	11
	AhSTP14	*6CD4KH*	A08	512	55.98	9.26	104.75	0.58	27–487	12
	AhSTP15	*JWC1SR*	A09	487	53.96	9.35	102.67	0.5	27–469	9
	AhSTP16	*QN8412*	A10	511	56.26	8.24	104.76	0.54	26–485	11
	AhSTP17	*RLH8R5*	A10	513	56.58	8.77	110.6	0.65	27–489	12
	AhSTP18	*UX2ZZF*	B01	502	54.84	9.46	105.12	0.54	31–488	11
	AhSTP19	*YZD8WZ*	B02	518	57.22	8.95	102.57	0.47	27–489	12
	AhSTP20	*5H82NF*	B03	1072	117.5	8.38	95.43	0.15	583–1046	10
	AhSTP21	*IG4CN6*	B03	397	44.02	9.18	98.01	0.56	1–376	9
	AhSTP22	*NIE1A8*	B03	511	56.38	9.01	105.93	0.49	29–488	12
	AhSTP23	*QP3LBN*	B03	461	50.58	8.62	108.11	0.59	9–435	10
	AhSTP24	*RIP12T*	B03	1069	117.14	8.31	95.43	0.14	580–1043	10
	AhSTP25	*I2HAHG*	B04	507	55.32	9.14	103.67	0.55	27–487	10
	AhSTP26	*H1FI0I*	B05	434	48.11	9	101.77	0.51	29–429	8
	AhSTP27	*HF3809*	B05	488	53.85	9.18	102.87	0.57	25–466	11
	AhSTP28	*P6VCNZ*	B05	524	57.5	9.23	107.92	0.52	28–490	12
	AhSTP29	*ANEC18*	B06	490	54.17	6.04	101.29	0.51	26–459	10
	AhSTP30	*U5XNSE*	B06	494	54.36	6.02	103.04	0.55	26–464	11
	AhSTP31	*ESBB0K*	B07	503	54.78	9.43	111.67	0.67	27–486	11
	AhSTP32	*F13FNW*	B07	509	55.62	9.27	105.36	0.6	27–487	12
	AhSTP33	*IX8CE2*	B09	458	51.03	9.18	99.17	0.47	27–440	9
	AhSTP34	*BV5KXL*	B10	526	59.11	9.33	103.61	0.42	51–502	11
	AhSTP35	*LAWA8U*	B10	511	56.23	8.21	104.19	0.56	26–485	11
	AhSTP36	*TN7L58*	B10	511	56.11	7.94	104.36	0.54	26–485	11
Arachis duranensis	*AdSTP1*	*Aradu.MFZ42*	A01	1208	135.36	8.57	102.48	0.42	737–1194	11
	AdSTP2	*Aradu.MI3PF*	A02	516	56.8	9.23	94.69	0.51	28–491	11
	AdSTP3	*Aradu.9EZ7Z*	A03	518	57.21	9.04	102.57	0.47	27–489	12
	AdSTP4	*Aradu.CR12A*	A03	511	56.67	9.1	106.3	0.49	29–488	12
	AdSTP5	*Aradu.ZK133*	A03	516	56.77	9.33	95.62	0.5	28–491	11
	AdSTP6	*Aradu.3CD7I*	A04	507	55.37	9.14	102.9	0.54	27–487	10
	AdSTP7	*Aradu.J9GQP*	A04	663	72.93	8.66	93.7	0.22	27–457	9
	AdSTP8	*Aradu.0GB26*	A05	524	57.47	9.23	108.47	0.52	28–490	12
	AdSTP9	*Aradu.6BH4S*	A05	510	56.42	9.24	104.75	0.58	25–488	11
	AdSTP10	*Aradu.EKK5E*	A06	494	54.41	6.89	103.83	0.5	26–464	10
	AdSTP11	*Aradu.HD3ZK*	A06	515	56.74	6.89	102.23	0.54	26–485	12
	AdSTP12	*Aradu.MEG9G*	A08	512	55.98	9.26	104.75	0.58	27–487	12
	AdSTP13	*Aradu.4FJ5L*	A09	508	56.35	9.41	104.57	0.52	27–490	11
	AdSTP14	*Aradu.EK0I3*	A10	508	55.89	8.21	103.27	0.53	26–482	11
	AdSTP15	*Aradu.YRM8T*	A10	513	56.58	8.65	110.6	0.65	27–489	12
Arachis ipaënsis	*AiSTP1*	*Araip.JJ9SB*	B01	1186	132.45	8.79	104.22	0.46	698–1172	11
	AiSTP2	*Araip.BY875*	B02	450	49.91	9.15	97.09	0.52	8–425	11
	AiSTP3	*Araip.HDT92*	B02	518	57.22	8.95	102.57	0.47	27–489	12
	AiSTP4	*Araip.ID0PF*	B03	511	56.38	9.01	105.93	0.49	29–488	12
	AiSTP5	*Araip.N7GAW*	B03	401	44.37	9.06	97.76	0.56	1–376	9
	AiSTP6	*Araip.VZ1YZ*	B03	476	52.41	8.71	106.34	0.56	1–450	11
	AiSTP7	*Araip.RBV5T*	B04	507	55.32	9.14	103.67	0.55	27–487	10
	AiSTP8	*Araip.MS5EY*	B05	510	56.26	9.2	105.31	0.6	25–488	11
	AiSTP9	*Araip.NI92H*	B05	548	60.31	9.23	94.14	0.32	27–447	10
	AiSTP10	*Araip.80YPT*	B06	517	56.72	5.92	103.37	0.49	26–487	11
	AiSTP11	*Araip.D4GZ4*	B06	516	57.08	6.37	102.02	0.53	26–485	11
	AiSTP12	*Araip.CN5UC*	B07	491	53.59	9.53	111.22	0.66	27–474	12
	AiSTP13	*Araip.NF9ZR*	B07	541	59.23	9.24	103.83	0.53	59–519	12
	AiSTP14	*Araip.4HV2H*	B09	454	50.53	9.11	98.55	0.47	27–436	9
	AiSTP15	*Araip.G26YQ*	B10	512	56.44	8.31	111.02	0.66	26–488	12
	AiSTP16	*Araip.I14ES*	B10	1181	129.97	8.38	106.4	0.58	26–429	12

aa: amino acid, MW: molecular weight, pI: theoretical isoelectric point, AI: aliphatic index, GRAVY: grand average of hydropathicity, TMD: protein transmembrane domain.

