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Genome-wide identification and expression analysis of *STP* gene family in *Malus domestica*

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Abstract

The sugar transporter protein (*STP*) gene family serves a pivotal role in monosaccharide translocation, plant growth, and developmental processes. Utilizing the *Arabidopsis thaliana STP* gene family as a reference, comprehensive analyses were conducted on apple (*Malus domestica*) *STP* genes, encompassing sequence alignment, physicochemical characterization, gene structure organization, conserved motif identification, and tissue-specific expression profiling. Quantitative real-time PCR (qRT-PCR) was employed to investigate the hormone-responsive expression patterns of the apple *STP* gene family. A total of 30 *STP* genes were identified, encoding polypeptides ranging from 235 to 655 amino acids in length. Gene structure analysis revealed variations in exon number and positional distribution among different *STP* genes, indicative of functional diversification. Cis-regulatory element analysis demonstrated the presence of core promoter elements, enhancer sequences, and regulatory motifs associated with hormone signaling, abiotic stress responses, organ-specific expression, and light regulation. Microarray-based expression profiling indicated that the majority of *STP* genes exhibited preferential expression in seeds, seedlings, roots, and stems. Notably, *MdSTP3*, *MdSTP13*, and *MdSTP23* were identified as the predominant isoforms expressed in apple fruits, with their transcriptional activity being positively modulated by *MdERF* transcription factors. qRT-PCR validation further elucidated the differential responsiveness of the 30 *MdSTP* genes to indole-3-acetic acid (IAA), gibberellic acid (GA), and abscisic acid (ABA) treatments, with IAA exerting a pronounced upregulatory effect on *STP* gene expression. Collectively, these findings underscore the functional heterogeneity of the apple *STP* gene family in terms of tissue-specific expression and hormonal regulation, thereby providing a robust foundation for elucidating the molecular mechanisms underlying STP-mediated growth and developmental processes in plants.

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Introduction

Sugars, including monosaccharides, sucrose, and polyols, serve as fundamental metabolic precursors for carbohydrate synthesis, energy substrates, cellular osmolytes, and signaling molecules that regulate developmental processes and stress responses in plants[1–4]. Functioning as central metabolic hubs, sugars integrate diverse biochemical pathways encompassing carbon metabolism, lipid metabolism, protein turnover, secondary metabolite biosynthesis, and nucleotide metabolism, thereby playing essential roles in plant growth and development. These compounds are primarily synthesized in source organs (e.g., leaves) and subsequently translocated through the phloem to sink tissues (e.g., seeds, fruits) via either symplastic (plasmodesmata) or apoplastic (transmembrane) pathways, with the latter requiring specialized sugar transporters[5,6]. The source-to-sink sugar flux is mediated by two major transporter families: monosaccharide transporters (MSTs) and sucrose transporters (SUTs)[7,8]. Following apoplastic sucrose hydrolysis, the resulting hexoses are imported into sink cells by sugar transporter proteins (STPs) and hexose transporters (HTs)[9,10]. STPs, belonging to the MST superfamily, function as H^+-coupled symporters typically containing 12 transmembrane domains that facilitate the transport of various monosaccharides, including fructose, glucose, galactose, mannose, and xylose across plasma membranes[11,12]. Since the initial cloning of an *STP* gene from Chlorella in 1989[13], genome sequencing has enabled the identification of *STP* gene families in numerous plant species, revealing both conserved features and species-specific adaptations in their expression patterns and substrate specificities[14–17]. For instance, while most STPs exhibit broad substrate ranges, certain isoforms like cassava *MeSTP4* and *MeSTP10* demonstrate exclusive specificity for maltose transport[18,19]. Tissue-specific expression patterns are particularly evident in *Arabidopsis*, where *AtSTP1* shows ubiquitous expression[20], *AtSTP4* localizes to root tips and pollen[21], and *AtSTP2/6/9/11* display pollen-specific expression. Similarly, rice *OsMST3* is uniquely expressed in root xylem, where it contributes monosaccharides for secondary cell wall biosynthesis[22]. These findings collectively highlight the functional diversification of STP family members in mediating spatial and temporal regulation of sugar allocation, with important implications for understanding carbon partitioning and sink strength modulation in plants.

Sugars constitute the fundamental carbon skeletons for plant metabolic processes and biomass accumulation. Under escalating environmental stresses, plants encounter substantial energetic challenges in maintaining homeostasis, necessitating a delicate trade-off between growth-related energy expenditure and stress adaptation mechanisms. Phytohormones, as master regulators of plant growth, development, and stress responses, play pivotal roles in modulating sugar metabolism under adverse conditions. The sugar transporter protein (STP) family, as crucial facilitators of monosaccharide transport, exhibits hormone-responsive regulation patterns. For instance, STP1 demonstrates significant induction upon treatment with defense-related phytohormones including salicylic acid (SA) and methyl jasmonate (MeJA)[23]. Comprehensive studies in pepper

(*Capsicum annuum* L.) reveal tissue-specific regulatory patterns: six-hour treatments with abscisic acid (ABA), indole-3-acetic acid (IAA), and gibberellins (GA) upregulate *CaSTP5/6/9/10/17* expression in root tissues, while foliar responses show contrasting regulation - with CaSTP6 being significantly induced by ABA and IAA, whereas *CaSTP5/8/9/10* are markedly suppressed[24]. These findings underscore the potential involvement of STP-mediated sugar transport in plant adaptive responses to environmental perturbations, highlighting their importance in stress-responsive metabolic reprogramming.

Sugars represent critical determinants of fruit quality in apples, with their accumulation being regulated through a coordinated series of physiological processes, including photosynthetic carbon fixation in source leaves, phloem-mediated translocation, apoplastic/symplastic transport within fruit tissues, and vacuolar sequestration[25]. As such, sugar transporter proteins (STPs) serve as pivotal regulators governing fruit sugar accumulation dynamics. The present investigation employs integrated bioinformatics approaches coupled with qRT-PCR-based hormone induction assays to systematically characterize the STP gene family, with a particular focus on gene architecture, subcellular localization, cis-regulatory elements, and spatiotemporal expression profiles. These comprehensive analyses establish a robust molecular foundation for elucidating the functional specialization of STP family members and provide a scientific framework for targeted genetic improvement of fruit quality traits.

Materials and methods

Materials and processing

The experiment was carried out from October 2023 to April 2024, and the test material was tissue-cultured 'gala' apple seedlings. Connect apple single bud stems to MS medium (MS + 30 g·L^{-1} sucrose + 6 g·L^{-1} agar + 0.5 mg·L^{-1} 6-BA + 0.05 mg·L^{-1} NAA, PH adjusted to 5.8–6.0), placed in an artificial climate box (RDN-1000D-4 type) pre-cultured for 40 d (temperature 25 °C, light intensity 8,000 lx, photoperiod 16/8 h), and then choose to grow robust and without contaminated apple tissue culture seedlings are used for treatment. This experiment was treated with 10 mmol·L^{-1} IAA, 50 mmol·L^{-1} GA, 50 mmol·L^{-1} ABA solution stress for 0, 4, 8, 12, and 24 h, and each treatment was repeated three times[26]. A sample of 0.1 g (apple leaves) was collected and weighed, wrapped in tin foil and quickly frozen in liquid nitrogen, and then the total RNA of apple leaves was extracted by the CTAB method[27,28].

Identification of apple *STP* gene family members

Fourteen *Arabidopsis STP* gene accession numbers were obtained from known literature[12]. Enter the accession number of *Arabidopsis STP* in NCBI and obtain the CDS sequence and amino acid sequence corresponding to each gene. Enter the obtained CDS fragments into the Rosaceae database GDR (www.rosaceae.org) for BLAST alignment, the screening condition E ≤ 1e -10, and perform DNAMAN alignment on the obtained genes to remove redundant sequences. Using the characteristic domain of the *STP* gene family in the literature (Pfam: PF00083), through SMART (http://smart.embl-heidelberg.de) and Pfam (http://pfam.sanger.ac.uk) online software, all predicted proteins are tested for structural integrity, and members that do not contain characteristic domains are removed to obtain apple *STP* gene family members. In addition, this study identified *STP* gene family members in sweet orange (*Citrus sinensis* (L.) Osbeck), tomato (*Solanum lycopersicum* L.), cassava (*Manihot esculenta* Crantz), Chinese cabbage (*Brassica rapa* L.), sorghum (*Sorghum bicolor* (L.) Moench), and grape (*Vitis vinifera* L.) to infer the phylogenetic relationships of apple *STP* gene family members. The HmmerSearch tool was employed to screen their genomes using the PF00083 model, while SMART and Pfam were utilized to validate the candidate genes.

Analysis of physicochemical properties, gene structure, motif sequence and subcellular location of *STP* gene family in apple

Through the bioinformatics online software ExPASy (https://web.expasy.org/protparam) to analyze the physical and chemical properties of the apple STP family members, such as the number of amino acids, molecular weight, fat index, and hydrophilicity[29]. GSDS (http://gsds.cbi.pku.edu.cn/) was used for gene structure analysis[30]. TMHMM Server v. 2.0 (www.cbs.dtu.dk/services/TMHMM) was used for analyzing protein transmembrane structure. Using the online website MEME (http://meme.nbcr.ne-t/meme/cgi-bin/meme.cgi) to analyze protein-conserved motifs[31]. Using WoLF PSOT (www.genscript.com/wolf-psort.html) to analyze the subcellular localization of Apple STP family members.

