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Evolution and amplification of the trehalose-6-phosphate synthase gene family in Theaceae

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Abstract

Background Trehalose-6-phosphate synthase (TPS) is an essential enzyme involved in the production of trehalose, and the genes associated with *TPS* are crucial for various processes such as growth, development, defense mechanisms, and resistance to stress. However, there has been no documentation regarding the evolution and functional roles of the *TPS* gene family within Theaceae.

Results Here, we uncovered the lineage-specific evolution of *TPS* genes in Theaceae. A total of 102 *TPS* genes were discovered across ten Theaceae species with sequenced genomes. Consistent with the previous classification, our phylogenetic analysis indicated that the *TPS* genes in Theaceae can be categorized into two primary subfamilies and six distinct clades (I, II-1, II-2, II-3, II-4, II-5), with clade I containing a greater number of introns compared to those found in clade II. Segmental duplication served as the main catalyst for the evolution of *TPS* genes within Theaceae, and numerous *TPS* genes exhibited inter-species synteny among various Theaceae species. Most of the *TPS* genes were ubiquitously expressed, and expression divergence of *TPS* paralogous pairs was observed. The *cis*-acting elements found in *TPS* genes indicated their involvement in responses to phytohormones and stress.

Conclusion This research enhanced our understanding of the lineage-specific evolution of the *TPS* gene family in Theaceae and offered important insights for future functional analyses.

Keywords TPS, Theaceae, Lineage-specific evolution, Expression pattern

Introduction

Trehalose, a disaccharide that does not reduce, is found widely in nature and can be observed in a variety of organisms, including bacteria, plants, and mammals [1]. In plants, trehalose influences carbon distribution and is crucial for growth, development, and resilience to stress [2, 3]. In response to challenging environmental conditions like cold, drought, heat, and salinity, trehalose is produced in large quantities and serves as a protective agent to preserve cellular integrity and viability [3, 4].

Trehalose contains two glucose molecules connected by an α,α−1,1-glycosidic linkage [1]. Trehalose is produced from UDP-glucose (UDPG) and glucose 6-phosphate (Glc6P), involving two enzymatic reactions during its biosynthesis in plants [5, 6]. Initially, trehalose-6-phosphate synthase (TPS) facilitates the formation of trehalose-6-phosphate (T6P) from UDPG and Glc6P [6]. Subsequently, trehalose-6-phosphate phosphatase (TPP) removes the phosphate group from T6P to generate trehalose and inorganic phosphate [1]. Plant TPS proteins possess two crucial domains: Glyco_transf_20 (TPS domain, Pfam: PF00982) and Trehalose_PPase

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(TPP domain, Pfam: PF02358), whereas TPP proteins consist solely of the TPP domain [7]. Plant TPP proteins demonstrate TPP activities; however, the TPP domain in TPS proteins has lost its enzymatic function through the course of evolution [8].

TPS genes are widely distributed in the genomes of all major plant taxa [3, 9–11]. The *TPS* genes are highly abundant and diverse in higher plants [10]. So far, many *TPS* genes have been identified in plants, including 11 in *A. thaliana* [12], 11 in rice [8, 12], 12 in *Populus* [9], 13 in Quinoa [13], 13 in apple [14], 9 in peach [15], 53 in cotton [16], and 12 in wheat [17].

Based on sequence similarity, *TPS* genes are divided into two distinct classes [12]: class I and class II, which display different characteristics in gene numbers, gene structures, expression profiles, enzyme activities, and physiological functions [3]. Only AtTPS1 and OsTPS1 encode an enzyme with TPS activity, respectively [7, 18], whereas Class II TPS proteins lack the TPS activity [3, 19]. *TPS* genes are crucial for a range of biological processes, such as embryo development, flower induction, seed filling, and tolerance to both biotic and abiotic stresses. For instance, *AtTPS1* plays a pivotal role in glucose, abscisic acid (ABA), and stress signaling. Mutation of *AtTPS1* leads to embryonically lethal [20], while overexpressing *AtTPS1* in *A. thaliana* and tobacco enhanced tolerance to drought, temperature and osmotic stresses, respectively [21]. Similarly, overexpression of *OsTPS1* in rice increased tolerance to drought, salt, and cold stresses [22]. The a*ttps9* mutants displayed salt sensitivity, while overexpressing *AtTPS9* increased salt tolerance by enhanced suberin lamellae deposition in root endodermis [23]. *AtTPS5* acts as a negative regulator of ABA signaling, and its mutation leads to increased sensitivity to ABA during seed germination [24]. Mutation of *AtTPS6* reduced trichome branching and increased stomatal density [25]. Overexpression of *OsTPS8* in rice improved salt tolerance [26] and heterogeneous expression of wheat *TPS11* in *A. thaliana* enhanced cold tolerance [27].

Tea (*Camellia sinensis* (L.) Kuntze) and oil-tea (*Camellia oleifera* Abel) have great economic values, and are widespread in temperate, subtropical, and tropical regions [28]. They belong to the Theaceae, which contains more than 300 species [29]. During the past several years, the genomes of many tea and oil-tea were sequenced [30–39]. So far, TPSs have been identified in several species, such as *A. thaliana*, rice, *Populus*, apple, and cotton [7, 12, 14]. However, a comprehensive genome-wide identification or functional prediction of TPSs has not yet been conducted in tea and oil-tea plants. In this study, we examined the evolutionary relationships of *TPSs* in various tea species. This research identified 102 *TPS* genes from ten Theaceae species, and categorized them into two subfamilies. We analyzed its gene and protein structure, investigated the expression patterns in different tissues as well as the transcriptional responses under stress conditions. Our study offers a theoretical foundation for future research on the biological roles of *TPS* gene family members in tea and oil-tea plants.

Results

Identification and characteristics analyses of *TPS* genes in Theaceae plants

To identify all TPS homologs in Theaceae species, we conducted both BLAST and HMM searches using TPS sequences from *A. thaliana* and rice. All obtained sequences were filtered based on E values and subsequently analyzed with the SMART and Pfam databases to verify the existence of the conserved TPS domains PF00982 and PF02358. Finally, 15, 10, 11, 9, 9, 11, 11, 11, 8 and 7 *TPS* genes were identified from, *Camellia sinensis* var. *sinensis* cv. Shuchazao (Scz), Tieguanyin (Tgy), Biyun (By), Huangdan (Hd), Longjing-43 (Lj), *Camellia sinensis* var. *assamica* cv. Yunkang-10 (Yk), *Camellia lanceoleosa* (Cla), *Camellia chekiangoleosa* (CCH), *Camellia oleifera* (Col) and wild tea tree (DASZ), respectively (Table 1). The tea *TPS* genes that were identified were named based on their homologs in *A. thaliana* and the results of the phylogenetic analysis (Fig. 1, Table 1).

The physicochemical properties of tea TPS members were summarized in Table 1, including amino acid sequence length, isoelectric point (pI), and molecule weight (MW) (Table 1). The size of tea TPS proteins was highly variable from 282 (By-TPS7) to 1048 amino acids (Lj-TPS9), with an average length of 828 aa. The MW ranged from 31.18 (By-TPS7) KDa to 119.73 KDa (Lj-TPS9), and their pIs ranged from 4.87 (Lj-TPS9) to 9.04 (Yk-TPS10). These physicochemical parameters of tea TPS are comparable to those of TPS identified in *A. thaliana*, and rice.

Phylogenetic analysis of *TPS* gene families

To classify TPS family members within Theaceae, an unrooted phylogenetic tree was created using the complete protein sequence alignment of TPSs from *A. thaliana* (11 members), rice (11 members), and Theaceae (102 members) (Fig. 1). Based on the branches of the phylogenetic tree, the 124 TPS proteins could be distinctly categorized into two major subfamilies: Group I and Group II. The group II subfamily could be further assigned to 5 subclades (II-1, 2, 3, 4, and 5) with high bootstrap support (Fig. 1). This outcome is in agreement with prior studies conducted on *A. thaliana*, rice, and *Populus* [9, 12]. The number of TPS genes in *A. thaliana*, rice, and various Camellia species (Yk, Scz, By, Lj, Hd, Tgy, Col,