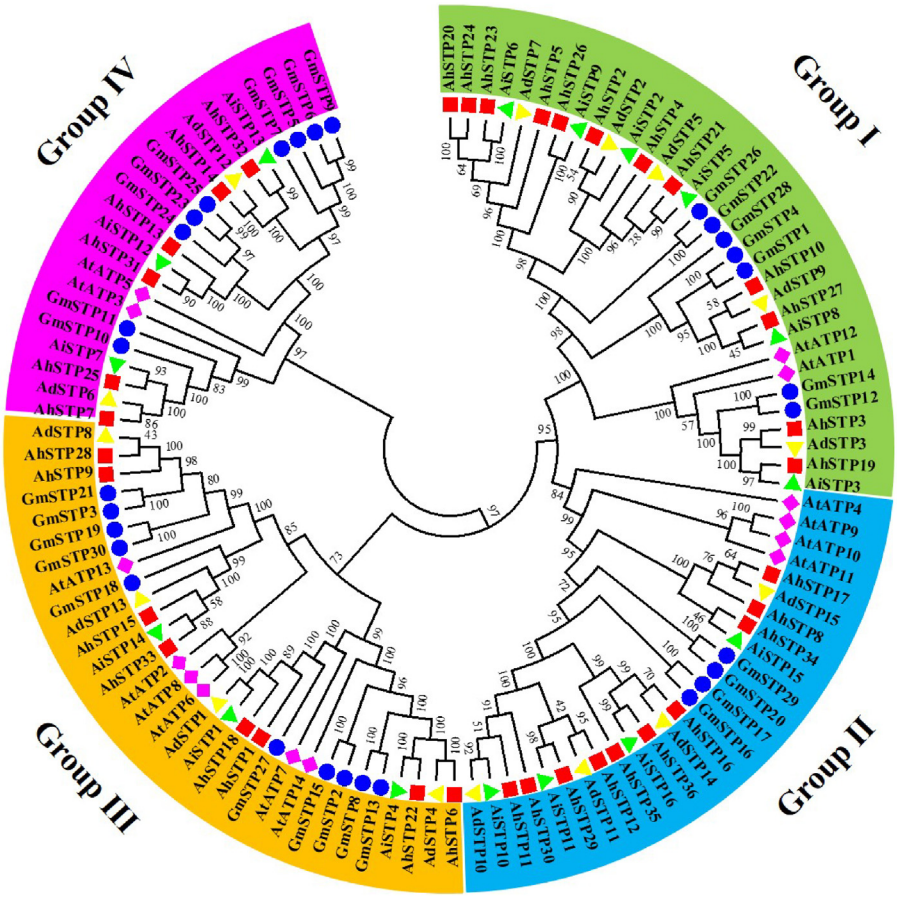

Fig. 1. Phylogenetic relationships among *STP* genes in peanut. A total of 111 STP proteins from peanut (AhSTP, 36), *Arabidopsis thaliana* (AtSTP, 14), *Glycine max* (GmSTP, 30), *Arachis duranensis* (AdSTP, 15), and *Arachis ipaënsis* (AiSTP, 16) were used to construct a neighbor-joining phylogenetic tree with MEGA7.0 software with 1000 bootstrap replicates. The tree was divided into four groups (Group I–Group IV). The STP proteins from peanut, *A. thaliana*, *G. max*, *A. duranensis*, and *A. ipaënsis* are indicated by red squares, yellow triangles, green triangles, purple diamonds, and blue circles, respectively.

Table 2
Twelve different motifs commonly observed in STP proteins in peanut.

Motif ID	Length	Protein squences	Pfam Domain
1	41	ICJYVAGFAWSWGPLGWLVPSEIFPLEIRSAGQSINVSVNM	Sugar_tr
2	29	VGFANQSVPJYLSEMAPYKYRGALNIGFQ	Sugar_tr
3	47	RQYEGKVTLFVIITCIVAAMGGLJFGYDJGISGGVTSMDPFLKKFFP	Sugar_tr
4	46	NQYCKFDSQVLTLFTSSLYLAALVASLFASTVTRAFGRRLSMILGG	
5	40	AAVPAILJCIGAJFLPDTPNSLIERGQHEKAKKMLQKIRG	Sugar_tr
6	41	LSMLCHFKFGLFIFFAAWVLVMTIFIYFLLPETKGVPIEEM	Sugar_tr
7	41	TLVSIFTVDKFGRRKLFLEGGAQMFICQIVIGAAIGSKFGD	Sugar_tr
8	49	DEEFQDLVDASEAASKVKHPWKNJLKRRYRPQLVMAIFIPFFQQLTGIN	Sugar_tr
9	29	FYAPVLFTTJGFGSBASLMSAVITGGVNV	
10	29	JFFLVGALLNGFAQNVAMLIIGRILLGFG	
11	21	ITIGILVANLJNYFTAKIKNG	
12	21	NYVWKSHWFWNKFVPSDSVLV	

Six *AhSTP* genes (*AhSTP3/15/19/32/33/35*) were collinear among all four species. Six *AhSTP* genes (*AhSTP5/16/18/20/26/36*) were not found collinear *STP* genes in the genomes of *A. duranensis* and *A. ipaënsis*, while Some *STP* genes in *A. duranensis* and *A. ipaënsis*, such as *AdSTP7/14* and *AiSTP9*, were not observed collinear STP genes in *A. hypogaea*.