Multiple sequence alignment of apple STP protein and construction and analysis of phylogenetic tree

Using ClustalX software to perform multiple sequence alignment of apple STP protein[32]. The phylogenetic tree was constructed using the amino acid sequences of STPs from *Arabidopsis*, sweet orange, tomato, cassava, Chinese cabbage, sorghum, grape, and apple with the MEGA7.0 software. The neighbor-joining (NJ) method was employed for tree construction, and the Bootstrap value was set to 1,000 to ensure the robustness and reliability of the phylogenetic analysis[33].

Analysis of cis-acting elements and microarray expression profile of *STP* gene family in apple

Search for apple tissue expression data (GSE42873) in the GEO database of NCBI (National Center for Biotechnology Information). The expression data of apple *STP* is obtained according to its gene accession number[34]; tissue types include different organs of apples, such as flowers and fruits, leaves, roots, stems, seeds and seedlings, etc. Use Excel software to convert the log2 transform of the obtained data, and finally, use the heat map creation tool HemI (www.hemi.biocuckoo.org) to complete the visual analysis of the expression of the *STP* gene chip.

First, obtain the gene sequence 2,000 bp upstream of the apple STP promoter in the Rosaceae database, and then submit the obtained sequence to the PlantCARE website (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) for analysis and activation The cis-acting element of the subregion.

Transcriptional regulation analysis of the *STP* gene family members in apple

Extract the upstream 2,000 bp regulatory region sequences of the apple *STP* gene family members and use PlantTFDB (https://planttfdb.gao-lab.org) to predict their transcription factor binding sites. Use Cytoscape (3.10.0) to construct the transcription regulatory network. Employ PlantTFDB to predict the DNA-binding domains of transcription factors and use JASPAR (https://jaspar.elixir.no/inference) to predict transcription factor binding sites on the DNA sequences. Utilize the AlphaFold 3 to predict the docking conformations of transcription factor-DNA interactions. Analyze the interaction interfaces between transcription factors and DNA using PDBePISA (www.ebi.ac.uk/pdbe/pisa) and perform visualization using PyMOL 3.1.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Enter the CDS sequence of the apple *STP* family gene in the website of Sangong Bioengineering (Shanghai) Co., Ltd., conduct

online primer design, and submit the designed primers (Supplementary Table S1) to the company for synthesis. Prime Script RT reagent Kit (Perfect RealTime) kit (TaKaRa) for cDNA synthesis. The reverse transcription product should be stored at −20 °C for later use. For qRT-PCR, the Swiss LightCycler 96 real-time fluorescent quantitative PCR instrument was used for quantitative analysis, with the apple *GAPDH* gene as an internal reference gene[35], and specific expression analysis of the apple *STP* gene family. The amplification system contains 1.5 μL cDNA, 1 μL upstream and downstream primers, 10 μL SYBR, 6.5 μL ddH2O, and 20 μL total system. The reaction program is: 95 °C for 30 s, 95 °C for 5 s, 60 °C for 34 s, 95 °C for 15 s, 60 °C for 60 s, 95 °C for 15 s, a total of 40 cycles. There are three biological replicates for each treatment, and the test data is converted by log10 transform. Finally, a heat map is made with HemI.

Results

Physicochemical characterization of the apple *STP* gene family

Genome-wide analysis identified 30 *STP* genes in apples, designated *MdSTP1* through *MdSTP30*, which were unevenly distributed across ten chromosomes (Table 1). The encoded polypeptides exhibited considerable variation in length, ranging from 235 amino acid residues (*MdSTP25*, the shortest isoform) to 655 residues (*MdSTP13*, the longest isoform). Bioinformatic characterization revealed substantial physicochemical diversity among family members: instability indices ranged from 27.77 to 43.10, indicating variable protein stability; aliphatic indices spanned 23.51 to 112.00, reflecting differences in thermostability; and consistent negative GRAVY values confirmed the hydrophilic nature of all STP proteins. Molecular weights varied from 26,332.08 to 254,819.64 Da, while theoretical isoelectric points (pI) showed a broad distribution from 4.97 to 9.43, suggesting functional diversification in cellular microenvironments.

Secondary structure and subcellular localization analysis of apple STP proteins

Secondary structure prediction of the 30 apple STP proteins revealed consistent structural features across all family members (Table 2). The composition analysis demonstrated that these proteins contain four major structural elements: α-helices (45.75%–56.60%), β-turns (4.46%–7.66%), extended chains (14.02%–19.83%), and random coils (20.85%–31.68%). Notably, the relative abundance of these structural elements followed the order: α-helix > random coil > extended chain > β-turn, indicating that α-helices and random coils dominate the secondary structure of apple STP proteins.

Subcellular localization predictions showed that apple STP proteins primarily localize to the plasma membrane, with additional members targeting the vacuole, endoplasmic reticulum, cytoskeleton, and Golgi apparatus. A smaller subset of STP proteins was predicted to localize to mitochondria, chloroplasts, and peroxisomes, suggesting functional specialization among family members within different cellular compartments.

Homology alignment and evolution analysis of apple STP protein sequences

Comprehensive phylogenetic analysis of STP proteins across multiple plant species revealed seven well-defined subfamilies (I–VII), each demonstrating distinct species distribution patterns (Fig. 1). The subfamilies varied significantly in size: subfamily I

Table 1. Physical and chemical property analysis of *STP* genes in apple.

Gene name	Gene accession no.	Chromosome location	Peptide (aa)	Mw (kD)	PI	II	AI	GRAVY	CDs (bp)	FULL (bp)
MdSTP1	MDP0000137412	Chr 02	516	185,529.61	7.14	38.78	25.58	0.603	1,551	2,189
MdSTP2	MDP0000146340	–	453	49,132.05	8.95	30.30	106.75	0.581	1,359	2,270
MdSTP3	MDP0000154362	Chr 15	522	56,747.72	9.09	33.43	111.34	0.535	1,569	2,943
MdSTP4	MDP0000175148	Chr 13	459	254,819.64	6.72	39.01	26.77	0.616	1,380	3,033
MdSTP5	MDP0000177942	Chr 01	479	214,554.17	7.53	35.91	26.45	0.604	1,440	2,522
MdSTP6	MDP0000185402	Chr 15	514	–	–	27.77	104.11	0.616	1,545	1,947
MdSTP7	MDP0000187438	Chr 02	518	56,544.23	9.34	34.07	103.92	0.452	1,557	2,357
MdSTP8	MDP0000190194	Chr 13	454	157,635.95	6.41	42.94	26.49	0.655	1,365	1,876
MdSTP9	MDP0000190996	Chr 09	430	162,749.64	7.27	43.10	25.51	0.533	1,293	1,917
MdSTP10	MDP0000191620	Chr 07	497	53,868.39	9.39	31.32	105.94	0.574	1,494	1,634
MdSTP11	MDP0000192591	Chr 15	*605*	66,120.31	8.96	33.13	102.83	0.401	1,818	2,841
MdSTP12	MDP0000193050	Chr 13	528	57,859.72	9.24	39.08	106.91	0.426	1,587	5,069
MdSTP13	MDP0000193898	Chr 05	655	72,422.79	9.20	34.86	100.15	0.453	1,968	2,993
MdSTP14	MDP0000201154	Chr 03	574	62,630.85	9.03	30.94	98.17	0.352	1,725	3,341
MdSTP15	MDP0000204929	Chr 13	551	205,076.15	6.62	41.55	28.20	0.658	1,536	2,447
MdSTP16	MDP0000216376	Chr 01	450	124,547.01	6.97	38.52	23.51	0.608	1,353	1,497
MdSTP17	MDP0000231284	Chr 09	509	55,673.94	9.06	34.07	110.51	0.606	1,530	1,958
MdSTP18	MDP0000236607	Chr 10	428	155,806.89	7.08	40.50	24.13	0.654	1,287	1,840
MdSTP19	MDP0000240921	Chr 02	518	56,580.07	9.34	34.11	103.92	0.457	1,557	2,357
MdSTP20	MDP0000258256	Chr 15	518	56,293.23	9.23	33.39	108.26	0.505	1,557	2,642
MdSTP21	MDP0000266249	Chr 14	479	51,146.79	9.28	32.64	106.47	0.568	1,440	1,538
MdSTP22	MDP0000282726	Chr 09	509	55,818.17	8.97	31.36	110.67	0.599	1,530	1,916
MdSTP23	MDP0000288533	Chr 10	519	228,109.15	6.55	35.69	25.74	0.641	1,560	2,712
MdSTP24	MDP0000289098	Chr 13	510	56,313.63	9.00	29.22	112.00	0.571	2,415	1,533
MdSTP25	MDP0000294725	–	235	26,332.08	9.33	39.69	110.81	0.376	708	708
MdSTP26	MDP0000485591	Chr 13	371	161,539.07	4.97	38.02	26.82	0.647	1,116	1,920
MdSTP27	MDP0000721688	Chr 13	524	57,363.86	9.43	41.79	99.56	0.306	1,575	7,162
MdSTP28	MDP0000735707	Chr 15	563	61,159.36	8.89	29.65	97.14	0.477	1,692	3,273
MdSTP29	MDP0000831221	Chr 13	511	55,997.23	9.10	33.31	107.03	0.565	1,536	1,951
MdSTP30	MDP0000906893	Chr 02	521	223,660.09	6.98	38.53	27.14	0.641	1,566	2,649

Table 2. The secondary structure and subcellular location of STP proteins in apple.