Table 1 *TPS* genes identified in ten Theaceae plants

Species name	Number of Caleosin	Gene name	Subfamily	Num of Exon	Number of Amino Acid	Molecular Weight (Da)	Theoretical pI
Camellia sinensis var. sinensis cv. Biyun	11	By-TPS9	Clade II-4	3	833	94355.18	5.98
		By-TPS8	Clade II-4	3	855	96398.52	5.82
		By-TPS10	Clade II-5	3	862	97848.92	5.8
		By-TPS4	Clade II-2	3	863	97318.26	5.64
		By-TPS5	Clade II-2	3	865	97429.23	5.7
		By-TPS3	Clade II-1	3	856	97336.75	5.65
		By-TPS6	Clade II-3	4	749	85799.88	8.88
		By-TPS2	Clade I	13	836	93838.11	8.36
		By-TPS11	Clade II-5	6	648	73008.86	6.41
		By-TPS1	Clade I	10	493	54880.81	6.96
		By-TPS7	Clade II-3	2	282	31189.19	5.84
Camellia nanyongensis	8	Col-TPS8	Clade II-5	4	860	97498.34	5.69
		Col-TPS7	Clade II-4	4	863	97368.71	5.85
		Col-TPS2	Clade II-1	4	857	97444.95	5.76
		Col-TPS1	Clade II-1	4	859	97681.23	5.8
		Col-TPS3	Clade II-2	4	857	96516.35	5.65
		Col-TPS6	Clade II-3	4	889	100204.52	6.02
		Col-TPS5	Clade II-3	3	855	96590.77	6.47
		Col-TPS4	Clade II-3	4	746	85307.35	8.84
Camellia chekiangoleosa	11	Cch-TPS9	Clade II-4	3	854	96637.61	5.82
		Cch-TPS8	Clade II-4	3	855	96448.6	5.73
		Cch-TPS10	Clade II-5	3	862	97721.64	5.61
		Cch-TPS3	Clade II-1	3	856	97365.9	5.74
		Cch-TPS4	Clade II-2	3	865	97370.16	5.65
		Cch-TPS7	Clade II-3	3	855	96333.96	5.8
		Cch-TPS1	Clade I	17	944	105899.98	8.29
		Cch-TPS6	Clade II-3	3	855	96605.78	6.29
		Cch-TPS5	Clade II-3	1	706	80941.82	8.79
		Cch-TPS2	Clade I	16	939	105222.64	6.6
		Cch-TPS11	Clade II-5	4	685	78076.93	6.8
Camellia lanceoleosa	11	Cla-TPS1	Clade I	17	932	104988.6	7.41
		Cla-TPS10	Clade II-4	3	854	96622.65	5.91
		Cla-TPS11	Clade II-5	3	862	97695.62	5.65
		Cla-TPS2	Clade I	17	935	104818.97	6.52
		Cla-TPS9	Clade II-4	3	855	96444.64	5.77
		Cla-TPS4	Clade II-1	3	856	97337.85	5.74
		Cla-TPS5	Clade II-2	3	865	97398.21	5.7
		Cla-TPS8	Clade II-3	3	855	96275.88	5.75
		Cla-TPS7	Clade II-3	1	679	77603.41	8.49
		Cla-TPS6	Clade II-3	3	865	98008.91	5.62
		Cla-TPS3	Clade II-1	2	429	48700.57	8.47
wild tea tree DASZ	7	DASZ-TPS6	Clade II-4	3	854	96663.79	6.06
		DASZ-TPS5	Clade II-4	4	818	92633.59	5.82
		DASZ-TPS7	Clade II-5	4	916	104155.44	6.32
		DASZ-TPS4	Clade II-3	3	855	96216.8	5.8
		DASZ-TPS1	Clade I	16	869	97463.5	6.74
		DASZ-TPS3	Clade II-3	10	868	97685.03	6.47
		DASZ-TPS2	Clade II-2	7	603	67473	5.38

Table 1 (continued)

Species name	Number of Caleosin	Gene name	Subfamily	Num of Exon	Number of Amino Acid	Molecular Weight (Da)	Theoretical pI
Camellia sinensis var. sinensis cv. Huangdan	9	Hd-TPS8	Clade II-4	3	854	96644.74	6.03
		Hd-TPS7	Clade II-4	3	855	96444.65	5.82
		Hd-TPS2	Clade I	17	935	104640.78	6.57
		Hd-TPS4	Clade II-2	3	863	97318.26	5.64
		Hd-TPS9	Clade II-5	3	862	97804.86	5.74
		Hd-TPS3	Clade II-1	3	856	97308.8	5.69
		Hd-TPS5	Clade II-2	3	865	97429.23	5.7
		Hd-TPS6	Clade II-3	2	681	78150.06	8.53
		Hd-TPS1	Clade I	10	545	60772.17	8.37
Camellia sinensis var. sinensis cv. Longjing-43	9	Lj-TPS1	Clade I	17	932	104904.45	7.18
		Lj-TPS8	Clade II-4	3	854	96644.74	6.03
		Lj-TPS9	Clade II-5	4	1048	119732.79	4.87
		Lj-TPS2	Clade I	17	935	104599.77	6.57
		Lj-TPS5	Clade II-2	3	865	97429.23	5.7
		Lj-TPS3	Clade II-1	3	856	97336.75	5.65
		Lj-TPS7	Clade II-3	3	855	96274.83	5.75
		Lj-TPS4	Clade II-2	5	839	94586.25	5.37
		Lj-TPS6	Clade II-3	3	862	97911.74	5.36
Camellia sinensis var. sinensis cv. Shuchazao	15	Scz-TPS1	Clade I	17	932	104904.45	7.18
		Scz-TPS13	Clade II-4	3	854	96644.74	6.03
		Scz-TPS12	Clade II-4	3	855	96398.52	5.82
		Scz-TPS11	Clade II-4	3	855	96444.65	5.82
		Scz-TPS15	Clade II-5	3	862	97875	5.8
		Scz-TPS3	Clade I	17	935	104599.77	6.57
		Scz-TPS4	Clade II-1	2	700	80307.6	6.65
		Scz-TPS5	Clade II-2	3	865	97429.23	5.7
		Scz-TPS10	Clade II-3	3	855	96274.83	5.75
		Scz-TPS9	Clade II-3	3	855	96274.83	5.75
		Scz-TPS2	Clade I	18	924	103413.4	6.51
		Scz-TPS7	Clade II-3	3	855	96580.75	6.29
		Scz-TPS6	Clade II-3	3	866	98147.93	5.35
		Scz-TPS8	Clade II-3	4	790	90210.4	7.6
		Scz-TPS14	Clade II-5	5	461	51849.74	8.85
Camellia sinensis var. sinensis cv. Tieguanyin	10	Tgy-TPS1	Clade I	17	932	104904.45	7.18
		Tgy-TPS8	Clade II-4	3	854	96663.79	6.06
		Tgy-TPS7	Clade II-4	3	855	96447.72	5.77
		Tgy-TPS2	Clade I	17	935	104640.78	6.57
		Tgy-TPS10	Clade II-5	3	862	97862.99	5.84
		Tgy-TPS4	Clade II-2	3	865	97429.23	5.7
		Tgy-TPS3	Clade II-1	3	856	97350.78	5.65
		Tgy-TPS6	Clade II-3	3	855	96540.69	6.32
		Tgy-TPS5	Clade II-3	3	842	95441.93	5.54
		Tgy-TPS9	Clade II-5	6	596	66845.83	7.03

Table 1 (continued)

Species name	Number of Caleosin	Gene name	Subfamily	Num of Exon	Number of Amino Acid	Molecular Weight (Da)	Theoretical pI
Camellia sinensis var. assamica cv. Yunkang-10	11	Yk-TPS1	Clade I	17	932	104904.45	7.18
		Yk-TPS9	Clade II-4	3	854	96669.79	6.06
		Yk-TPS8	Clade II-4	3	771	87668.81	5.89
		Yk-TPS2	Clade I	18	951	106343.73	6.57
		Yk-TPS11	Clade II-5	3	862	97751.76	5.75
		Yk-TPS4	Clade II-2	3	865	97429.23	5.7
		Yk-TPS3	Clade II-1	4	858	97618.11	5.61
		Yk-TPS7	Clade II-3	3	855	96273.85	5.8
		Yk-TPS6	Clade II-3	3	855	96582.77	6.32
		Yk-TPS5	Clade II-3	6	1005	113311.27	5.23
		Yk-TPS10	Clade II-5	5	461	51879.76	9.04
Arabidopsis thaliana	11	AtTPS5	Clade II-2	3	862	97454.49	5.76
		AtTPS8	Clade II-4	3	856	97561.18	5.6
		AtTPS1	Clade I	17	942	105975.73	6.7
		AtTPS10	Clade II-4	3	861	97324.38	6.16
		AtTPS9	Clade II-4	3	867	98495.79	5.92
		AtTPS11	Clade II-5	3	862	98275.23	6.96
		AtTPS2	Clade I	17	822	93004.88	5.61
		AtTPS6	Clade II-1	3	860	97703.22	5.9
		AtTPS7	Clade II-3	3	851	96688.46	5.59
		AtTPS4	Clade I	17	795	89474.44	6.09
		AtTPS3	Clade I	17	783	89466.57	7.34
Oryza sativa	11	OsTPS1	Clade I	17	980	108425.95	6.22
		OsTPS10	Clade II-1	3	885	99445.48	5.83
		OsTPS4	Clade II-1	3	860	96634.66	5.76
		OsTPS11	Clade II-4	3	863	97809.63	5.96
		OsTPS6	Clade II-3	3	899	100533.68	5.48
		OsTPS7	Clade II-4	3	862	97645.3	6.19
		OsTPS2	Clade II-3	3	913	102269.72	5.37
		OsTPS3	Clade II-3	3	878	99392.68	5.5
		OsTPS5	Clade II-5	3	847	93883.35	6.71
		OsTPS8	Clade II-5	3	824	91504.41	5.76
		OsTPS9	Clade II-5	3	886	96286.41	7.38