Ka/Ks ratios of *STP* gene pairs were determined to further clarify the evolution of *STP* genes in peanut. Ka/Ks values were less than 1 for all segmental and tandem duplicated *AhSTP* gene pairs and most orthologous *STP* gene pairs between peanut and its two ancestors, suggesting that *STP* genes in peanut might have experienced strong purifying selection (Table 3, Table 4, and Table S7). Accoding to KS value, the divergence time of the *STP* gene pairs was estimated to clarify the origin of peanut *STP* genes. The divergence time of the majority orthologous *STP* gene pairs was predicted to nearly 0.81–9.95 MYA (Table 3 and Table S7). Meanwhile, some orthologous *STP* gene pairs were predicted to diverge much earlier. For example, the divergence time of six gene pairs (AhSTP9/AhSTP15, AhSTP9/AhSTP33, AhSTP13/AhSTP32, AhSTP13/AhSTP14, AhSTP15/AhSTP28, and AhSTP28/AhSTP33) was estimated to be around 93.17 to 103.25 MYA. Five orthologous *STP* gene pairs in the A sub-genome and 10 orthologous *STP* gene pairs in the B sub-genome were conserved during the evolution of allotetraploid peanut from its wild diploid ancestors (Table 4). *AhSTP25* (B04) and *AhSTP33* (B09) in the B sub-genome most recently diverged from the orthologous STP pairs *AdSTP6/AhSTP7* (A04) and *AdSTP13/AhSTP15* (A09) in the A sub-genome, respectively (Table 3, Fig. S1). No *STP* genes were detected on chromosome A07 in *A. duranensis*; however, *AhSTP13* was detected on chromosome A07 in *A. hypogaea*, and it most recently diverged from the orthologous *STP* pair *AiSTP12/AhSTP31* (B07) in the B sub-genome (Table 3, Fig. S1). The divergence between the tandem duplicated gene pair *AhSTP35/36* and the orthologous pair *AiSTP16/*

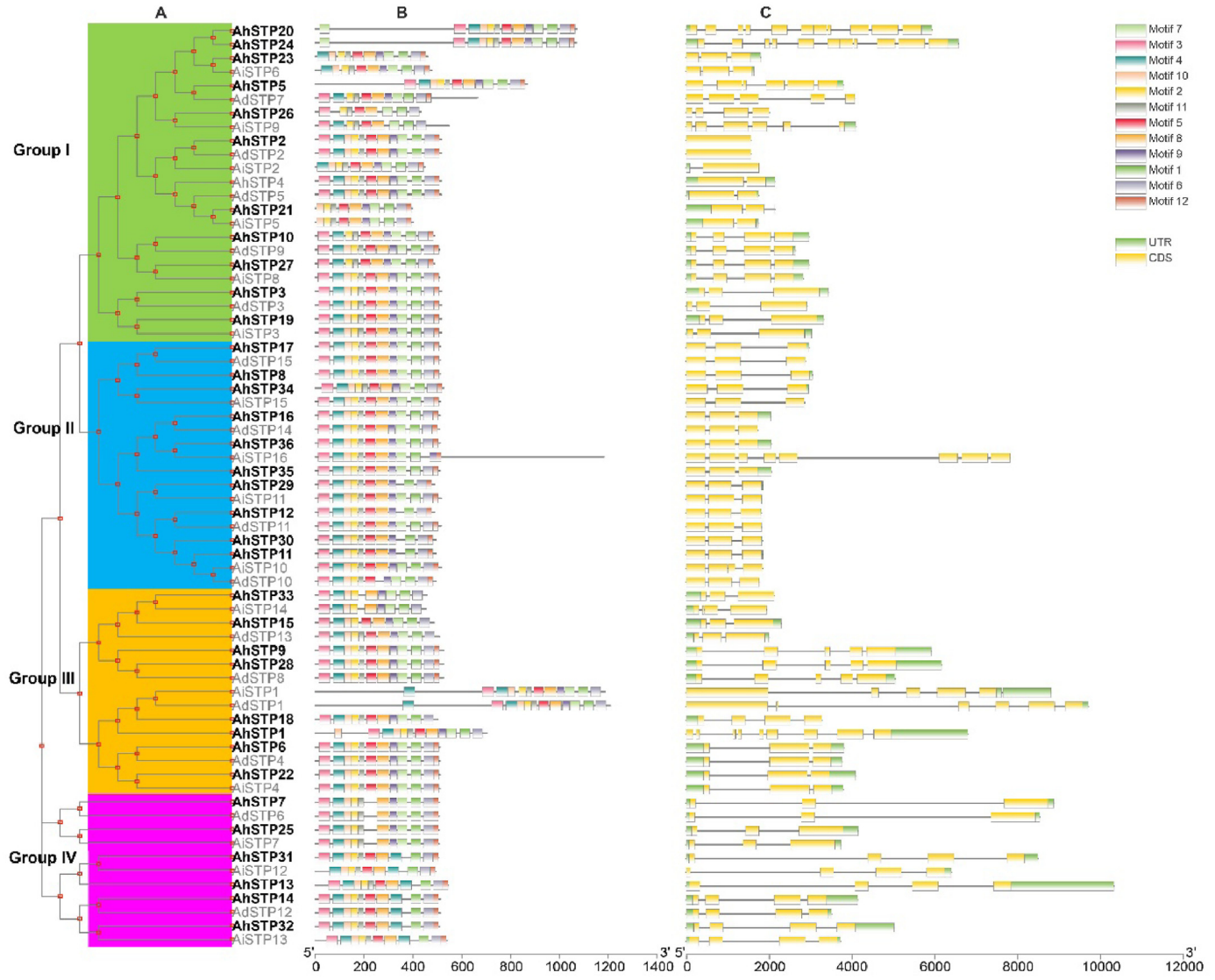

Fig. 2. Conserved motifs of STP proteins and the structure of *STP* genes in peanut. (A) An unrooted phylogenetic tree was built using MEGA 7.0 with the full-length STP protein sequences of peanut and its two diploid ancestors *Arachis duranensis* and *Arachis ipaënsis*. AhSTPs are shown in bold font. (B) Conserved motifs of peanut STP proteins. The motifs were predicted by the MEME program (http://meme-suite.org/tools/meme), and the maximum number of motifs was set to 12. Twelve conserved motifs are shown by different color blocks, with one color corresponding to one motif. (C) The exon–intron structure of peanut *STP* genes was analyzed using TBtools software. Green blocks indicate the 5′- and 3′-untranslated regions. Yellow blocks indicate exons, and black lines correspond to introns.

AhSTP35 occurred much earlier than the divergence between the A and B sub-genome according to estimates in previous studies (Yin et al., 2020; Zhuang et al., 2019), which indicates that the origin of these genes is complex.