Protein	Alpha helix	Extended strand	Beta turn	Random coil	Subcellular localization
MdSTP1	50.58%	15.70%	5.23%	28.49%	plas, vacu, golg, cyto
MdSTP2	50.77%	14.79%	4.64%	29.80%	plas, vacu, cyto, E.R., golg
MdSTP3	47.89%	15.71%	5.75%	30.65%	plas, vacu, E.R.
MdSTP4	45.75%	18.08%	5.66%	30.50%	plas, vacu, golg, E.R.
MdSTP5	55.95%	15.03%	4.80%	24.22%	plas, vacu
MdSTP6	49.61%	15.76%	5.06%	29.57%	plas, vacu, cyto, E.R.
MdSTP7	47.30%	16.41%	5.02%	31.27%	plas, vacu, golg, cyto
MdSTP8	54.63%	14.98%	5.07%	25.33%	plas, E.R., golg
MdSTP9	51.63%	15.58%	6.05%	26.74%	plas, vacu
MdSTP10	52.92%	16.30%	4.63%	26.16%	plas, vacu, cyto, E.R.
MdSTP11	48.43%	16.86%	4.46%	30.25%	plas, vacu, golg, cyto
MdSTP12	49.05%	15.34%	5.68%	29.92%	plas, vacu, E.R., cyto, mito
MdSTP13	48.09%	19.24%	7.48%	25.19%	plas, vacu, golg, cyto, E.R.
MdSTP14	47.74%	15.85%	5.92%	30.49%	plas, vacu, cyto, mito, E.R.
MdSTP15	49.71%	15.46%	5.48%	29.35%	vacu, plas, cyto, E.R.
MdSTP16	48.89%	17.33%	4.67%	29.11%	plas, vacu, cyto
MdSTP17	51.67%	15.52%	5.70%	27.11%	plas, vacu
MdSTP18	49.71%	15.46%	5.48%	29.35%	plas, vacu, E.R., cyto, mito, extr
MdSTP19	48.65%	16.80%	4.83%	29.73%	plas, vacu, golg, cyto
MdSTP20	48.46%	16.22%	5.02%	30.31%	vacu, plas, golg, cyto
MdSTP21	46.14%	19.83%	5.64%	28.39%	vacu, plas, cyto
MdSTP22	50.49%	15.13%	5.70%	28.68%	plas, vacu, E.R., chlo, pero
MdSTP23	49.13%	16.96%	5.59%	28.32%	vacu, plas, golg, cyto
MdSTP24	47.65%	17.06%	6.67%	28.63%	vacu, plas, E.R., chlo, cyto, pero
MdSTP25	56.60%	14.89%	7.66%	20.85%	plas, E.R., golg, chlo, vacu
MdSTP26	52.29%	14.02%	4.58%	29.11%	plas, vacu, golg
MdSTP27	47.14%	16.41%	4.77%	31.68%	plas, vacu
MdSTP28	47.78%	17.41%	6.04%	28.77%	plas, E.R., golg
MdSTP29	48.92%	14.68%	6.07%	30.33%	plas, vacu, E.R., chlo, pero
MdSTP30	47.60%	16.12%	5.37%	30.90%	plas, vacu, cyto, E.R.

contained ten members, subfamilies II and III comprised 27 and 24 members respectively, while Subfamilies IV–VII included 27, 25, 18, and 31 members respectively. Notably, apple STP proteins (MdSTPs) exhibited unique evolutionary trajectories, with subfamily VI containing the highest proportion of MdSTPs (9/30) - a striking contrast to basal species like *Arabidopsis* and grape that possess only one STP member in this subfamily. Conversely, MdSTPs were completely absent from subfamily VII despite its broad representation across other species, suggesting apple-specific gene duplication and deletion events during evolution. The analysis further revealed lineage-specific distribution patterns: sorghum showed remarkable concentration in subfamily III (46.15% of its STPs), grape STPs (VvSTPs) predominantly clustered in Subfamily VII, while citrus STPs (CsSTPs) were mainly distributed in subfamily II. These findings highlight substantial divergence in STP family evolution even among closely related fruit-bearing species, including apple, grape, and citrus, underscoring the dynamic nature of *STP* gene family expansion and specialization across plant lineages.

Chromosome localization and evolutionary history of apple *STP* gene family

Chromosomal mapping analysis identified 30 *MdSTP* genes unevenly distributed across the apple genome (Fig. 2a), with 28 genes localized to ten chromosomes and two genes residing on unplaced scaffolds. Chromosome 13 emerged as a major hotspot, harboring seven *MdSTP* genes (*MdSTP8/12/15/24/26/27/29*). Phylogenetic analysis revealed these genes cluster into distinct clades, with *MdSTP12/15/8/24/29* grouping in clade IV, while *MdSTP26* and 27 belong to clade I. Although these genes are not arranged in tandem, their phylogenetic relationships and chromosomal positions suggest derivation from ancestral segmental duplication events (Fig. 1). The study identified several tandem duplication pairs, including *MdSTP11/20* on chromosome 15, *MdSTP7/19*, *MdSTP17/22*, and *MdSTP6/28* (Supplementary Table S2), indicating tandem duplication has significantly contributed to STP family expansion in apple. Furthermore, synteny analysis (Fig. 2b) detected six pairs of syntenic *MdSTP* genes distributed across 17 chromosomes, providing compelling evidence for the role of polyploidization events in shaping the evolutionary trajectory of the apple *STP* gene family. These findings collectively demonstrate that both tandem and segmental duplication mechanisms have driven the diversification of sugar transporters in apples during their evolutionary history.

Structural analysis of apple *STP* gene family

Comprehensive structural analysis of the 30 apple *STP* genes (Fig. 3) revealed significant variation in genomic organization. The exon count ranged from one to six, with *MdSTP13* containing the maximum number of exons (six) and *MdSTP25* representing the only intronless member. Gene length varied substantially from < 1 kb (MdSTP25) to 7 kb (*MdSTP27*), with most members (66.7%) exhibiting either four or five exons. Notably, phylogenetic conservation was evident in gene structure organization. Members within the same phylogenetic branch demonstrated remarkable structural similarity: (1) Subfamily II members MdSTP10 and MdSTP21 both contained two exons with comparable gene lengths; (2) Subfamily V members (MdSTP3/30/20/19/7) uniformly possessed four exons each. These conserved structural patterns strongly suggest functional and regulatory coherence among evolutionarily related *STP* genes, potentially reflecting conserved roles in sugar transport mechanisms across different apple tissues and developmental stages. The observed structural conservation, particularly in exon-intron organization, provides important insights into the evolutionary constraints shaping this critical transporter family.