Cch, Cla, and DASZ) across different groups are as follows: Group I (4, 1, 2, 3, 2, 2, 2, 2, 0, 2, 2, 1), Group II-1 (1, 2, 1, 1, 1, 1, 1, 1, 2, 1, 2, 0), Group II-2 (1, 0, 1, 1, 2, 2, 2, 1, 1, 1, 1, 1), Group II-3 (1, 3, 3, 5, 2, 2, 1, 2, 3, 3, 3, 2), Group II-4 (3, 2, 2, 3, 2, 1, 2, 2, 1, 2, 2, 2), and Group II-5 (1, 3, 2, 2, 2, 1, 1, 2, 1, 2, 1, 1) (Table 2). Rice had only one Group I TPS, whereas most Theaceae plants had at least two members, such as Yunkang-10 (two members), Shuchazao (three members), Biyun (two members), Longjing-43 (two members), Huangdan (two members), Tieguanyin (two members), *Camellia chekiangoleosa* (two members), *Camellia lanceoleosa* (two members). AtTPS1 and OsTPS1 were proved to have TPS activity [7, 18], suggesting that the Theaceae *TPS* genes in group I might have TPS activity. No Group I TPS was identified in *Camellia oleifera,* probably due to its incomplete genome annotation [38]. In most subgroups (II-1, 2, 3, 4, and 5), at least one number from all 12 species was present, with the exception of *OsTPS* and *DASZ-TPS* genes in group II-2, and II-1, respectively (Table 1, Fig. 1). The

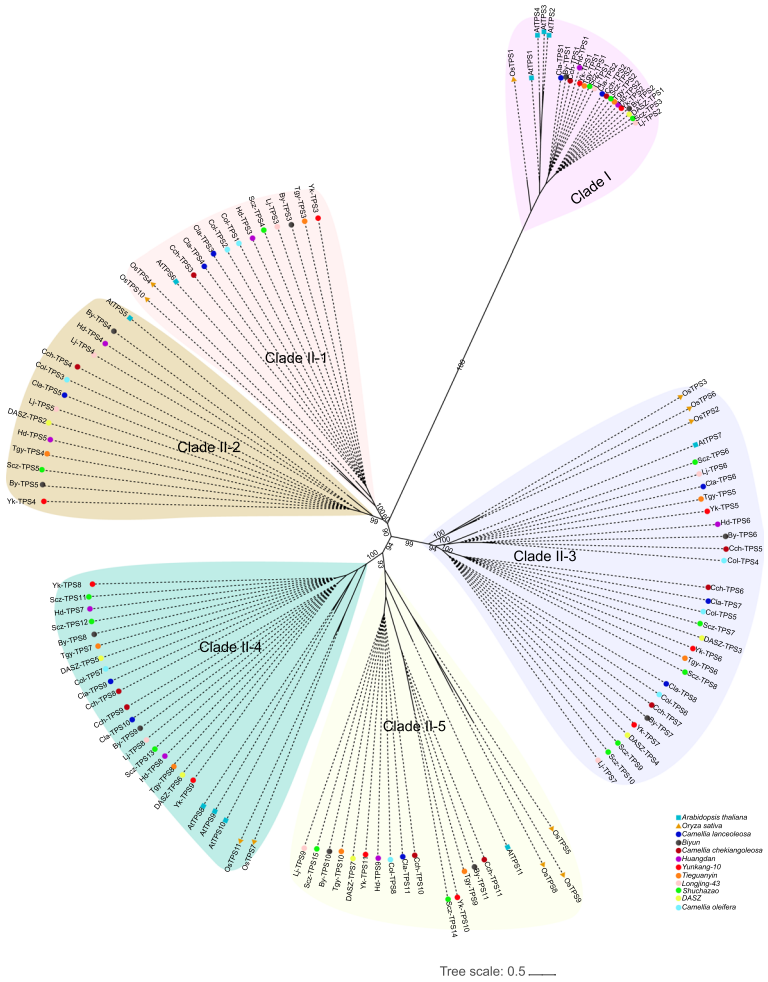

Fig. 1 The phylogenetic tree of TPS genes from *Arabidopsis thaliana* (*AtTPS*), *Oryza sativa* (*OsTPS*), *Shuchazao* (*Scz-TPS*), *Biyun* (*By-TPS*), *Longjing-43* (*Lj-TPS*), *Huangdan* (*HdTPS*), *Yunkang-10* (*Yk-TPS*), *Tieguanyin* (*Tgy-TPS*), wild tea *DASZ* (*DASZ-TPS*), *Camellia oleifera* (*Col-TPS*), *Camellia chekiangoleosa* (*Cch-TPS*), and *Camellia lanceoleosa* (*Cla-TPS*). Colored circles represent different species. Gene subfamilies are indicated with different colors

Theaceae *TPS* genes often clustered closer to *A. thaliana TPS* genes than to rice, that was expected since *A. thaliana* and Camellia are dicots whereas rice is a monocot. This result suggesting that *A. thaliana* and Camellia *TPS* genes share more similar functions.

Gene structures and protein profiles of Theaceae TPSs

To gain a deeper insight into the molecular features of Theaceae *TPS* genes, we analyzed their gene structures, including exons, introns, and conserved motifs (Fig. 2). Most class I *TPS* genes possessed more than16 introns, whereas most class II *TPS* genes contained only two introns (Fig. 2A-B). Compared with group I *TPSs*, the average exon length was longer in group II *TPSs*, whereas the average gene length was shorter in group II *TPSs*. These differences in gene structures of Theaceae *TPS* are comparable to those of *TPS* existed in *A. thaliana*, and rice, indicating the evolutionary divergence between group I and II *TPSs.*

We next performed structural feature analysis of the conserved domains of Theaceae TPS proteins (Fig. 2C). All the TPS proteins consisted of two common conserved domains, one N-terminal glycosyltransferase 20 family (Glyco_transf_20) domin and one C-terminal trehalose phosphatase (Trehalose_PPase) domain. Additionally, the majority of Group II TPS proteins (80%, 68/84) contained a haloacid dehalogenase-like hydrolase (Hydrolase_3) domain in the C-terminal region. In addition, 10 distinct conserved motifs were identified using the MEME website (Fig. 2B), and these motifs were almost conserved in Theaceae TPS proteins. Motifs 1, 3, 4, 6, 7, 9, and 10 together composed the TPS domain (Glyco_transf_20), and motifs 2, 5, and 8 composed the TPP domain (Trehalose_PPase). The majority of the members of clades II-2 and II-4 harbored 10 motifs, while the majority of

Table 2 The distribution of the *TPS* genes in different subfamilies

Species name	Clade I	Clade II-1	Clade II-2	Clade II-3	Clade II-4	Clade II-5	Sum
Arabidopsis thaliana	4	1	1	1	3	1	11
Oryza sativa	1	2	0	3	2	3	11
Camellia sinensis var. assamica cv. Yunkang-10	2	1	1	3	2	2	11
Camellia sinensis var. sinensis cv. Shuchazao	3	1	1	5	3	2	15
Camellia sinensis var. sinensis cv. Biyun	2	1	2	2	2	2	11
Camellia sinensis var. sinensis cv. Longjing-43	2	1	2	2	1	1	9
Camellia sinensis var. sinensis cv. Huangdan	2	1	2	1	2	1	9
Camellia sinensis var. sinensis cv. Tieguanyin	2	1	1	2	2	2	10
Camellia nanyongensis	0	2	1	3	1	1	8
Camellia chekiangoleosa	2	1	1	3	2	2	11
Camellia lanceoleosa	2	2	1	3	2	1	11
wild tea tree DASZ	1	0	1	2	2	1	7
Sum	23	14	14	30	24	19	124

the members of clades II-1, II-3, and II-5 possessed two motif 7. Clade I TPSs display a different motif conservation, and all members of clade I lacked motif 8 (Fig. 2). In addition, 4 and 10 members of clade I contained two numbers of motif 7 and motif 3, respectively. The findings from the structural analysis validated the reliability of the phylogenetic tree, indicating functional distinctions between clades I and II.