3.5. Expression profiles of STP genes in peanut

The expression profiles of *AhSTP* genes were determined using transcriptome data from 'Tifrunner' to clarify the potential functions of AhSTPs (Clevenger et al., 2016). The relative expression levels of *AhSTP* genes varied extensively among tissues and developmental stages; expression patterns were similar among most *AhSTP* genes within the same subgroup of phylogenetic tree (Fig. 4, Table S8). In Group I, *AhSTP* genes showed distinct expression patterns. For example, seven *AhSTP* genes (*AhSTP*2/4/10/2124/26/27), showed relatively high expression in reproductive shoot tip, and three (*AhSTP*2/26/27) of them also exhibited substantial high expression in vegetative shoot tip. While, the remaining five genes (*AhSTP3/5/19*/20/23) were differentially expressed. *AhSTP* genes in Group II showed highly similar expression patterns and were preferentially expressed in pistil tissue, but these *AhSTP* genes were expressed at low levels in other tissues (Fig. 4). In Group III and IV, most of the *AhSTP* genes grouped in pairs exhibited similar expression. For instance, *AhSTP* 15 and *AhSTP* 33 in the same clade of Group III, showed similar expression across most tissues, as well as *AhSTP* 14 and *AhSTP* 32 in Group IV. In addition, the four most highly expressed *AhSTP* genes across all 22 tissues were *AhSTP3*, *AhSTP9*, *AhSTP19*, and *AhSTP28*, which had similar expression patterns across all tissues and developmental stages and belonged to Group I and Group III, respectively. (Fig. S2 and Fig. 4).

Data on the expression levels of all 36 *AhSTP* genes were used to identify genes associated with pod development. In 'Tifrunner' (subsp. *hypogaea*), the four highly expressed genes *AhSTP3*, *AhSTP9*, *AhSTP19*, and *AhSTP28* were highly expressed in the fruit pod (Peg tip Pat., Fruit Pat.1, and Fruit Pat. 3) and pericarp (Pericarp Pat. 5 and Pericarp Pat. 6); however, the expression of these genes was down-regulated during mature pod (seed) formation (Fig. 4). RNA-seq data of the four genes in the other peanut cultivar 'ICGV 91114' (subsp. *fastigiate*) revealed that the expression patterns of these four *AhSTP* genes were distinct (Sinha

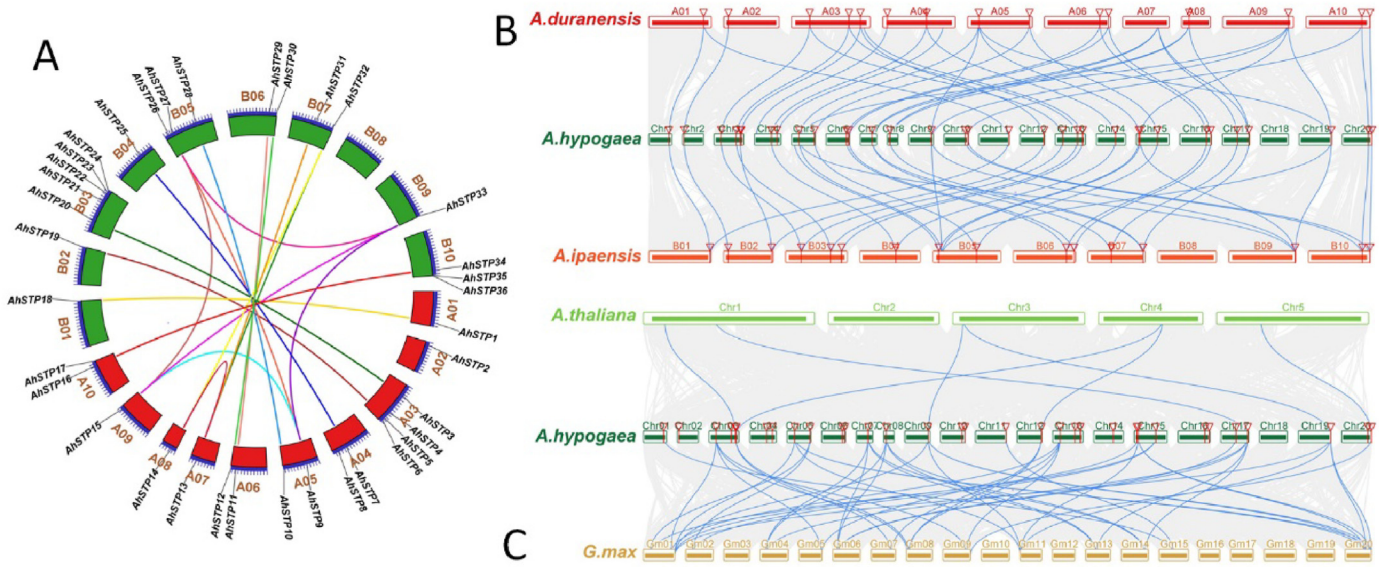

Fig. 3. Gene duplication and synteny analysis of *STP* genes in peanut. (A) Duplication events of *STP* genes in peanut. Red and green bars indicate chromosomes of the A sub-genome and B sub-genome, respectively. Gene names are labeled based on their position in the peanut genome. Segmental duplicated gene pairs are linked by lines of different color, with one color corresponding to one gene pair. The colinear blocks between different chromosomes are indicated by gray lines in the background. (B) Synteny analysis of *STP* genes between cultivated peanut and its two diploid ancestors. *STP* genes are indicated by red inverted triangles based on their positions in the genome. Blue lines correspond to syntenic *STP* gene pairs, and gray lines in the background correspond to collinear blocks between peanut and its two diploid ancestors. (C) Synteny analysis of *STP* genes in peanut, *Arabidopsis thaliana*, and *Glycine max*. The red inverted triangles correspond to *AhSTP* genes. Blue lines indicate syntenic *STP* gene pairs, and gray lines in the background indicate collinear blocks between cultivated peanut and *A. thaliana* and between peanut and *G. max*.

Table 3
Rate of non-synonymous substitutions (Ka) and synonymous substitutions (Ks) of orthologous *STP* gene pairs in peanut.