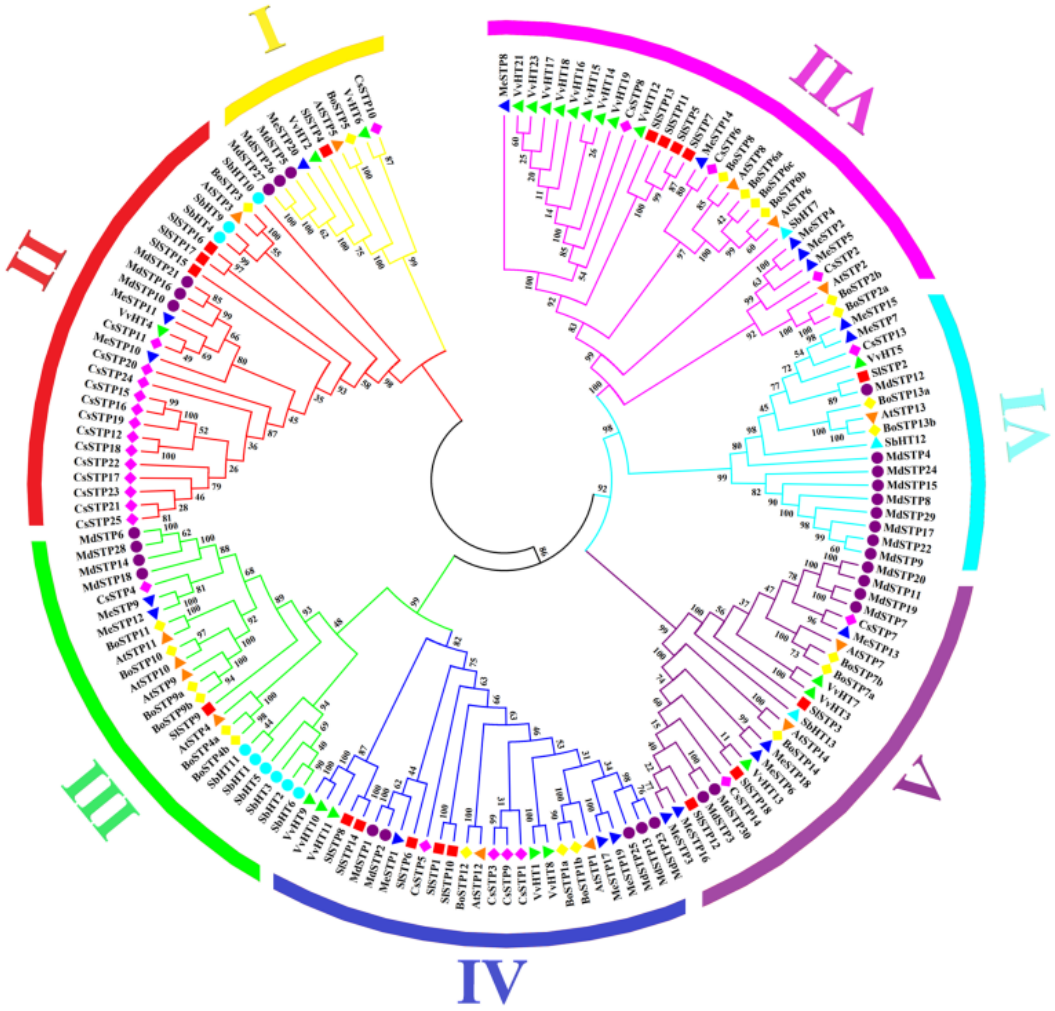

Fig. 1 Phylogenetic tree of STP proteins based on multiple species. The outermost ring of the phylogenetic tree is color-coded to represent different subfamilies, while distinct symbols on the branches denote various species. The numbers above the branches indicate bootstrap values. The abbreviations At, Cs, Sl, Me, Br, Sb, and Vv correspond to *Arabidopsis*, sweet orange, tomato, cassava, Chinese cabbage, sorghum, and grape, respectively.

Apple STP protein conservative motif analysis

Bioinformatic analysis of the apple STP family identified ten conserved protein motifs (designated Motifs 1–10) with distinct structural characteristics (Fig. 4). These motifs exhibited considerable variation in length, ranging from 15 amino acids (Motif 8) to 41 amino acids (Motif 7). Five motifs (3/4/6/8/10) were universally conserved across all MdSTP family members, suggesting their fundamental role in maintaining structural integrity and transporter function. The C-terminal domain was characterized by three conserved motifs: Motif 2 (29 aa), Motif 5 (21 aa), and Motif 6 (20 aa), while the N-terminal region contained Motif 7, 9, and 10. Phylogenetic analysis revealed striking clade-specific motif conservation patterns: Branch I members (MdSTP5/6/27) consistently lacked N-terminal Motif 9; Branch II members (MdSTP21/16/10) were deficient in Motif 4; and Branch V members (MdSTP20/19/7/10/11) retained complete motif complements. Notably, the identical motif composition observed in MdSTP5/6/27 (6 motifs) and similar profiles in MdSTP16/10 strongly suggest functional conservation within phylogenetic clades. These findings demonstrate that while the apple STP family has diversified into six distinct phylogenetic branches, members within each clade maintain conserved motif architectures.

Cis-acting regulatory element analysis of the apple *STP* gene family

The 2,000 bp promoter sequences upstream of apple *STP* genes (Fig. 5) identified a sophisticated regulatory architecture comprising four functionally distinct categories of cis-acting elements. The promoter regions contained: (1) hormone-responsive elements, including gibberellin-sensitive motifs (P-box, GARE-motif, TATC-box), methyl jasmonate-responsive elements (CGTCA-motif, TGACG-motif), auxin-related motifs (TGA-element, AuxRR-core), salicylic acid-responsive TCA-element, and abscisic acid-sensitive ABRE; (2) stress-inducible elements such as drought-responsive MBS and low temperature-sensitive LTR; (3) tissue-specific regulatory components including the meristem-associated CAT-box; and (4) light-responsive elements (ACE, G-box, MRE, Box 4, I-box). These findings demonstrate that apple STP genes are regulated by an intricate network of cis-regulatory elements that integrate hormonal signals (gibberellins, jasmonates, auxins, abscisic acid, and salicylic acid), environmental stresses (drought and low temperature), tissue-specific developmental cues, and light-mediated transcriptional control. The combinatorial presence of these regulatory motifs strongly suggests that the STP gene family serves as a crucial regulatory node coordinating multiple physiological processes, including hormone-mediated growth regulation, stress adaptation

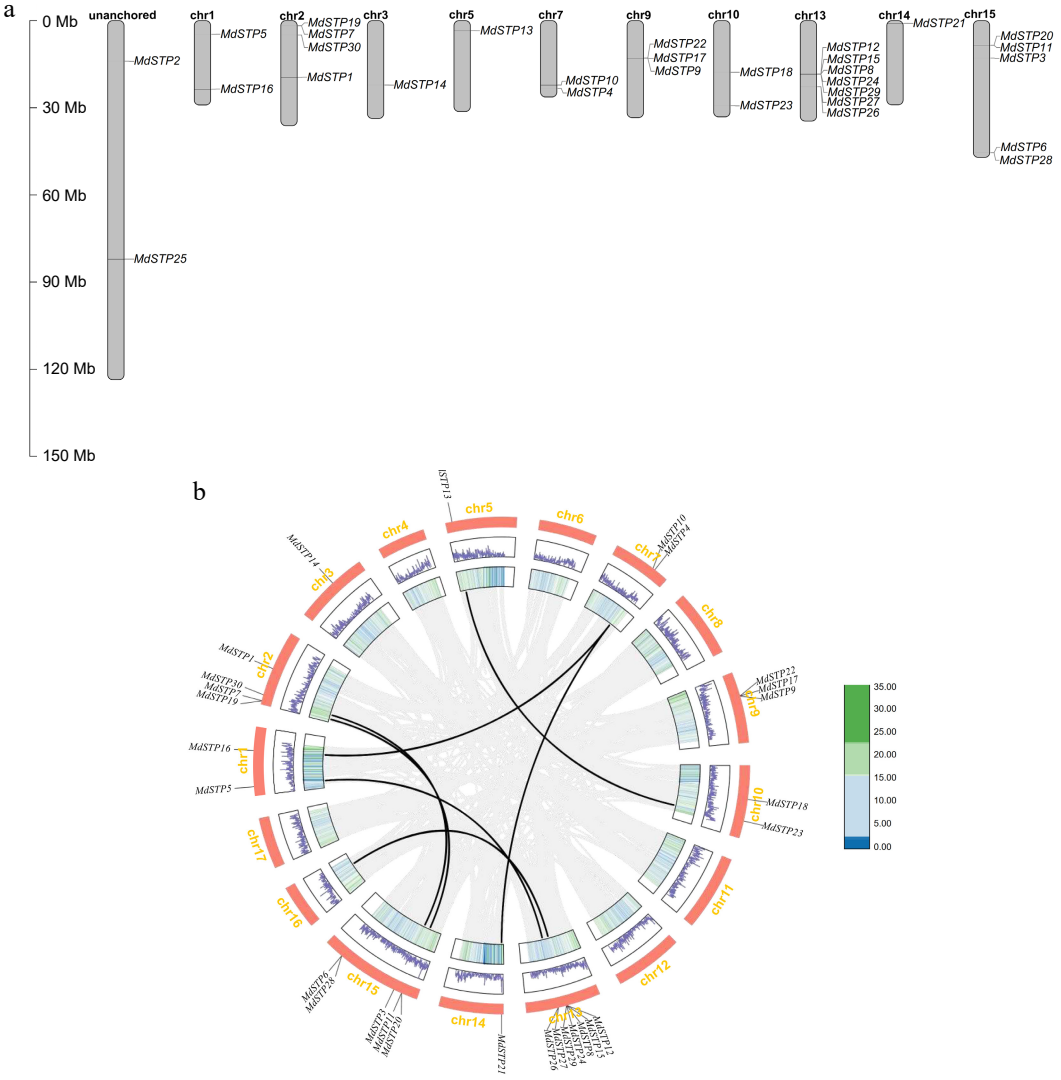

Fig. 2 Chromosomal localization and intra-species synteny of MdSTPs gene family members in apple. (a) Illustrates the chromosomal localization of *MdSTPs* gene family members in apple. (b) Demonstrates the syntenic relationships within the apple genome. In (a), the positions of individual genes are marked by black lines, with corresponding chromosome numbers displayed at the top of each chromosomal bar. In (b), grey lines represent syntenic blocks within the apple genome, while black lines specifically indicate syntenic gene pairs of *MdSTPs*. The innermost first and second concentric circles display gene density distributions across corresponding chromosomes, presented as heatmaps and line graphs, respectively.

mechanisms, organ-specific development, and photomorphogenic responses, highlighting their pivotal role as integrators of both endogenous developmental programs and environmental stimuli in apple.

Chip expression profile of apple *STP* genes

Gene chip analysis showed that 30 *MdSTP* genes had different expression patterns in different tissues of different apple varieties (Fig. 6). Among the 30 *MdSTP* genes, 24 genes (except *MdSTP3/4/7/10/13/23*) showed relatively high expression levels in seeds and seedlings of different apple varieties; 27 genes (except *MdSTP3/13/23*) showed relatively high expression levels in the roots and stems of Golden Delicious GD and X8877. In contrast, most genes that keep high expression were found in flowers of M69, young fruits of M74, leaves of M14 and fruits of M20. The expression levels in M74 flowers, M20 young fruits, M49 leaves and M74 fruits were all lower. Interestingly, some *MdSTP* genes are expressed in all tissues, but their expression levels vary greatly in the same tissues of different apple varieties. For example, *MdSTP8* is more highly expressed in flowers of M69 than in flowers of M74. *MdSTP24* was expressed higher in mature fruits of M20 but lower expressed in young fruits of M20. *MdSTP3/13/23* exhibit low expression levels across all tissues of the apple cultivars, including seedlings and seeds, where other *MdSTPs* are highly expressed. These findings highlight the remarkable functional diversification of *MdSTP* genes, with distinct expression profiles reflecting their specialized roles in different developmental stages and tissue types.