Gene duplication analysis of TPS proteins

Previous analysis identified duplicate pairs of *TPS* genes created by segmental duplication in *A. thaliana* and rice [12], and segmental duplication contribute most to the expansion of *TPS* genes. To understand the expansion pattern of Theaceae *TPS* genes, a collinear relationship was examined (Fig. 3, Figure S1). 8 (*TPS1/TPS2, TPS1/TPS3, TPS2/TPS3, TPS8/TPS9, TPS8/TPS10, TPS9/TPS10, TPS12/TPS13, TPS14/TPS15*), 3 (*TPS1/TPS2, TPS4/TPS5, TPS8/TPS9*), 3 (*TPS1/TPS2, TPS6/TPS7, TPS8/TPS9*), 3 (*TPS1/TPS2, TPS7/TPS8, TPS9/TPS10*), 3 (*TPS1/TPS2, TPS4/TPS5, TPS7/TPS8*), 2 (*TPS1/TPS2, TPS7/TPS8*), 1 (*TPS5/TPS6*), and 1 (*TPS3/TPS4*) pairs of *TPSs* were identified in Shuchazao (SCZ), Biyun (By), *Camellia chekiangoleosa* (Cch), *Camellia lanceoleosa* (Cla), Huangdan (HD), Tieguanyin (Tgy), *Camellia oleifera* (Col), and wild tea (DASZ), respectively. In addition, 4 (*TPS1/TPS2, TPS8/TPS9, TPS8/TPS10, TPS9/TPS10*), and 6 (*TPS2/TPS3, TPS2/TPS6, TPS5/TPS8, TPS5/TPS9, TPS8/TPS9, TPS1/TPS11*) pairs of *TPSs* were identified in *A. thaliana*, and rice, respectively (Fig. 3). All these duplicated gene pairs were located in paralogous blocks, indicating that these duplicated gene pairs were formed by a segmental duplication event. The Ka/Ks ratios for all *TPS* pairs were found to be below 1 (Table S2), suggesting that these TPS genes have undergone purifying selection.

To investigate the evolutionary mechanism of Theaceae *TPS* genes, the inter-species synteny was analyzed among Theaceae species, *A. thaliana*, and rice (Fig. 4). The results showed syntenic gene pairs were extensively present among these species. The number of gene pairs orthologous to *A. thaliana TPS* genes were 14 (Biyun), 8 (Shuchazao), 8 (*Camellia chekiangoleosa*), 11 (*Camellia lanceoleosa*), 10 (Huangdan), 10 (Tieguanyin), *Camellia oleifera* (Col), 7 (Longjing), and 7 (DASZ) (Fig. 4). One-to-many, or many-to-one homozygosity were identified among Theaceae species and *A. thaliana*. More orthologoue gene pairs were found between Theaceae species and *A. thaliana* than between Theaceae species and rice (Fig. 4), which is consistent with the result of phylogenetic analysis that the *TPS* genes of theaceae clustered closer to the *TPS* genes of Arabidopsi than to the *TPS* genes of rice (Fig. 1).

Analysis of *cis*-acting elements

To determine the possible regulatory mechanisms of *TPS* genes in Theaceae, we predicted the cis-acting elements of their promoters using Plant CARE (Fig. 5, Table S3). A total of 41 distinct types of cis-acting elements were identified and classified into four primary categories: light-responsive elements, plant growth-related elements, stress-responsive elements, and phytohormone-responsive elements. The light-responsive cis-acting elements were the most prevalent, followed by those related to stress response and phytohormones, while the plant growth-related elements were the least represented. 21 types of light responsive elements were identified, with Box4 and G-box account for 25% and 21%, respectively. In stress and phytohormone responsive elements, ARE (an anaerobic induction element), MBS (a drought-inducible element), ABRE (ABA-responsive element),

Fig. 2 Illustrates the phylogenetic relationships (**A**), gene structure (**B**), and domain architecture (**C**, **D**) of TPS genes. In panel **A**, a protein IQ tree shows different TPS subfamilies represented in various colors. Panel **B** displays the exon/intron structures of TPS genes, where black lines indicate introns, green boxes represent exons, and blue boxes denote UTRs. The scale at the bottom provides an estimate of exon and intron sizes. Panel **C** highlights the conserved motifs found in TPS proteins, with ten putative motifs shown in differently colored boxes. Finally, panel **D** presents the domain architecture of full-length TPS proteins, featuring conserved domains such as glycosyltransferase family 20, trehalose-phosphatase, and haloacid dehalogenase-like hydrolase domains highlighted in distinct colors

ERE (ethylene-responsive element), and CGTCA-motif (methyl jasmonic acid [MeJA]-responsive element) were observed in most Theaceae *TPS* genes. The presence of these elements implied that Theaceae *TPS* genes could participate in the plant response to environmental stimuli and phytohormone signaling. Notably, 8 *TPS* genes (*Cla-TPS8, Col-TPS6, Cch-TPS7, Yk-TPS7, DASZ-TPS4, Scz-TPS9, Scz-TPS10*, and *Lj-TPS7*) belonging to clade II-3 contained the most elements of G-box and ABRE, indicating their possible function in light and phytohormone signal transduction (Fig. 5).

Transcriptional profiling of *TPS* genes

To explore the expression pattern of Theaceae *TPS* genes, we acquired the public RNA-seq data and created a heat map with the TPM values of the *TPS* genes (Fig. 6, Table S4). In Shuchazao, eight different tissues were analyzed, including root, stem, young leaf, mature

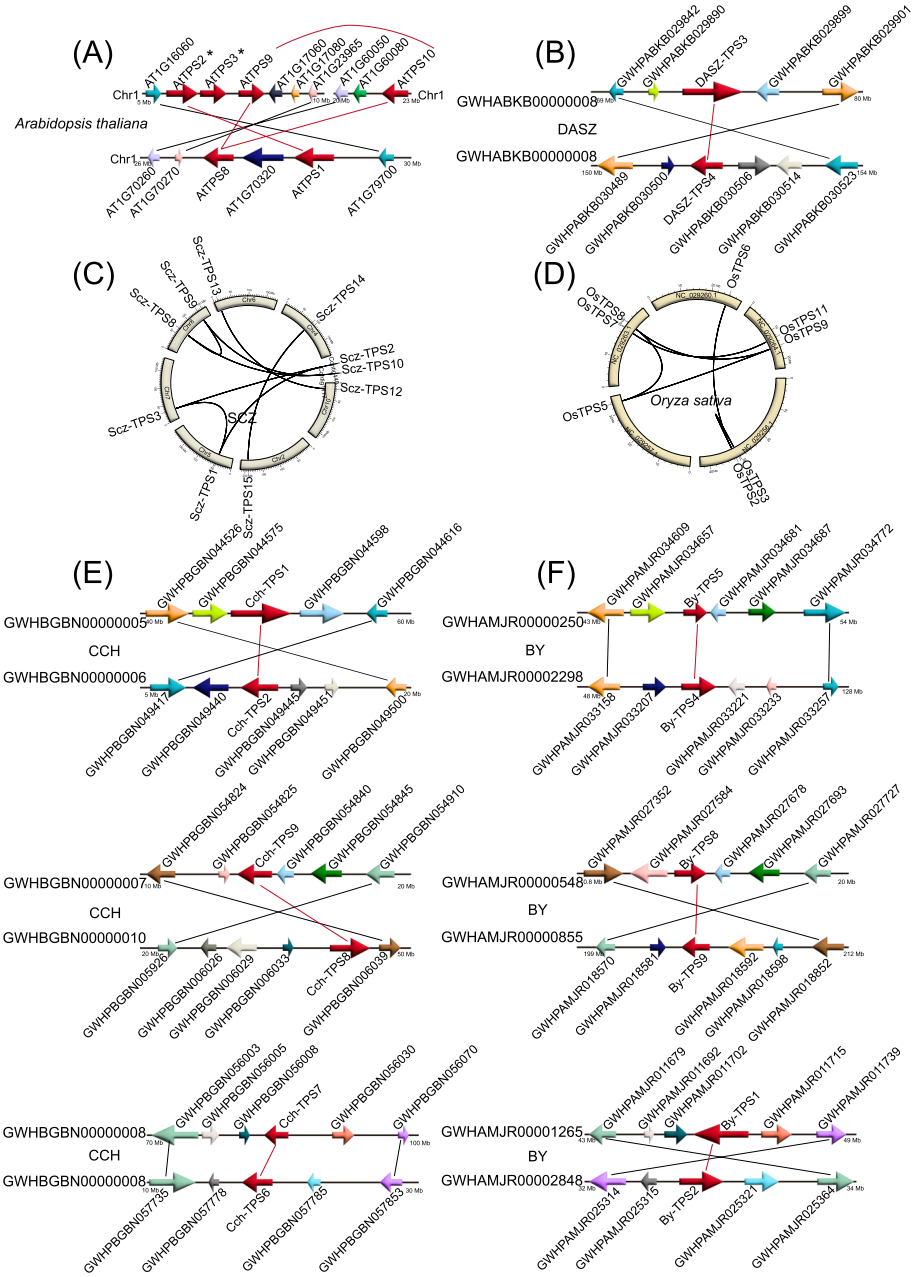

Fig. 3 The intraspecies syntenic relationship pattern diagram of *TPS* genes in *A. thaliana* (**A**), DASZ (**B**), Shuchazao (**C**), rice (**D**), *Camellia chekiangoleosa* (**E**), and Biyun (**F**). The *TPS* genes were displayed with red colors. The collinearity genes were lined by lines and the tandem genes were displayed with black stars. Other colors indicate genes located around the *TPS* and on the collinear blocks