Orthologous pairs		Ks	Ka	Ka/Ks	Divergence time (MYA)
AhSTP1	*AhSTP18*	0.0403	0.0021	0.0521	2.4543
AhSTP3	*AhSTP19*	0.1619	0.0062	0.0383	9.8599
AhSTP6	*AhSTP22*	0.1634	0.0127	0.0777	9.9513
AhSTP7	*AhSTP25*	0.0276	0.0042	0.1522	1.6809
AhSTP9	*AhSTP15*	1.6317	0.2230	0.1367	99.3727
AhSTP9	*AhSTP28*	0.0332	0.0021	0.0633	2.0219
AhSTP9	*AhSTP33*	1.6955	0.2183	0.1288	103.2582
AhSTP10	*AhSTP27*	0.0478	0.0063	0.1318	2.9111
AhSTP11	*AhSTP30*	0.0655	0.0062	0.0947	3.9890
AhSTP12	*AhSTP29*	0.1133	0.0104	0.0918	6.9001
AhSTP13	*AhSTP14*	1.5043	0.2242	0.1490	91.6139
AhSTP13	*AhSTP31*	0.0326	0.0107	0.3282	1.9854
AhSTP13	*AhSTP32*	1.5451	0.2225	0.1440	94.0987
AhSTP14	*AhSTP32*	0.0541	0.0085	0.1571	3.2948
AhSTP15	*AhSTP28*	1.5299	0.2258	0.1476	93.1730
AhSTP15	*AhSTP33*	0.0134	0.0042	0.3134	0.8161
AhSTP17	*AhSTP34*	0.0597	0.0021	0.0352	3.6358
AhSTP28	*AhSTP33*	1.5847	0.2210	0.1395	96.5104
AhSTP35	*AhSTP36*	0.1501	0.0199	0.1326	9.1413

et al., 2020). The expression of these genes was low in peg, pod shell, seed_5, and seed_15 and high during the mature stage of the seed (Fig. S3). These findings suggest that these genes might play a role in the construction and development of the pod. qRT-PCR analysis of these genes in five tissues (peg, pod shell, seed_5, seed_10, and seed_25) of two cultivars (subsp. *hypogaea* 'ZPG13' and subsp. *fastigiate* 'Xiaobaisha') revealed that the expression levels of the four genes were different, and these differences were consistent with the expression patterns of these genes in 'Tifrunner' and 'ICGV 91114', respectively. (Fig. 5). The different expression patterns of AhSTP genes among cultivars suggest that these genes might participate in pod construction and development of different cultivars via different ways.

Table 4
Rate of non-synonymous substitutions (Ka) and synonymous substitutions (Ks) of nearest orthologous *STP* gene pairs between peanut and its two diploid ancestors (*Arachis duranensis* and *Arachis ipaënsis*).

Genome	orthologous pairs		Ks	Ka	Ka/Ks	Nearest divergence time (MYA)
AA	*AdSTP1*	*AhSTP1*	0.0000	0.0000	–	0.0000
	AdSTP2	*AhSTP2*	0.0286	0.0021	0.0734	1.7418
	AdSTP3	*AhSTP3*	0.0140	0.0021	0.1500	0.8526
	AdSTP4	*AhSTP6*	0.0203	0.0000	0.0000	1.2363
	AdSTP5	*AhSTP4*	0.0000	0.0000	–	0.0000
	AdSTP6	*AhSTP7*	0.0000	0.0000	–	0.0000
	AdSTP6	*AhSTP25*	0.0276	0.0042	0.1522	1.6809
	AdSTP8	*AhSTP9*	0.0263	0.0021	0.0798	1.6017
	AdSTP8	*AhSTP28*	0.0197	0.0042	0.2132	1.1998
	AdSTP9	*AhSTP10*	0.0202	0.0021	0.1040	1.2302
	AdSTP10	*AhSTP11*	0.0201	0.0066	0.3284	1.2241
	AdSTP11	*AhSTP12*	0.0286	0.0041	0.1434	1.7418
	AdSTP12	*AhSTP14*	0.0000	0.0000	–	0.0000
	AdSTP13	*AhSTP15*	0.0000	0.0000	–	0.0000
	AdSTP13	*AhSTP33*	0.0134	0.0042	0.3134	0.8161
	AdSTP15	*AhSTP17*	0.0441	0.0021	0.0476	2.6857
BB	*AiSTP3*	*AhSTP19*	0.0000	0.0000	–	0.0000
	AiSTP4	*AhSTP22*	0.0000	0.0000	–	0.0000
	AiSTP5	*AhSTP21*	0.0000	0.0000	–	0.0000
	AiSTP6	*AhSTP23*	0.0000	0.0000	–	0.0000
	AiSTP7	*AhSTP25*	0.0000	0.0000	–	0.0000
	AiSTP8	*AhSTP27*	0.0000	0.0000	–	0.0000
	AiSTP10	*AhSTP30*	0.0284	0.0041	0.1444	1.7296
	AiSTP11	*AhSTP29*	0.0070	0.0021	0.3000	0.4263
	AiSTP12	*AhSTP31*	0.0000	0.0000	–	0.0000
	AiSTP12	*AhSTP13*	0.0326	0.0107	0.3282	1.9854
	AiSTP13	*AhSTP32*	0.0000	0.0000	–	0.0000
	AiSTP14	*AhSTP15*	0.0134	0.0042	0.3134	0.8161
	AiSTP14	*AhSTP33*	0.0000	0.0000	–	0.0000
	AiSTP15	*AhSTP34*	0.0000	0.0000	–	0.0000
	AiSTP16	*AhSTP35*	0.1501	0.0199	0.1326	9.1413