Transcriptional regulatory relationships of the apple *STP* gene family members in fruit

To elucidate the transcriptional regulatory mechanisms of *MdSTPs* in apple fruit, this study analyzed the expression levels of *MdSTPs* during fruit ripening and identified the transcription factors that modulate their activity. The results revealed that *MdSTP3*, *MdSTP13*, and *MdSTP23* are the predominantly expressed *MdSTPs* during fruit maturation, with *MdSTP3* exhibiting the highest expression levels in both cultivars (Fig. 7a). Notably, in cultivar M20, the expression levels of most *MdSTPs*, including *MdSTP3*, *MdSTP13*, and *MdSTP23*, decreased during the ripening stage compared to their levels at 100 days post-anthesis. In contrast, in cultivar M74, the expression levels

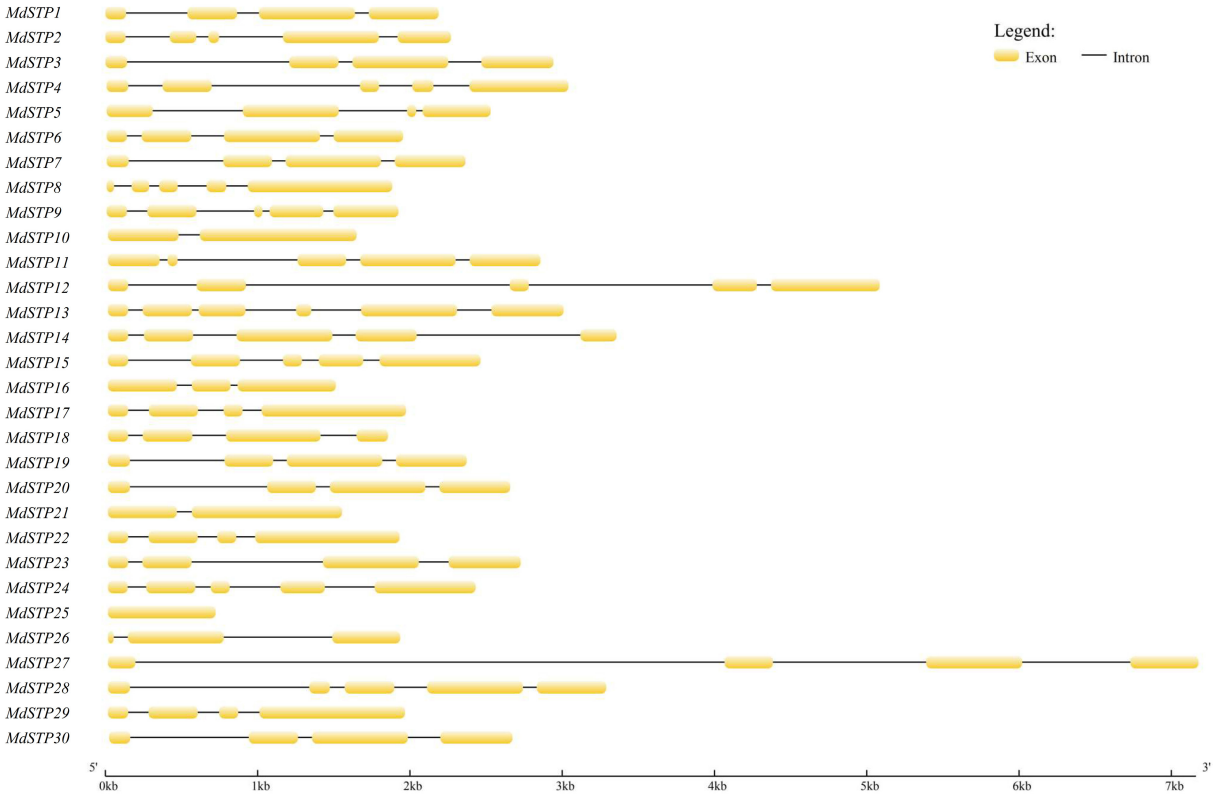

Fig. 3 Gene structure of *MdSTPs*. Exons are depicted as yellow boxes, while introns are represented by black lines.

of most *MdSTPs* increased during ripening relative to their levels at 100 days post-anthesis. Predictions of transcription factor interactions suggest that *MdSTPs* can be activated or repressed by members of 39 TFs families, including ERF, MYB, and DOF (Fig. 7b). The ERF transcription factor family was found to have the highest number of binding sites. This study identified 214 *ERF* genes in the apple genome and found that 93 *MdSTPs* were expressed in the fruit of both cultivars. Among these, *MdERF44*, *MdERF50*, *MdERF71*, and *MdERF86* were highly expressed in the fruit (Fig. 7d). Correlation analysis indicated that the expression levels of most *MdERFs* were positively correlated with the expression levels of *MdSTP3*, *MdSTP13*, and *MdSTP23* (Fig. 7c), suggesting that *MdERFs* may positively regulate the expression of these *MdSTPs*.

To verify whether the ERF transcription factor can activate the expression of the *MdSTPs* gene, this study utilized molecular docking to analyze the binding conformation between the AP2 domain of *MdERF50* and the promoter of *MdSTP13*. The results (Fig. 8) show that the AP2 domain of *MdERF50* (5'-VAGPPVKHRKQHRXKYAQNQ EPSLLRGVYFKNMKWHAAIKVDKKQIHLGTVGSQEEAAHLYDRAAF MCGREPNFELSEEEKQELRKFKWDEFLVMTRHAITNKKHMRRQGA ESEKRSESPQLEDSDWEDDXEEV-3') can bind to the 5'-GATAAAGAG AAGAAAAATGA-3' region of the *MdSTP13* promoter, forming a total of 19 hydrogen bonds involving 14 amino acid residues. This suggests that MdSTPs are likely subject to transcriptional regulation by MdERFs.

Response analysis of apple *STP* gene family to different hormone stress

The response of 30 *MdSTP* family members to different hormone stresses was analyzed based on qRT-PCR. The results showed that apple *STP* family members had different expression levels of *MdSTP* when treated with IAA, ABA and GA for 4, 8, 12, and 24 h, respectively (Fig. 9). When *STP* family members were induced by IAA for 4 h, *MdSTP8* and *MdSTP26* were significantly up-regulated. Interestingly, when the *MdSTP* gene was induced by IAA at 8, 12 and 24 h, most of the genes were also significantly up-regulated, such as *MdSTP2/9/16/17/18/21/22/26/27/28* genes. It is speculated that IAA is regulating the expression of *STP* family members. When *STP* family members were induced by ABA for 4 and 12 h, some genes were up-regulated, such as *MdSTP10*, *MdSTP12*, and *MdSTP23*. However, *MdSTP8*, *MdSTP18* and *MdSTP30* were all down-regulated after being induced by ABA at different times. When *STP* family members were induced by GA for 8 h, the expression of *MdSTP1*, *MdSTP8*, *MdSTP29* and *MdSTP30* were significantly up-regulated. When GA was induced for 4 h, *MdSTP1*, *MdSTP6*, and *MdSTP18* were significantly down-regulated. At the same time, when GA was induced for 24 h, *MdSTP18* was also significantly down-regulated.

Discussion

The *STP* gene family plays fundamental roles in regulating plant carbon allocation, floral organ development, crop yield, and environmental adaptation through its involvement in sugar transport processes[36,37]. While functionally conserved across species, this gene family exhibits nomenclature variations, being designated as MST in rice (*Oryza sativa*), HT in grape (*Vitis vinifera*), and HXT in sugarcane (*Saccharum officinarum*)[15,16,36]. Through comprehensive genome analysis using HMMER and BLAST algorithms, the study identified 30 STP family members in apple, which are unevenly distributed across ten chromosomes. Comparative genomic analysis revealed that apple possesses a notably larger *STP* gene family compared to other plant species, including *Arabidopsis* (14 members)[12], tomato (*Solanum lycopersicum*, 18)[28], cassava (*Manihot esculenta*, 20)[14], sweet orange (*Citrus sinensis*, 25)[38], Chinese cabbage (*Brassica oleracea*, 22)[39], grape[15], and pear (*Pyrus bretschneideri*, 20)[18], suggesting potential functional expansion and diversification in the apple genome.