leaf, old leaf, apical bud, flower, and fruit (Fig. 6A). Most of the *TPS* genes were ubiquitously expressed, with the exception of *TPS7/8/14* which were particularly expressed in the flower, albeit in very low level. Furthermore, both analogous and distinct expression patterns were observed among the *TPS* genes in each clade. *TPS1*, *TPS2*, and *TPS3* (Clade I) were ubiquitously expressed, with *TPS1* showing higher expression than *TPS2* and *TPS3*. It is worth noting that *TPS8* and *TPS9* (Clade II-3), *TPS14* and *TPS15* (Clade II-5), as four duplication genes, displayed different expression patterns. *TPS9* and *TPS15* display a high expression in the tissues tested, whereas the transcripts of *TPS8* and *TPS14* were barely detected (Fig. 6A).

Similar expression patterns were also identified in *TPS* genes of other Theaceae species (Fig. 6B-I). As for

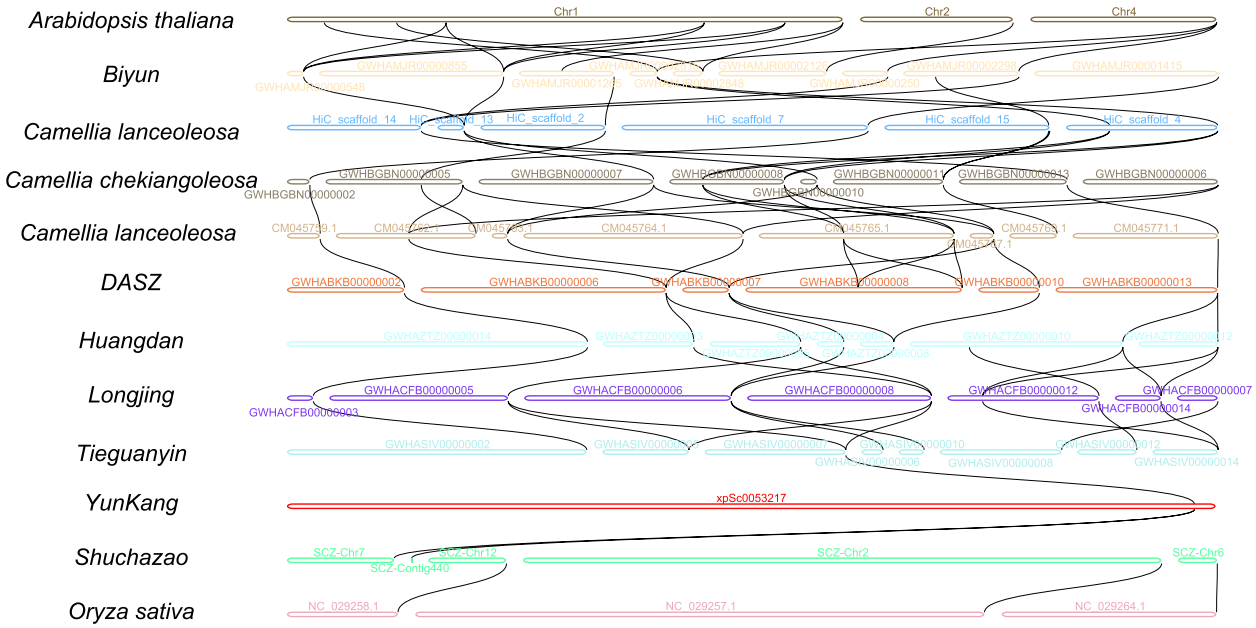

Fig. 4 The interspecies syntenic relationships of *TPS* genes among Theaceae, rice, and *A. thaliana* are depicted. The collinearity of orthologous *TPS* genes is represented by black lines

the members of the Shuchazao clade I *TPS* genes, the clade I *TPS* genes of other species showed a similar expression pattern in the leaf, stem, flower and root tissues. For example, *Cch-TPS1* and *Cch-TPS2*, *Cla-TPS1* and *Cla-TPS2*, *Hd-TPS1* and *Hd-TPS2*, *Lj-TPS1* and *Lj-TPS2*, *Tgy-TPS1* and *Tgy-TPS2*, *By-TPS1* and *By-TPS2*, as twelve duplication genes, showed similar expression levels in the tissues tested. *Cch-TPS7, Cla-TPS8*, *DASZ-TPS4, Hd-TPS8* were highly expressed in all the tissues, whereas the expression of their paralog genes *Cch-TPS6, Cla-TPS7*, *DASZ-TPS3*, *Hd-TPS7* were barely detected (Fig. 6B-I). These findings suggest that Theaceae *TPS* genes have experienced significant functional divergence throughout their evolutionary history.

It has been shown that TPS proteins play a crucial role in regulating gene expression. To investigate the possible functions of tea *TPS* genes, we examined their expression patterns in Shuchazao under conditions of cold, drought, salt stress, and MeJA treatment (Fig. 7). During periods of cold, the expression of the majority of *TPS* genes were increased (Fig. 7A), whereas their expression levels were decreased under salt stress (Fig. 7C). *TPS15* was upregulated under both drought and salt conditions, while *TPS5* displayed an opposite trend to drought and salt stresses (Fig. 7B, D). In addition, distinct patterns of expression were observed between TPS paralogs. For example, *TPS1* was upregulated under cold stress, while *TPS2* was downregulated under drought and salt stresses. *TPS15* was upregulated under cold, drought and salt stresses, while *TPS14* was only downregulated under MeJA condition. *TPS9* and *TPS13* were differentially expressed under cold, salt and MeJA stresses, whereas the expressions of *TPS8* and *TPS12* were not changed.

We further examined the expression of *TPS* genes under both abiotic (heat, cold, drought, salt) and biotic stress (*Ectropis obliqua*) conditions in the local tea variety with quantitative RT-PCR (Fig. 8). The qPCR showed that *TPS15* was significantly upregulated under heat, cold, drought, salt and *Ectropis obliqua* stresses, while *TPS5* was significantly decreased under drought, salt and *Ectropis obliqua* stresses. *TPS1* was upregulated under cold stress; *TPS2*, *TPS3*, *TPS4*, and *TPS13* were upregulated under heat stress; *TPS4* and *TPS9* were upregulated under *Ectropis obliqua* stress. *TPS10* and *TPS11* were specially upregulated under salt and cold treatments, respectively. The findings reveal that different *TPS* genes exhibit distinct expression levels when subjected to various stresses, indicating their participation in stress response mechanisms.

Discussion

Molecular evolution of TPS proteins in Theaceae

TPS genes play vital roles in plant growth, development, and stress responses [40, 41]. *TPS* genes have been identified and characterized in many plants, including *A. thaliana* [12], rice [12], wheat [17], cotton [16], and potato [42]. However, the *TPS* gene family in tea and