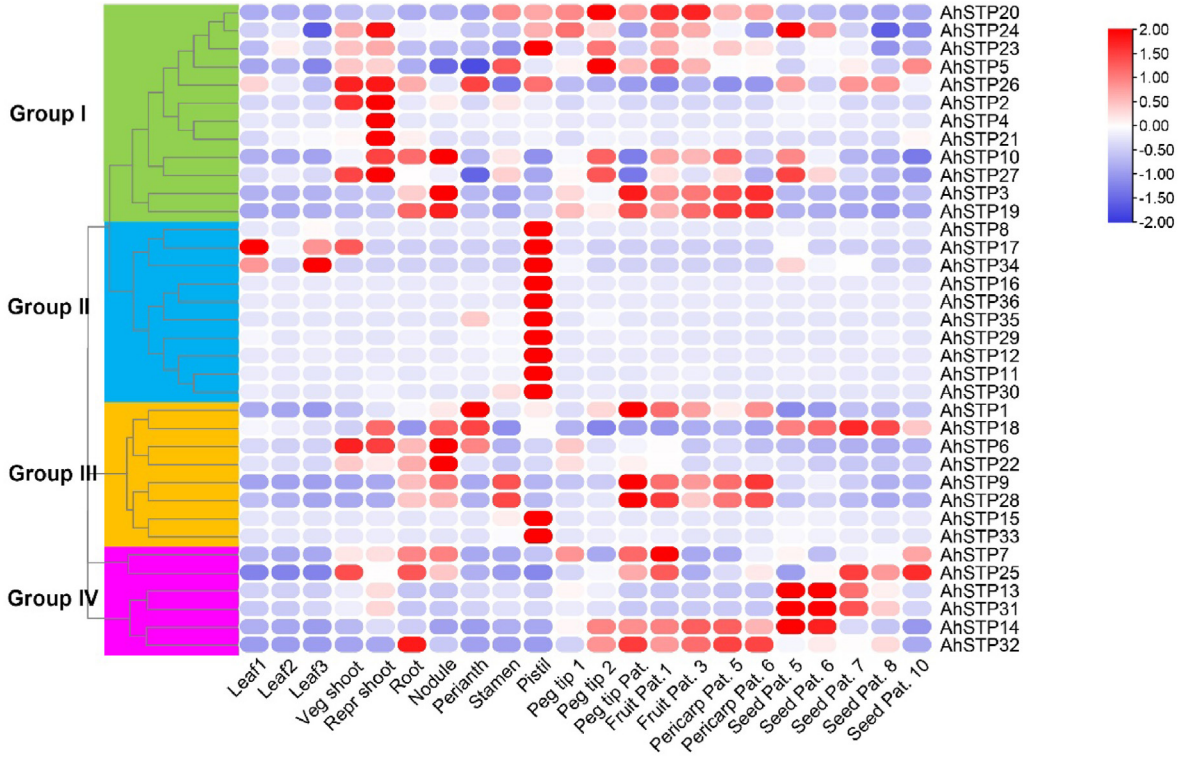

Fig. 4. Expression profiles of peanut *STP* genes in various tissues and at different development stages. The heatmap was built using log2 based FPKM values, which were scaled per row using TBtools software. Red indicates high expression, and blue indicates low expression. An unrooted phylogenetic tree was built using MEGA 7.0 with the full-length STP protein sequences of peanut. Detailed information on the samples is provided in Table S3 and Table S8.

3.6. Expression patterns of peanut STP genes in response to hormone and abiotic stress treatments

The expression patterns of *AhSTP* genes under different hormonal and abiotic stress treatments were analyzed using transcriptome data downloaded from the Peanut Genome Resource (Zhuang et al., 2019). The 36 *AhSTP* genes exhibited distinct expression patterns in response to seven treatments (ABA, BR, PAC, ethephon, SA, low temperature, and drought) (Fig. 6). The expression of three *AhSTP* genes (*AhSTP2/16/35*) was not observed in all treatments. Under low-temperature treatment, the expression of 18 *AhSTP* genes was induced obvious up-regulated, and the expression of 10 *AhSTP* genes was substantial down-regulated. The expression of some genes was up-regulated under ABA, BR, PAC, ethephon, and drought treatment (Fig. 6). For example, the expression of six *AhSTP* genes (*AhSTP1/11/12/23/24/30*) was specifically up-regulated by drought, and the expression of two (*AhSTP21/36*), one (*AhSTP31*), four (*AhSTP8/17/26/34*), five (*AhSTP4/10/14/30/31*), and five (*AhSTP13/1723/24/29*) *AhSTP* genes was distinct up-regulated by ABA, BR, ethephon, PAC, and SA, respectively. The expression of some *AhSTP* genes was down-regulated by these treatments. For example, the expression of *AhSTP5/20* was significantly down-regulated under ABA, BR, and PAC treatment.

3.7. Gene variation analysis of AhSTP3/9/18/28

Allelic variation, sequence variation, and promoter sequence variation of *AhSTP3/9/19/28* were studied to explore the causes underlying variation in their expression profiles during pod construction and development in different peanut cultivars. Genic-SSR markers were developed for *AhSTP9/19/28* and used to genotype the 37 cultivars of the subspecies *hypogaea* and *astigiate*. No allelic variation or substantial structural variation in the three genes among the different cultivars was detected. *AhSTP9/19/28* sequences cloned from 'Xiaobaisha' and 'ZPG13' were similar; however, two single-base non-synonymous substitutions were identified in the coding region sequence, and these substitutions resulted in changes in amino acids and the secondary structures of proteins (Fig. S4 and Fig. S5). Promoter region analysis revealed that the single-base substitution in the *AhSTP3* promoter region was the cause of the AT1-motif deletion in subsp. *fastigiate*, which is an important component of the light-responsive module, and a single-base substitution and four-base insertion and deletion in *AhSTP19* resulted in the deletion of the CAAT-box, TATA-box, and AT–TATA-box (Table S9). No variation was detected in the promoter regions of *AhSTP9* and *AhSTP28*. These findings indicate that the regulation of the expression of these genes is complex.