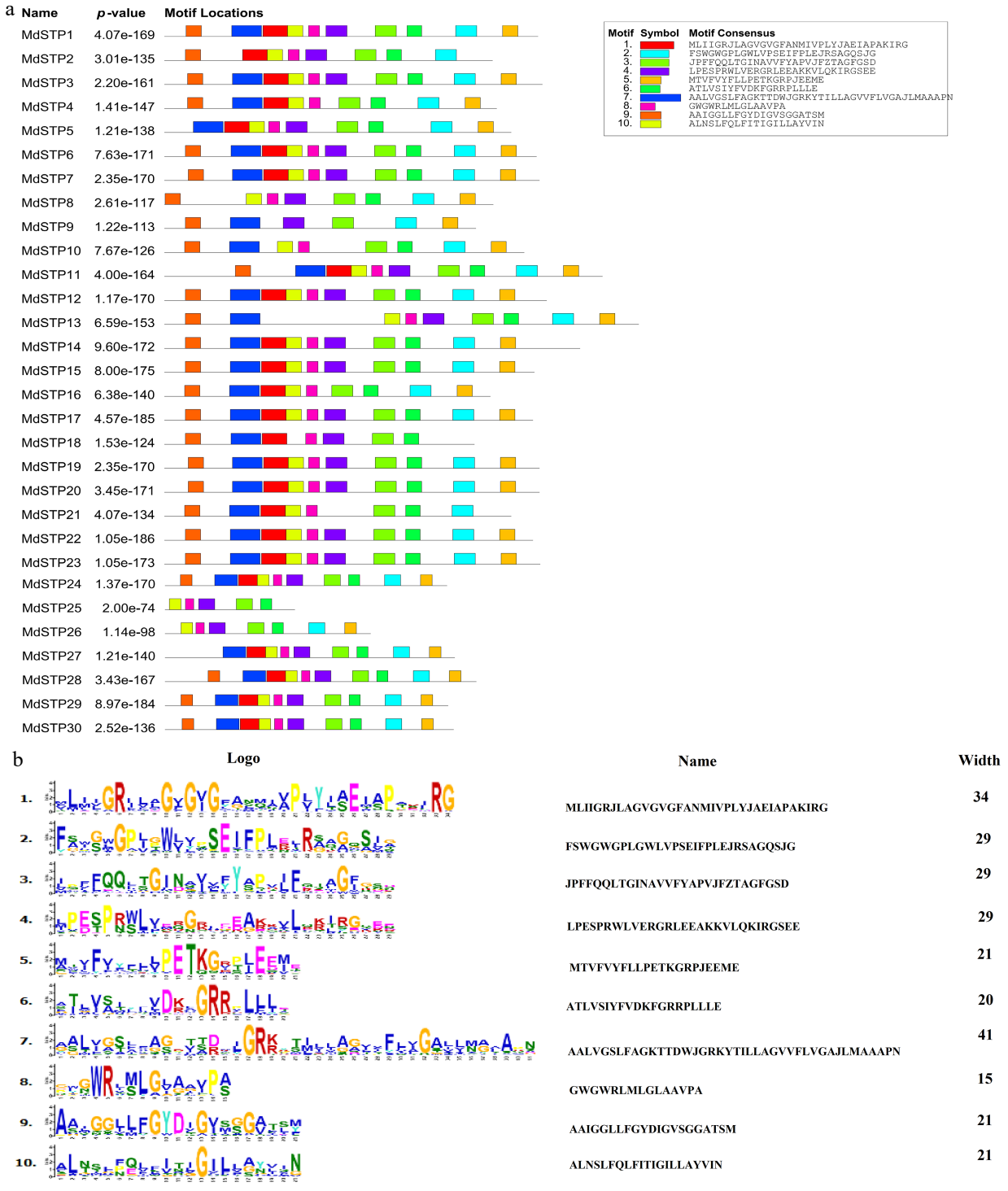

Fig. 4 Analysis of motifs in MdSTPs Proteins. (a) A total of 10 motifs were identified. (b) Sequence characteristics of the motifs. In (a), the 10 putative motifs are sequentially numbered from 1 to 10 and represented by distinct colors.

Bioinformatic analysis revealed that all apple STP proteins contain the conserved Sugar_tr domain (PF00083), characteristic of the Major Facilitator Superfamily (MFS). Plant MFS transporters typically possess 12 transmembrane domains (TMD1–TMD12) organized into N-terminal (TMD1–TMD6) and C-terminal (TMD7–TMD12) segments[11]. The majority of these proteins comprise 400–600 amino acid residues with highly conserved N- and C-terminal regions, suggesting evolutionary derivation from an ancestral 6-TMD protein through duplication events[40]. Comparative structural analysis demonstrated that while 14 MdSTPs maintain the complete 12-TMD architecture, others exhibit domain deletions - most notably MdSTP25 with only five TMDs. This pattern of TMD loss, consistent with observations in sorghum, cassava, grape and tomato[11,14,15,28], indicates frequent N- or C-terminal domain deletion during STP family evolution. Gene structure analysis further revealed considerable exon number variation (1–6 exons, predominantly 2–4) among MdSTPs, mirroring patterns observed in *Arabidopsis*, grape and pear[11,12,15]. These structural variations likely

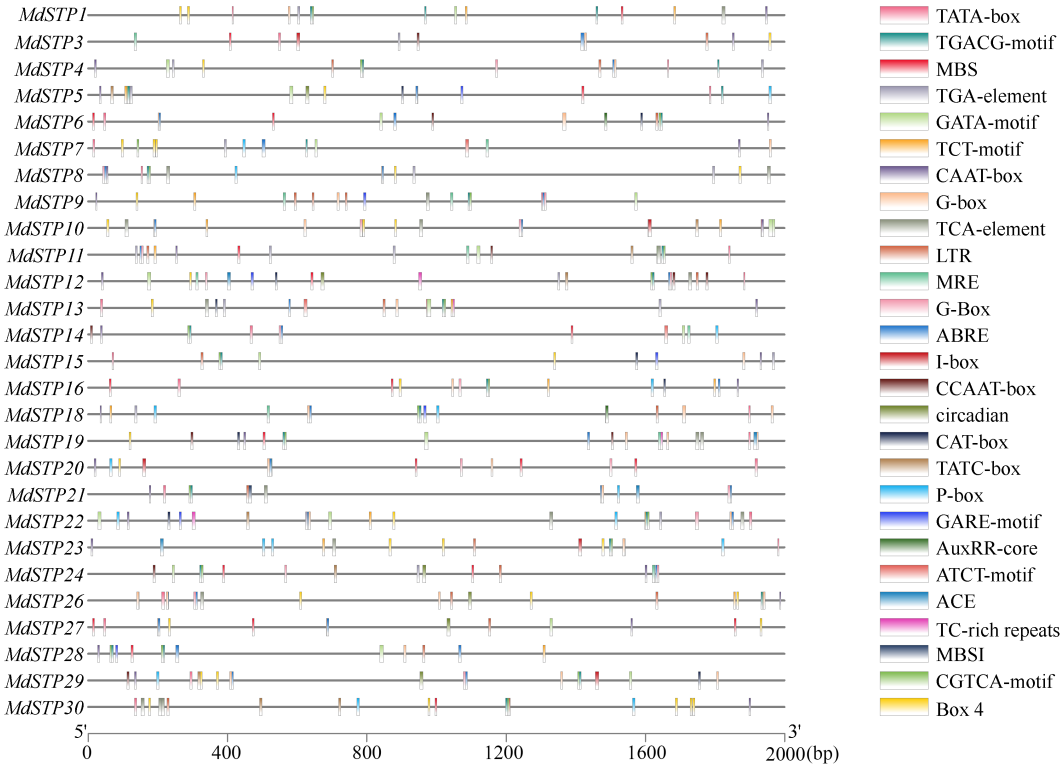

Fig. 5 Cis-acting regulatory elements of *MdSTPs* genes. A total of 27 cis-acting elements were identified within the promoter regions of the 30 *MdSTPs* genes, each represented by distinctively colored boxes.