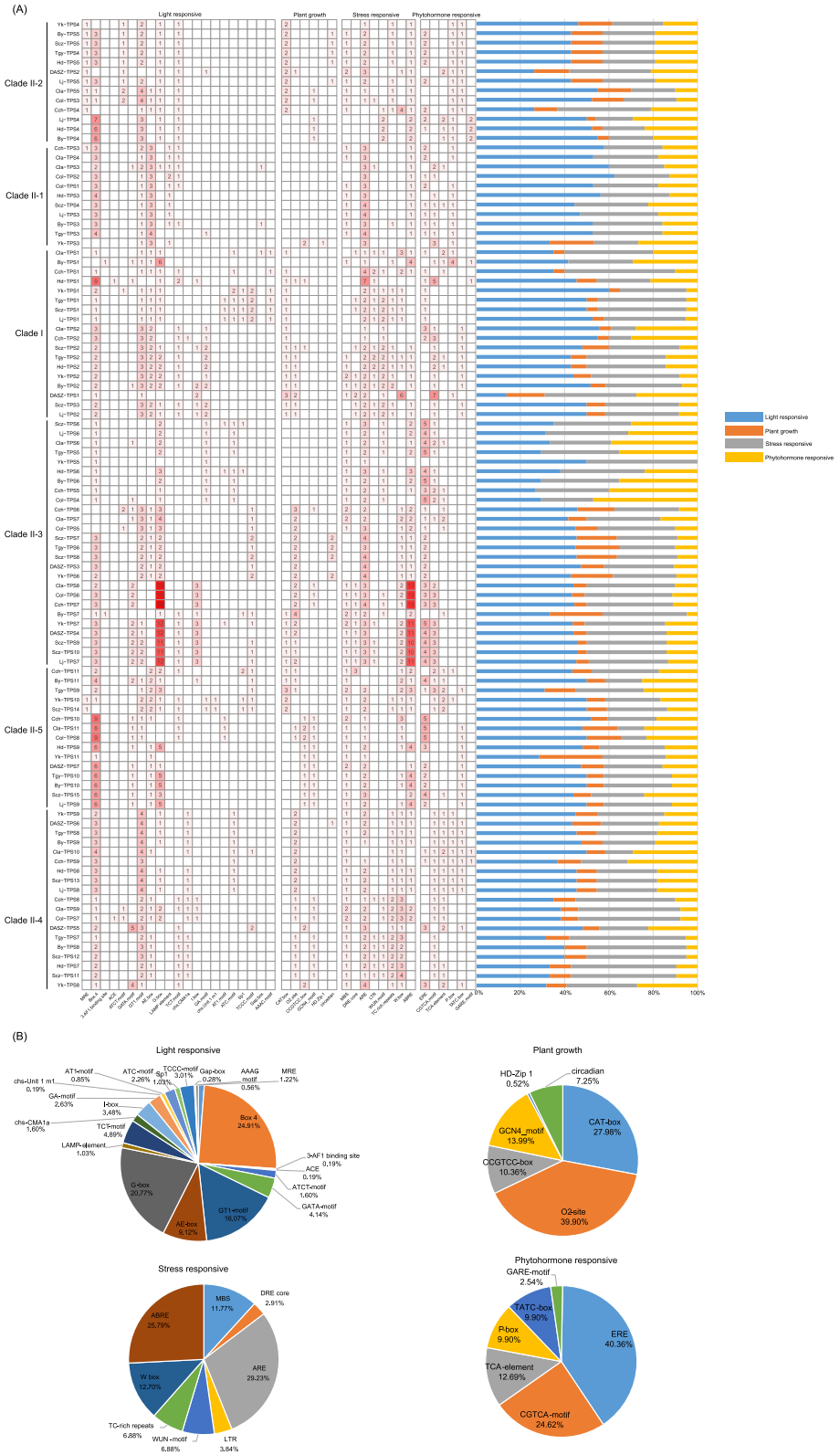

Fig. 5 Predicted cis-elements in the promoters of *TPS* genes are shown. **A** The count of cis-acting elements. **B** The distribution of various types of cis-acting elements within each category

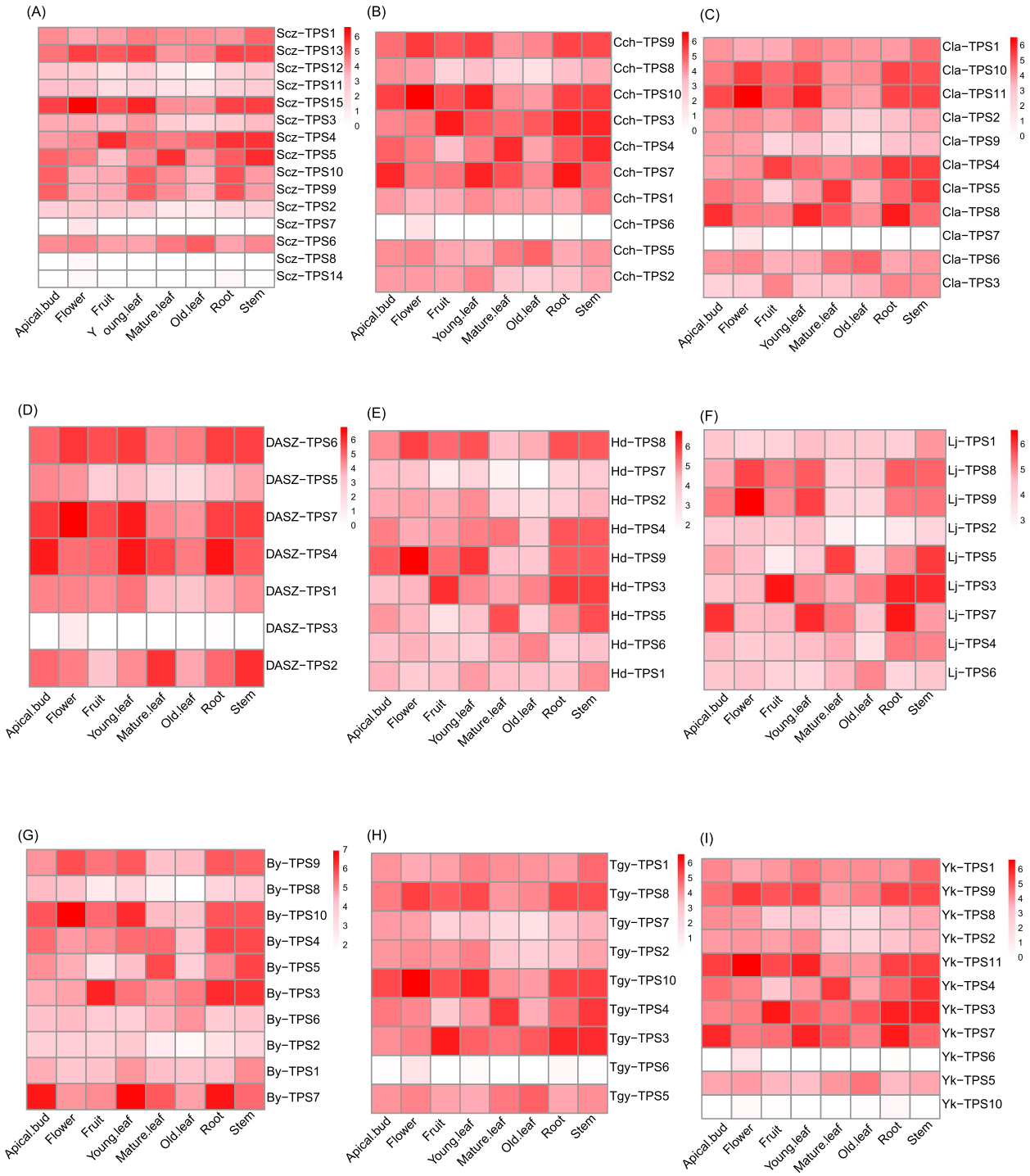

Fig. 6 Expression profiles of *TPS* genes. **A** Shuchazao. **B** *Camellia chekiangoleosa*. **C** *Camellia lanceoleosa*. **D** DASZ. **E** Huangdan. **F** Longjing-43. **G** Biyun. **H** Tieguanyin. **I** Yunkang. Gene expression values were calculated and normalized with $\log_2(\text{TPM}+1)$

oil tea has not been systematic studied. Herein, we present a first comparative evolutionary analysis of the *TPS* family in Theaceae species. *102* TPS genes were identified in ten Theaceae species, and the *TPS* gene numbers ranged from 7 to 15, indicating that *TPS* genes have experienced different gene duplication events in different Theaceae species (Fig. 1). Based on the phylogenetic analysis, the Theaceae *TPS* genes were divided into two