4. Discussion

Since the first *STP* gene was reported in *Chlorella* (Sauer et al., 1990), over 100 homologs have been identified and characterized in various plant species (Liu et al., 2018; Zhang et al., 2019). In our study, a total of 36 *STP* genes were identified in peanut genome, including 17 members in the A sub-genome and 19 members in the B sub-genome. The 36 *AhSTP* genes were clustered into four groups by phylogenetic analysis, and this was consistent with the classification of the *STP* gene family in other plants, such as Arabidopsis (Büttner, 2010), rice (Toyofuku et al., 2000), cassava (Liu et al., 2018), and cabbage (Zhang et al., 2019). *STP* genes have undergone an expansion in peanut relative to other dicot plants such as soybean; specifically, two and three *AhSTP* genes were gained in the A and B sub-genomes respectively, following the divergence of peanut from its diploid ancestors *A. duranensis* and *A. ipaënsis*. The number of *STP* genes in *A. duranensis* (15 *AdSTP* genes) and *A. ipaënsis* (16 *AiSTP* genes) is similar to the number of *STP* genes in the model plant Arabidopsis (14 *AtSTP* genes) but less than half the number of *STP* genes in peanut and soybean (Büttner, 2010). These findings indicate that the expansion of *STP* genes was driven by whole-genome duplication (WGD) and polyploidization events.

In this study, 18 segmental duplication events and one tandem

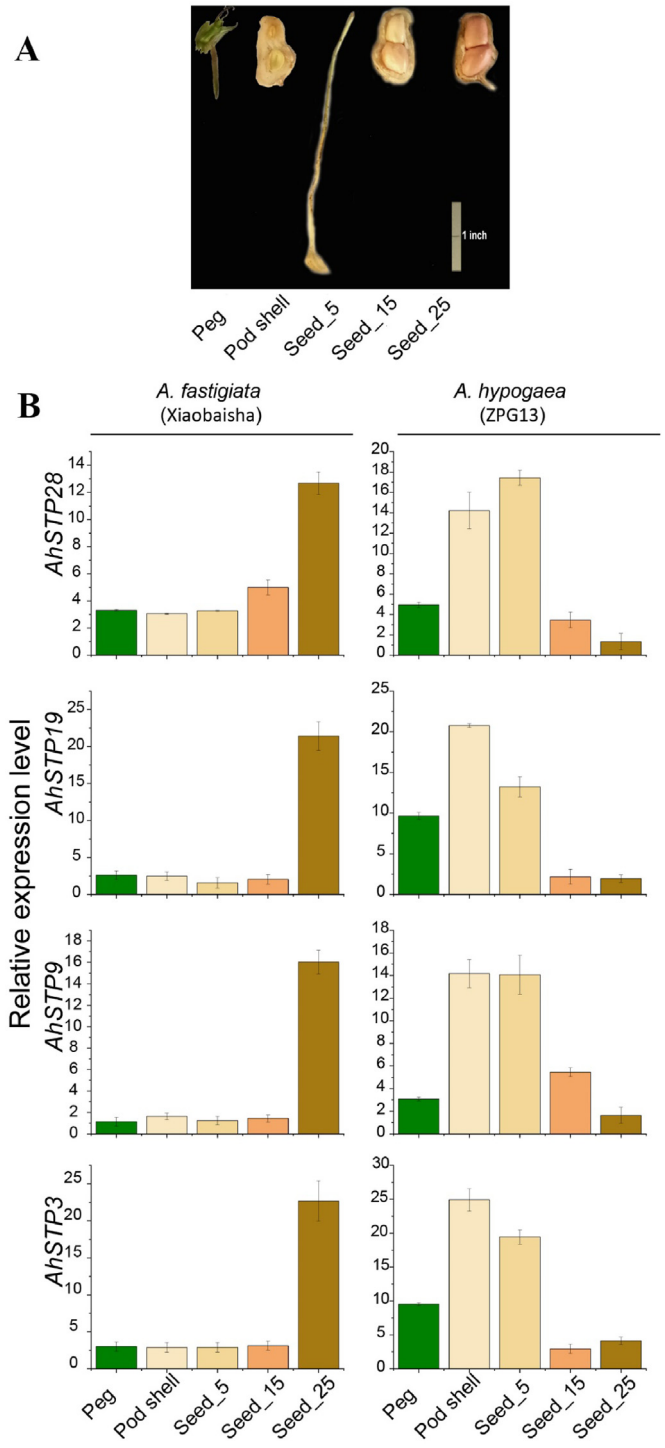

Fig. 5. qRT-PCR analysis of the relative expression of *AhSTP3/9/19/28* during pod formation and development in two peanut cultivars. (A) Five tissues during pod formation and development. (B) The two cultivars 'Xiaobaisha' and 'ZPG13' belong to subsp. *fastigiata* and subsp. *hypogaea*, respectively. The *actin* gene was used as the reference gene. The relative expression levels of the four genes were calculated using the $2^{-\triangle\triangle Ct}$ method. The value of each column corresponds to mean ± standard deviation based on three biological replicates.

duplication event were identified in peanut. The findings indicated that some *AhSTP* genes might have been generated by duplication events and that segmental duplication has played an important role in driving the evolution of *AhSTP* genes (Xie et al., 2018). Synteny analysis also provided evidence of gene gain and loss during polyploid speciation in *A. hypogaea*, as reporting in many other plants (Xie et al., 2018; Zhang et al., 2019). The Ka/Ks values of the 19 duplicated gene pairs were less than 1, indicating that *STP* genes in peanut have been subjected to purifying selection. Previous studies have indicated that at least three WGD events have occurred during the evolution of peanut based on measurements of the Ks values of orthologous gene pairs within collinear blocks (Wang et al., 2021). The WGD events include a core eudicot paleohexaploid event nearly 130 million years ago (Mya), a more recent pan-legume tetraploidization event shared with soybean that occurred approximately 58 Mya (Young et al., 2011; Schmutz et al., 2010), and an *Arachis* linkage-specific genome duplication event approximately 3.5 Mya (Chen et al., 2020; Bertioli et al., 2016). Analysis of *AhSTP* gene duplication events revealed 12 orthologous *STP* gene pairs that were associated with the *Arachis* linkage-specific genome duplication event approximately 3.5 Mya, and six gene pairs (*AhSTP9/AhSTP15*, *AhSTP9/AhSTP33*, *AhSTP13/AhSTP32*, *AhSTP13/AhSTP14*, *AhSTP15/AhSTP28*, and *AhSTP28/AhSTP33*) were associated with a core eudicot paleohexaploid event approximately 130 Mya. These findings indicate that *STP* genes in peanut have undergone expansions during the core eudicot paleohexaploid event and the *Arachis* linkage-specific duplication event.