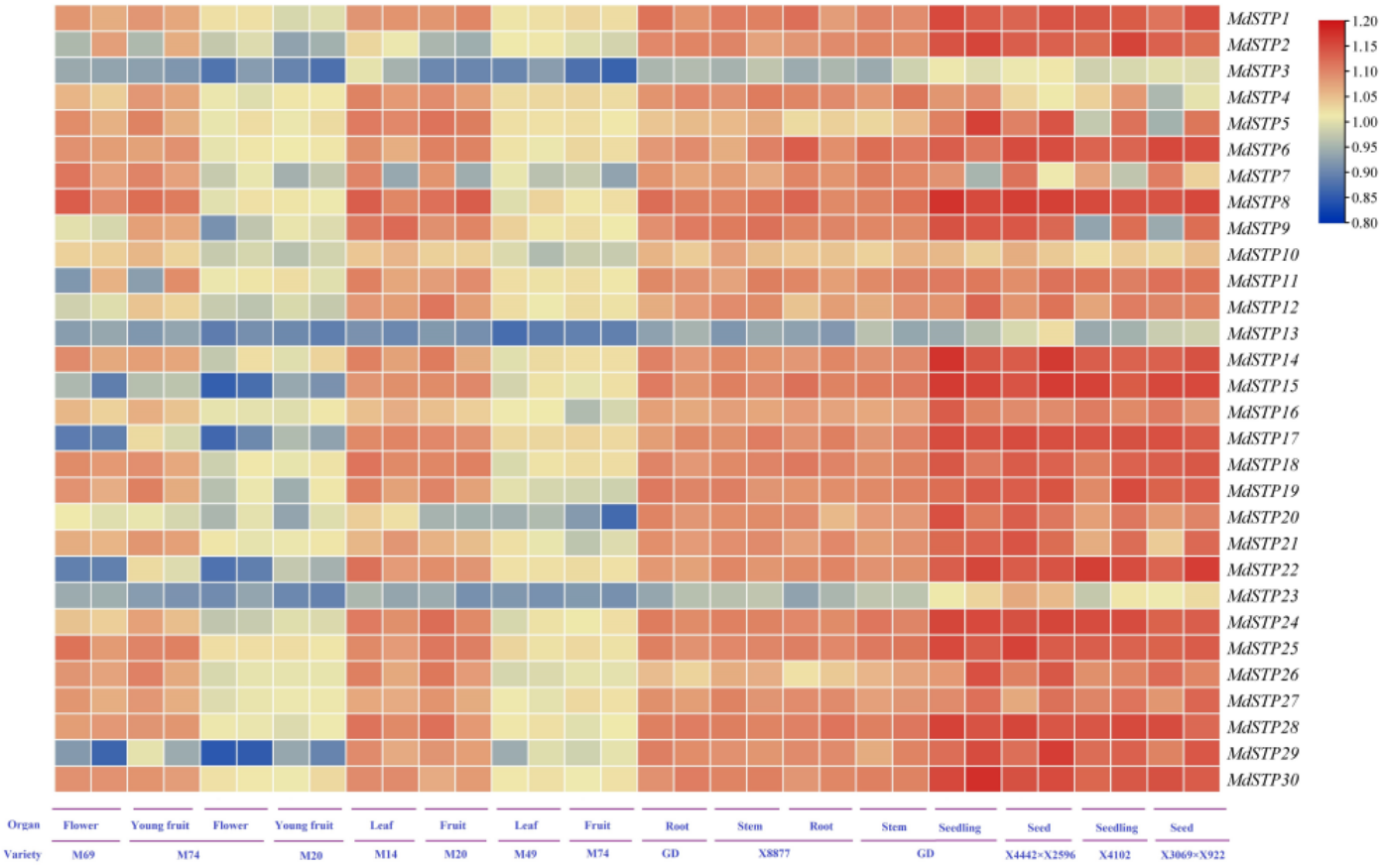

Fig. 6 Expression levels of *MdSTPs* across various cultivars and tissues. At the bottom of the heatmap, 'Variety' denotes different apple cultivars, while 'Organ' represents various tissue types. The heatmap employs a blue-yellow-red color gradient to indicate gene expression levels, ranging from low to high. The expression levels are comparatively analyzed based on the integrated data from both the horizontal and vertical axes.

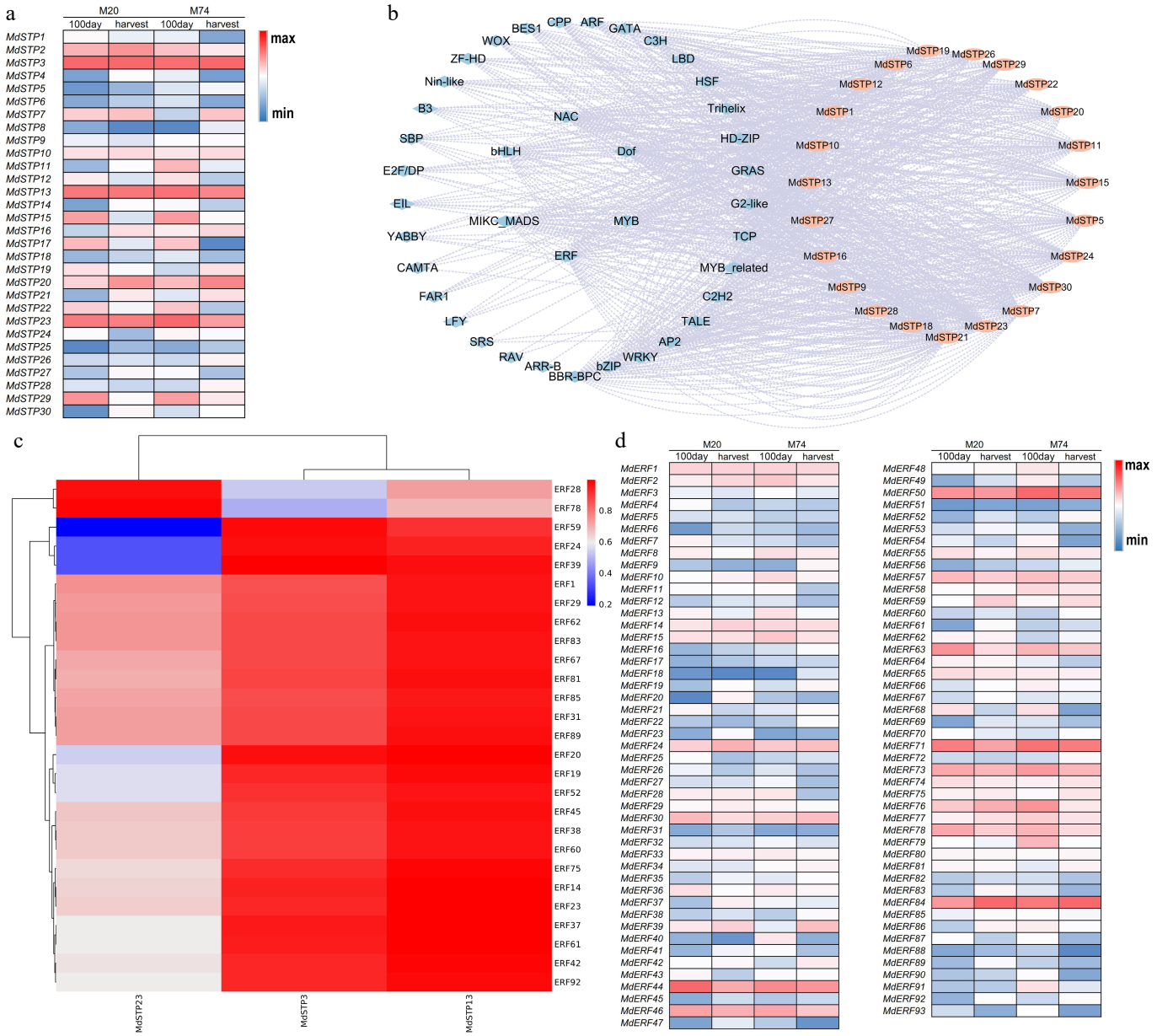

Fig. 7 The expression level of *MdSTPs* in fruits and its transcriptional regulatory mechanism. (a) Expression levels of *MdSTPs* in fruits. (b) Transcription factor regulatory network. (c) Correlation between the expression levels of *MdERFs* and *MdSTPs*. (d) Expression levels of *MdERFs* in fruits. In (a) and (d), '100 day' refers to 100 d post-flowering, while 'harvest' indicates the fruit ripening stage. The blue-white-red color gradient represents low-medium-high expression levels, respectively. The expression levels are comparatively analyzed based on the integrated data from both the horizontal and vertical axes. In (b), blue denotes transcription factors, while orange represents *MdSTPs*.

arose through evolutionary exon/intron gain/loss events, contributing to functional diversification within the *STP* gene family.

The prediction of subcellular localization revealed that all 30 apple STPs are targeted to the plasma membrane, consistent with their established role in mediating monosaccharide uptake from the extracellular space into plant cells[13]. Structural analysis of the *Arabidopsis* AtSTP13 homolog suggests that membrane localization signals are primarily encoded in C-terminal motifs[41], leading us to hypothesize that Motif 2 and Motif 5 may represent core domains critical for plasma membrane targeting of apple STPs. Phylogenetic classification divided the apple STP family into seven distinct subfamilies, with Subfamily VI containing the highest representation (9 MdSTPs), followed by Subfamily V (5 members), while Subfamily VII showed no apple representatives. This phylogenetic complexity contrasts with the simpler four-subfamily organization observed in gramineous species, including *Arabidopsis*, chestnut and cassava[42], highlighting significant evolutionary divergence in STP family expansion and specialization between plant lineages.

Comparative analysis of *STP* gene expression reveals distinct organ-specific patterns across diverse plant species[8]. In *Arabidopsis*, *AtSTP1* shows predominant expression in germinated seeds, seedlings, and guard cells[21,43], while *AtSTP2/6/9/11* exhibit pollen-specific expression and *AtSTP4* demonstrates root-preferential expression[44]. Citrus species display fruit-enriched expression of at least 9 *CsSTPs*[37], whereas tomato shows specialized patterns: *SlSTP1* in young fruits, *SlSTP3* exclusively in flowers and developing fruits, and *SlSTP2* in leaves, branches, and developing fruits[28]. Our study of apple STPs identified: (1) 24 genes (excluding *MdSTP3/4/7/10/13/23*) with high expression in seedlings and seeds across varieties; (2) 27 genes (excluding *MdSTP3/13/23*) showing elevated expression in