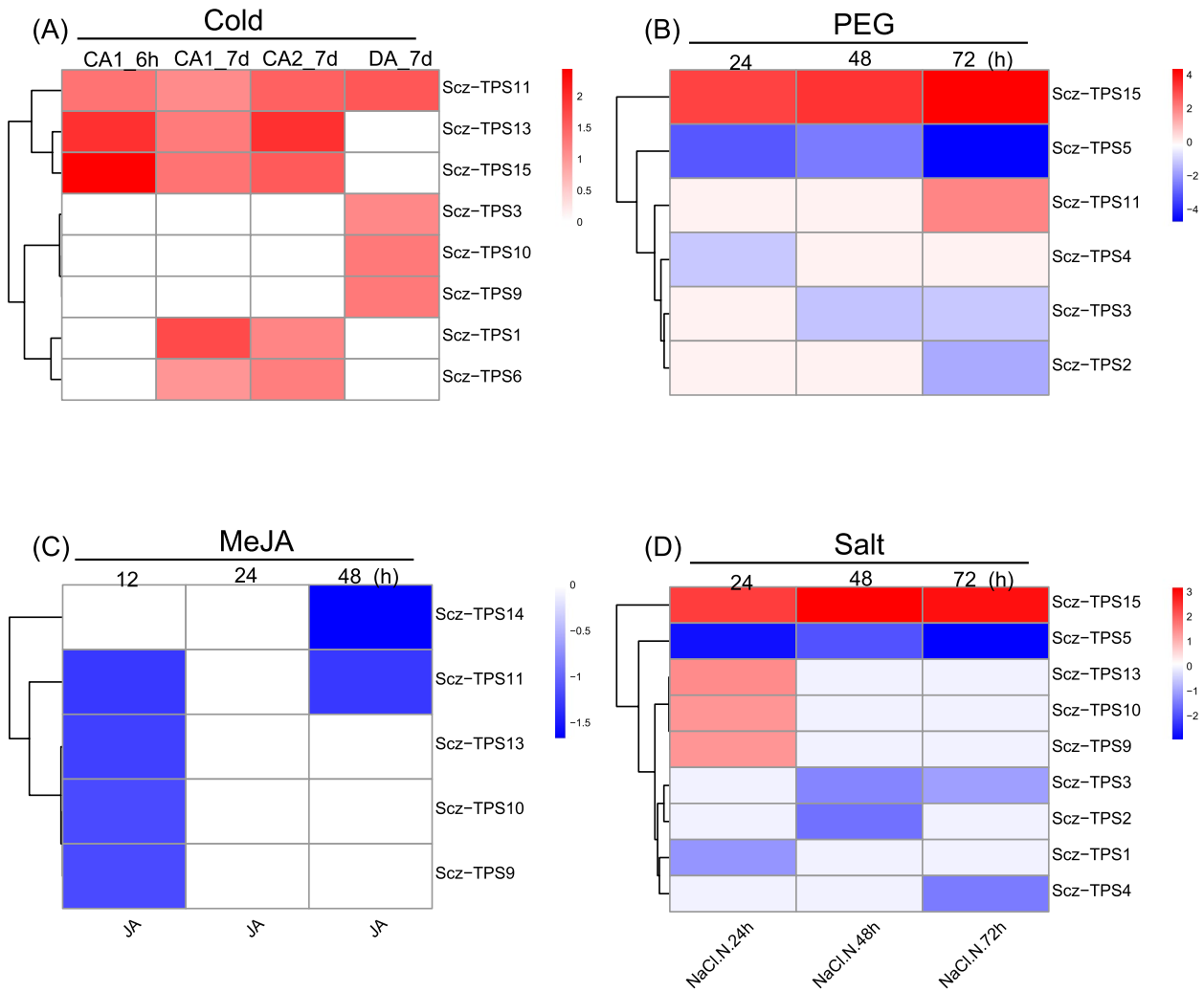

Fig. 7 Assessment of Shuchazao *TPS* genes expression under cold (**A**), drought (**B**), MeJA (**C**), and salt (**D**) conditions. Expression values were analyzed according to RNA-seq data. Under cold stress, all TPS genes were upregulated, while under MeJA treatment most TPS genes were downregulated

main subfamilies (clade I and clade II), which was consistent with the classification in *A. thaliana*, rice, and *populus* [12]. More *TPS* genes were classified in clade II, and clade II *TPSs* was further classified into five clusters: II-1, II-2, II-3, II-4, and II-5.

The two *TPS* subfamilies displayed distinct characteristics in terms of conserved motifs and gene structures (Fig. 2). In comparison to clade II TPSs, clade I *TPS* genes had a higher number of introns and exhibited more complex structures. The *TPS* genes in clade I typically contained 16 introns, while those in clade II generally had two introns (Fig. 2), which was consistent with the gene structures of *TPS* genes in *A. thaliana* and rice [12]. Domain analysis showed that motif 8 and the hydrolase_3 domain were lost in clade I TPS proteins, whereas the majority of clade II TPSs contained the hydrolase_3 domain, in agreement with the results of other studies [7, 11, 12, 16, 43]. Structural diversification has been reported to play an important role in the evolution of diverse gene family [44]. Consequently, the distinctive features of clade I and clade II Theaceae TPSs imply that these two groups of *TPS* genes experienced different pathways of molecular evolution and functional divergence.

There are more *TPS* members identified in clade II than in clade I (Fig. 1). In addition, many lineage-specific duplication events were identified in Theaceae *TPSs* (Fig. 1). For example, in clade I, clade II-2, clade II-4 and clade II-5, Theaceae *TPSs* underwent one duplication forming two branches, in clade II-3 two duplication

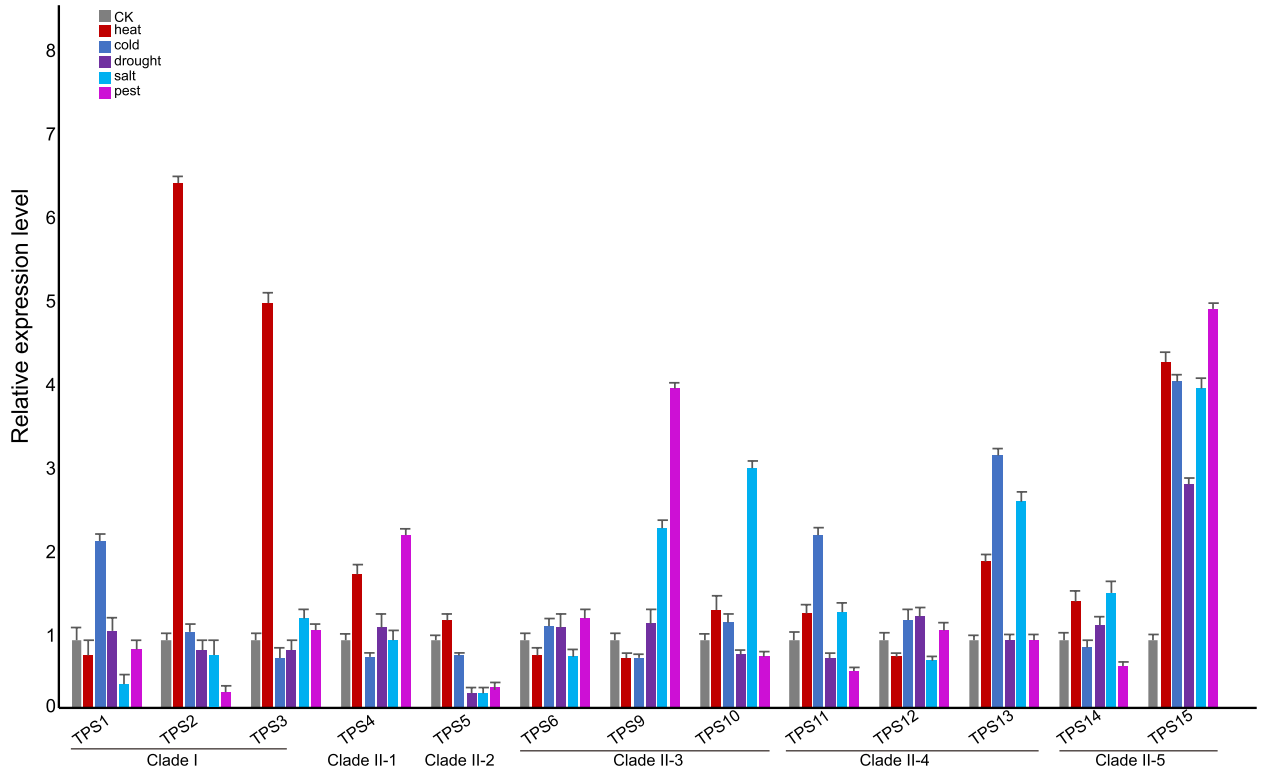

Fig. 8 The expression of *TPS* genes in response to heat, cold, drought, salt, and *Ectropis obliqua* Prout treatment was analyzed. Expression levels were normalized using *PTB* gene expression as a reference point, with the control group designated as '1'. Mean values were derived from three replicates, and error bars indicate the standard deviations of the biological replicates

events were identified, while in clade II-1 no duplication event was occurred. In clade I, clade II-4, clade II-5, and clade II-3, the duplicated TPSs were maintained in most Theaceae species, while in clade II-1, most Theaceae species lost one copy of TPS duplicates. Numerous segmental duplication events were identified in Theaceae species, suggesting that the proliferation of *TPS* genes within this family primarily resulted from segmental duplication. This expansion of the *TPS* gene family indicates its significant biological functions in tea and oil tea.

Expression divergence of *TPS* genes

Expression divergence was reported in *TPS* genes of *A. thaliana* and rice [12] (Figure S2). Most *TPS* genes were expressed in all tissues examined, while *AtTPS2, AtTPS2, AtTPS4*, and *OsTPS9* were selectively expressed at low levels in some specific tissues (Figure S2). It has been reported that clade I and clade II *TPS* genes displayed different characteristics in expression profiles, enzyme activities and physiological functions [3]. *A. thaliana* has four clade I *TPS* genes, with *AtTPS1* was ubiquitously expressed while *AtTPS2, AtTPS2,* and *AtTPS4* were low expressed. Only AtTPS1 was proved to have TPS activity [7, 8]. Most Theaceae species contained two clade I *TPS* genes (Fig. 1), and most of the duplicated paralogs displayed similar expression patterns (Fig. 6). Theaceae clade I *TPS* genes clustered closer and displayed higher sequence similarity to *A. thaliana TPS1*, indicating that Theaceae class I TPS proteins may have functions similar to AtTPS1. In addition, the low-copy numbers of *TPS* genes in clade I suggested a functional conservation during the long-term evolution.