Following the formation of allotetraploid peanut, the A and B subgenomes have undergone asymmetric exchanges of homoeologous sequences (or homeologous chromosome rearrangements), gene family expansions and contractions, homoeolog expression divergence, and selection (Yin et al., 2020; Zhuang et al., 2019; Bertioli et al., 2019). In our study, no non-synonymous substitutions were observed among five orthologous *AhSTP/AdSTP* pairs in the A sub-genome and 10 orthologous *AhSTP/AiSTP* pairs in the B sub-genome, indicating that *AhSTP* genes were more conserved in the B sub-genome than in the A sub-genome. This might stem from the effects of human domestication, which have stronger effects on homoeologous structural variation in genes of the A sub-genome in peanut according to Yin et al. (2020). Exchanges of genes (and chromosome rearrangements) between the sub-genomes were also observed in this study (Bertioli et al., 2019); the divergence time of *AhSTP25* (B04), *AhSTP33* (B09), and *AhSTP13* (A07) from the nearest orthologous pairs suggested that these genes were likely derived from the homeologous chromosomes A04, A09, and B07. All these findings indicate that *AhSTP* genes in the A sub-genome have undergone more changes since the divergence of peanut from its ancestor *A. duranensis*, suggesting that these genes play key roles in adaptation in plants (Bertioli et al., 2019; Yin et al., 2020).

Analysis of the expression profiles and evolution of *STP* genes could provide insights into their roles in pod formation and development. In our study, the expression of the generally highly expressed *AhSTP* genes (*AhSTP3/9/19/28*) in 'Tifrunner' and 'ICGV 91114' were the exact opposite. Specifically, the expression of these genes was opposite in the immature and mature pod stage in the two cultivars. In watermelon, the differential expression patterns of *STP* genes in different accessions was also observed during fruit development, and these genes mediate the transport and accumulation of sugars and organic acids (Umer et al., 2020). While, in Arabidopsis, *AtSTP12*, an ortholog of *AhSTP3* and *AhSTP19*, is high expressed in the seed immature stage, but its expression gradually decreases during the formation of mature seeds (Büttner 2010). The variable expression levels of the four *AhSTP* genes, which mediate the transport and accumulation of sugars, during pod construction and development suggest that they play important roles in pod development in peanut.

Gene expression patterns are affected by various factors and variation in genes likely has the most direct effect on gene expression patterns (Qin et al., 2009). For example, allelic variation, sequence variation, and promoter sequence variation all can alter the expression patterns and function of genes (Li et al., 2004; Shastry, 2009; Taylor et al., 2019). Gene variation analysis of four highly expressed genes in two cultivars ('Xiaobaisha' and 'ZPG13'), revealed base substitutions in the coding

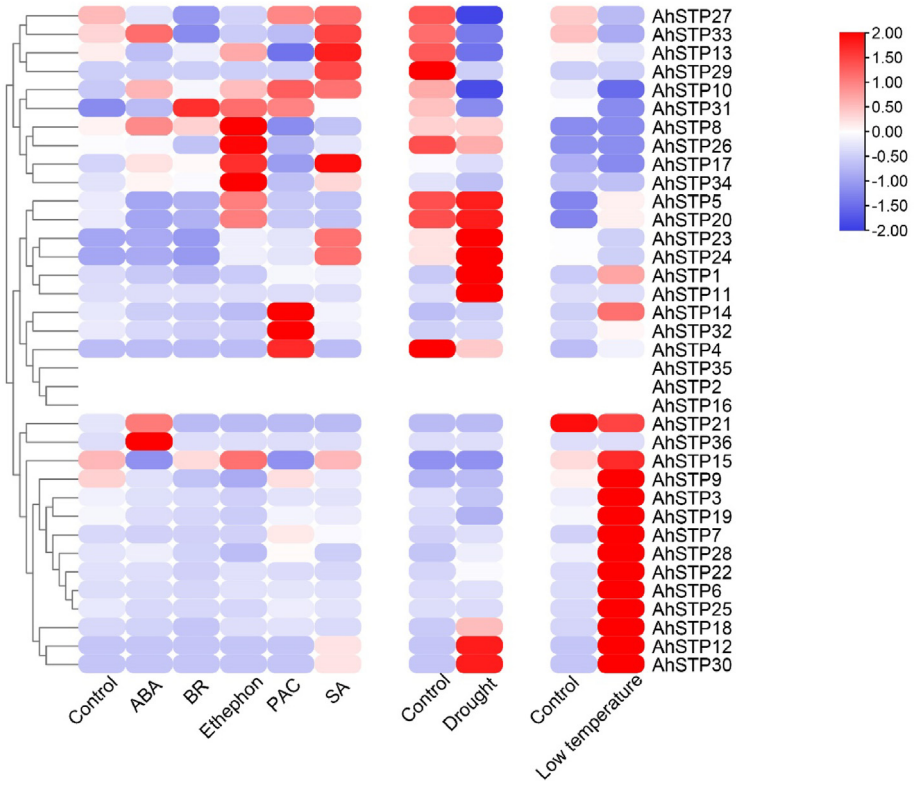

Fig. 6. Expression profiles of *STP* genes in peanut under different hormone and abiotic stress treatments. The heatmap was built using log2 based FPKM values, which were scaled per row using TBtools software. Red indicates high expression, and blue indicates low expression.

sequences and promoter regions of these four genes. However, these sequence changes have not occurred simultaneously in these genes, and these changes are unable to explain the distinct expression patterns among all four genes. Thus, sequence changes in the coding sequence, intron, and promoter regions are likely not the main drivers of the different expression patterns of these four genes in peanut pods. Additional studies are needed to determine the functions of these four genes, elucidate the regulatory mechanisms underlying the expression of these genes, as well as clarify whether the diverse expression patterns of these genes are associated with divergence among peanut subspecies.

Funding

This work was funded by Guangdong Basic and Applied Research Foundation, Grant Number 2020A1515010636, and Central public-interest Scientific Institution Basal Research Fund for Chinese Academy of Tropical Agricultural Sciences, Grant Number 1630102017002 and 1630102017006.

Declarations

The authors declare no conflict of interest.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgments

We thank TopEdit (www.topeditsci.com) for linguistic assistance during the preparation of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ocsci.2022.11.002.

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