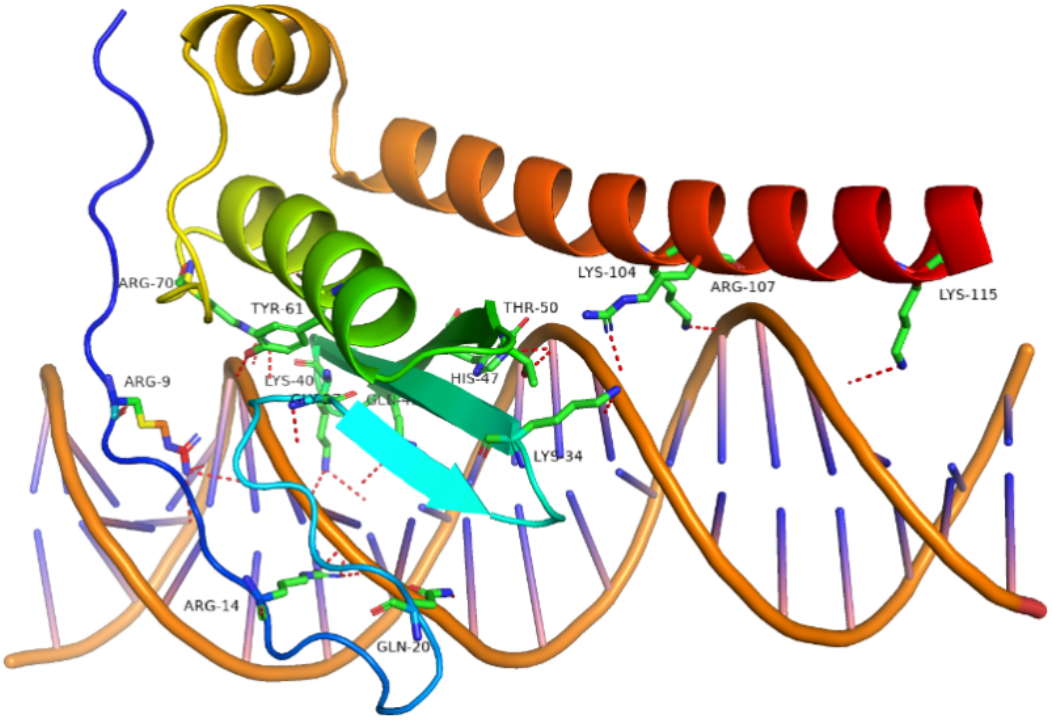

Fig. 8 The molecular conformation of the docking between MdERF and *MdSTP13* promoter region. The red dashed lines indicate hydrogen bonds between the AP2 domain and the *MdSTP13* promoter region.

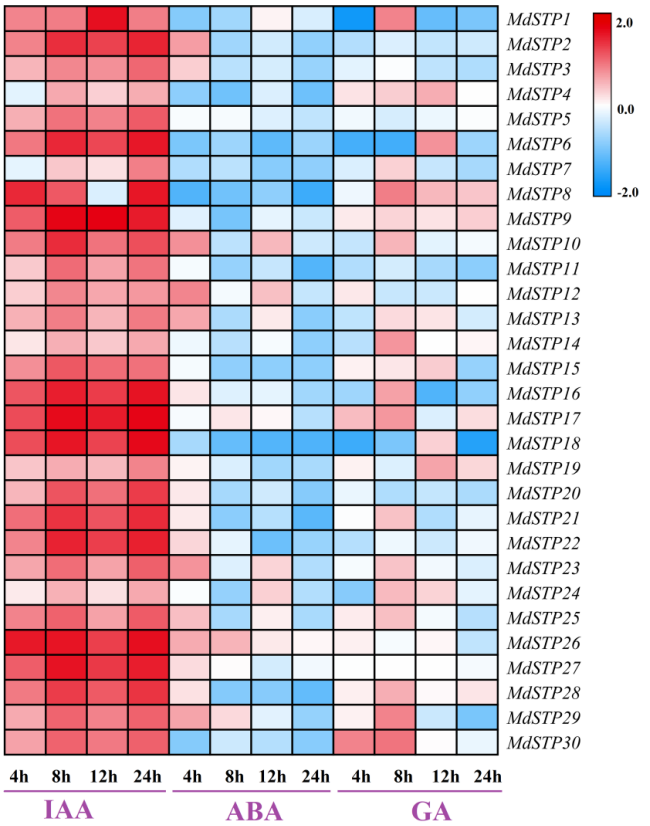

Fig. 9 Expression levels of *MdSTPs* gene under different hormone treatments. The horizontal axis represents different time points of plant hormone treatments, while the vertical axis indicates the relative expression levels of *MdSTPs*. The blue-white-red color gradient signifies low-medium-high gene expression levels, respectively. The expression levels are comparatively analyzed based on the integrated data from both the horizontal and vertical axes.

roots and stems of specific cultivars; and (3) a large number of *MdSTP* genes concentrated expression in M69 flowers, M74 young fruits, M14 leaves, and M20 mature fruits. These pronounced interspecies and interorgan expression differences strongly suggest *STP* genes play specialized roles in hexose accumulation, with apple MdSTPs likely contributing to carbohydrate partitioning in a tissue- and variety-specific manner.

The accumulation of sugars in fruits is a primary factor influencing their sweetness[45]. Typically, sugars are synthesized in the plant's photosynthetic tissues and then transported to fruits and other organs for storage via transport proteins[46,47]. This process involves multiple families of transporter proteins and is regulated by transcription factors[46]. The ERF transcription factor family is one of the largest in plants, participating in various biological processes[48]. Some experiments have confirmed the involvement of ERF transcription factors in the regulation of sugar accumulation in fruits[49,50]. Research on p*runus armeniaca* has revealed that two *STP* genes are significantly upregulated during fruit ripening, and this upregulation of *STP* expression appears to be associated with the increased expression of *ERF10*[26]. In this study, it was found that the expression levels of *MdSTP* genes exhibit significant differences during the ripening process and after harvest, suggesting that sugar accumulation in fruits is related to *MdSTPs*. The high expression of *MdSTP3*, *MdSTP13*, and *MdSTP23* may be closely associated with sugar transport during the fruit expansion stage. Additionally, it was observed that *MdSTPs* are likely regulated by *MdERFs*. In the fruit, the expression levels of *MdERFs* are significantly positively correlated with the expression levels of *MdSTPs*. Molecular docking analyses between proteins and DNA confirmed that ERF can bind to the promoter regions of *STP* genes. Therefore, the study concludes that *MdSTPs* are involved in sugar accumulation during apple fruit ripening, and *MdERFs* positively regulate sugar accumulation in apple fruits.

Sugar transporters are more sensitive to changes in the concentration of extracellular monosaccharides, and their expression can

be affected by biological and abiotic stresses, such as mechanical damage, nematode and bacterial infection, as well as hormone induction and sugar concentration regulation[41]. This means that the metabolic network that regulates sugar transporters is greatly affected by stimulus signals inside and outside the cell membrane. In order to identify the stress expression of hormones on apple *MdSTPs* gene, this study used 10 mmol·L^{-1} IAA, 50 mmol·L^{-1} GA, and 50 mmol·L^{-1} ABA to treat the leaves of apple tissue culture seedlings for 0, 4, 8, 12 and 24 h, respectively. The results showed that the *MdSTPs* gene was induced by IAA at different times, and there was no down-regulated expression of the *MdSTPs* gene. This study speculates that the *MdSTPs* gene is involved in the positive regulation process induced by IAA stress, which is consistent with the identification results of potato sugar transporter[51]. In sorghum, *SbSTP5* is highly expressed in roots induced by ABA, and *SbSTP4* is highly expressed in stalks induced by ABA[11]. In sugarcane, the expression of HXT2 is down-regulated after being stressed by ABA. When *HXT12* is under the stress of ABA, although the induction time is different, the expression of *HXT12* is up-regulated[26]. In this study, when the *MdSTPs* gene was induced by ABA at different times, some genes were up-regulated, and the other genes were down-regulated. At the same time, the most up-regulated genes, such as *MdSTP2/3/10/12/13/23/29*, etc., occurred at 4 h after ABA induction. It is speculated that these genes may be involved in the adversity response caused by ABA, and their function is strongest at 4 h. Zhang et al.[26] showed that after the sugarcane *HXT* gene was treated with GA at different times, the expression of different *HXT* genes was different, and the expression of *HXT2* gene was up-regulated in sugarcane leaves after 24 h GA treatment. In this study, when *MdSTP* family members were induced by GA for 8 h, *MdSTP1*, *MdSTP8*, *MdSTP29* and *MdSTP30* showed up-regulated expression. *MdSTP1*, *MdSTP6*, and *MdSTP18* showed down-regulated expression at 4 h of GA induction, and the most up-regulated genes occurred at 8 h of induction. It is speculated that these genes are most sensitive to GA treatment for 8 h, and they will play an important role at 8 h.

Conclusions

Through integrated bioinformatics and qRT-PCR approaches, comprehensive genome-wide identification and hormonal regulation analysis of the apple *STP* gene family was conducted. Our results demonstrate that the 30 identified *MdSTPs* phylogenetically cluster into seven distinct subfamilies exhibiting high structural conservation within each subgroup. Tissue-specific expression profiling revealed predominant *STP* activity in seeds, seedlings, roots, and stems, with *MdSTP3/13/23* emerging as key regulators of sugar accumulation during fruit ripening. Furthermore, this study established that MdERF transcription factors may positively regulate sugar accumulation process. qRT-PCR analysis showed that 30 *MdSTP* family members responded differently to IAA, GA and ABA stress induced at different times, and IAA positively regulated the expression of *STP* genes. These findings collectively provide novel insights into the structural evolution, spatial expression patterns, and hormonal regulation of STP-mediated sugar transport in apple.

Author contributions

The authors confirm their contribution to the paper as follows: study conception and design: Guo Z, Cao X; data collection: Guo Z, Zou Y, An X; analysis and interpretation of results: Guo Z, Zou Y, Zhou Y; draft manuscript preparation: Guo Z. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Conflict of interest

The authors declare that they have no conflict of interest.

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