The members of Class II TPS exhibit distinct expression patterns when exposed to different stress conditions (Fig. 7). Under cold stress, most of the *TPS* genes were upregulated, while under the MeJA condition, most of them were downregulated. *Scz-TPS15* was observed to be induced by multiple stresses, such as drought, cold, and salt, whereas *Scz-TPS5* was repressed by drought and salt (Figs. 7, 8). Expression divergence was also identified in the duplicate pairs of *TPSs* (Figs. 6, 7, 8). One copy of each seven duplicate gene (*Cch-TPS7/Cch-TPS6*, *Cla-TPS7/Cla-TPS8*, *DASZ-TPS3/DASZ-TPS4*, *Hd-TPS7/Hd-TPS8*, *Scz-TPS8/Scz-TPS10*, *Scz-TPS14/Scz-TPS15*, *Tgy-TPS7/Tgy-TPS8*) was highly expressed in all tissues examined, however, the transcript levels of the other paralog was low expressed only in a specific tissue (Fig. 6). Under stress conditions, expression divergence

was observed in the duplicate gene pairs (Figs. 7, 8). Specifically, one copy of each of the four duplicate gene pairs (*Scz-TPS1*/*Scz-TPS2*, *Scz-TPS9*/*Scz-TPS10*, *Scz-TPS12*/*Scz-TPS13*, *Scz-TPS14*/*Scz-TPS15*) exhibited differential expression under stress conditions, while the other paralog did not respond to various treatments. These findings suggest that these duplicate genes have undergone subfunctionalization or functional divergence. Further research is required to gain deeper insights into their expression patterns and functional divergence in relation to tea plant growth and stress responses.

Materials and methods

Data sources and sequence acquisition

TPS genes from *A. thaliana* and rice were obtained from TAIR11 (https://www.arabidopsis.org/) and RiceFREND (https://ricefrend.dna.affrc.go.jp), respectively [45]. Genomic and protein sequences for tea plants and oil tea plants were sourced from TeaPGDB (https://eplant.njau.edu.cn/tea/index.html), GitHub (https://github.com/Hengfu-Yin/CON_genome_data), NGDC (https://ngdc.cncb.ac.cn/), and NCBI (BioProject: PRJNA780224) [46]. Although the tea plants have annotated genomes, the *TPS* gene candidates might be mis-annotated during the automated genome annotation process. Two approaches were employed to identify TPS homologs in tea and oil tea. The conserved domains of TPS proteins, specifically Glyco-transf-20 (PF00982) and TPP (Trehalose_PPase, PF02358), were retrieved from Pfam [47]. HMMER was utilized for candidate TPS protein identification with an E-value cutoff of 1e-10 [48]. Additionally, the Basic Local Alignment Search Tool algorithms (BLASTP) were applied using the amino acid sequences of *A. thaliana* and rice TPS members against a protein database with the following parameters: maximum target sequence: 100, expected threshold: 13, word size: 10, scoring matrix: BLOSUM62, gap cost: existence 11 and extension 1, compositional adjustments: conditional compositional score matrix adjustment. The intersection of genes acquired using these two methods was used as a screening criterion for candidate TPS genes. The identified *TPS* genes were input to Pfam (http://pfam.xfam.org/) to establish the existence of TPS domains. The integrity of the domain was confirmed using online tools such as CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/) [49], HMM (https://hmmer.org/), Geneious (http://www.geneious.com) and SMART (https://smart.embl-heidelberg.de/) [48, 50]. The basic information on TPS proteins, including molecular weight (MW), isoelectric point (pI), and subcellular locations were predicted using the Expasy (https://web.expasy.org/protparam/) and GenScript (https://www.genscript.com/wolf-psort.html) websites [51]. The secondary structures of TPS proteins were predicted using the PRABI website (https://npsa-prabi.ibcp.fr/cgibin/npsa_automat.pl?page=npsa_sopma.html). The collinearity and selective evolutionary pressure of TPS genes were analyzed using the TBtools software [52].

Phylogenetic analyses, gene structure, motif analyses, and expression analysis

Multiple alignments of full-length TPS proteins were conducted using MAFFT software [53]. The maximum likelihood (ML) phylogenetic tree was generated using IQ-TREE2 with the parameters '-m MFP -B 1000' and included 1000 ultra-bootstrap replicates [54]. The gene structure was assessed and visualized through the Gene Structure Display Server (http://gsds.cbi.pku.edu.cn/) [55]. Conserved motifs were detected using the online MEME program (http://meme-suite.org/tools/meme) [56]. Transcript levels of *TPS* genes were investigated by using the public available RNA-seq data as shown in previous studies [30, 31, 36, 37]. Gene expression values were calculated and normalized with $\log_2(\mathrm{TPM}+1)$.

Synteny analysis

First, we utilized the BLAST tool to conduct sequence alignment. Specifically, we constructed a BLAST database using the protein sequence file of the target species as the reference. Subsequently, we employed the protein sequence file of the species to be compared as the query sequence and performed the alignment using blastp. The alignment parameters were set as follows: an E-value threshold of 1e-10, 12 threads, and output format 6 (standard table format). Upon completion of the sequence alignment, we merged the alignment result file with the genome annotation files of both species. We then executed the MCScanX tool, inputting the merged GFF file and the BLAST alignment result file, to generate collinearity analysis results. Following the completion of the MCScanX analysis, multiple output files were generated, including an HTML-formatted collinearity report, a collinearity alignment file, and a tandem repeat gene file. These files can be further utilized to construct collinearity dot plots, thereby providing a visual representation of the collinear regions between genomes.

RNA extraction and qRT-PCR analysis

For the qRT-PCR analysis, a 2-year-old local tea variety (Camellia sinensis cv. Xinyanghong 10) cultivated at Xinyang Normal University (23 ± 3 °C, 65 ± 5% room humidity, with a day/night cycle of 16/8 h) was selected for the experiments. In the drought treatment, plants were subjected to water deprivation for seven days. For salt stress, leaves were treated with a solution of 150 mM NaCl for six hours. Heat and cold treatments

involved exposing the plants to temperatures of 40 °C and 4 °C for six hours each. Additionally, twelve larvae from the third or fourth instar of *E. obliqua* were placed on three unfed tea plants for a duration of twenty-four hours to assess stress response. Plants that were not subjected to any stress served as the control (CK). Total RNA was extracted, and one microgram of this RNA was reverse transcribed into cDNA using the PrimeScript RT reagent Kit. Quantitative RT-PCR (qRT-PCR) was conducted with SYBR Premix EX Taq on an ABI StepOnePlus instrument. The relative expression levels were determined using the $2-\Delta Ct$ method, with CsPTB acting as the internal control gene. The experiments were conducted in triplicate. Specific primers were created using Primer 5 software (Table S5).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-025-11475-3.

Supplementary Material 1: Figure S1. The intraspecies syntenic relationship of *TPS* genes in *Huangdan* (A), *Camellia lanceoleosa* (B), *Tieguanyin* (C), Longjing (D), and *Camellia oleifera* (E),respectively.

Supplementary Material 2: Figure S2. The expression profiles of *TPS* genes in *Arabidopsis thaliana* and *Oryza sativa*.

Supplementary Material 3: Table S1. The identified TPS protein sequences. Table S2. The Ka/Ks analysis of *TPS* genes. Table S3. *Cis*-acting elements of *TPS* genes. Table S4. The expression of *TPS* genes. Table S5. Primers used to detect the expression of tea *TPS* genes in this study.

Authors' contributions

The study was conceived and directed by ZZB. ZZB and XT wrote the manuscript. XT, LKJ, HKX, LCX and LGQ performed the identification of *TPS* genes, protein structure, and evolution analysis. All the authors read and approved the final manuscript. All authors consent for publication.

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Data availability

The authors confirm that no new genes or proteins were generated in this study and all analyses were based on existing data in the databases. The data underlying the findings of this study are presented in the article and its supplementary materials. A. thaliana gene sequence data from this article can be found in the TAIR database (https://www.arabidopsis.org/). Oryza sativa gene sequence data from this article can be found in the NCBI database (https://www.ncbi.nlm.nih.gov/). Theaceae gene sequence data from this article can be found in the TPIA database (http://tpia.teaplants.cn/) and Github (https://github.com/Hengfu-Yin/CON_genome_data). The corresponding TPS accession numbers are in Table S1.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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